International Journal of

JAEFS

Agriculture, Environment and Food Sciences

Volume 7 • Issue 1 • March 2023

e-ISSN: 2618-5946

edit PUBLISHING

www.jaefs.com

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

EDITORIAL BOARD

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

REVIEWER BOARD

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

JOURNAL INFORMATION

Journal Name	International Journal of Agriculture, Environment and Food Sciences
Abbreviation	Int. J. Agric. Environ. Food. Sci.
Subjects	Agriculture, Environment and Food Sciences
e-ISSN	2618-5946
Publisher	Edit Publishing
Language	English
Frequency	Quarterly (March, June, September, December)
Price Policy	Editorial Processing Charges (EPCs) are paid by authors or their institution. This fee will be requested regardless of the acceptance/rejection condition of the article.
Type of Publication	International, Scientific, Open Access, Double blinded peer review, Widely distributed periodical
Manuscript Submission and Tracking System	JAEFS uses the submission system of TUBITAK ULAKBIM JournalPark Open Journal Systems - http://dergipark.gov.tr/jaefs
Licence	Journal is licensed under a Creative Commons Attribution 4.0 International License
Legal Responsibility	Authors are responsible for content of articles that were published in Journal.
Indexed and Abstracted in	TÜBİTAK ULAKBİM TR Dizin, Directory of Open Access Journal (DOAJ), AGORA (Access to Global Online Research in Agriculture), AGRIS (Agricultural Science and Technology Information), WorldCat, SOBIAD, Scilit, ROAD (Directory of Open Access Scholarly Resources), Neliti, International Citation Index, ROOT Indexing, ResearchBib, Index Copernicus International, ESJI, JournalTOCs, TEELS, ResearchGate, Microsoft Academic, Crossref, Google Scholar

Publisher Contact

Edit Publishing

Dicle Teknokent Yiğit Çavuş Mah. Silvan Yolu Üzeri Kat:2 No:26, Diyarbakır, Türkiye

Web: https://editpublishing.com

E-mail: info@editpublishing.com

Phone: +90 850 309 59 27

Journal Contact

International Journal of Agriculture, Environment and Food Sciences

Prof.Dr. Gültekin Özdemir (Editor-in-Chief)

Dicle University Faculty of Agriculture Department of Horticulture, Diyarbakir, Türkiye

Web: www.jaefs.com - dergipark.gov.tr/jaefs

E-mail: editor@jaefs.com

Phone: +90 532 545 07 20

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit
PUBLISHING

AIM AND SCOPE

"International Journal of Agriculture, Environment and Food Sciences" (JAEFS) is an international

journal, which publishes original research and review articles dealing with Agriculture, Environment and Food Sciences.

JAEFS Journal is an international, scientific, open access periodical published in accordance with independent, unbiased, and doubleblinded peerreview principles.

Journal publishes Quarterly (March, June, September, December).

The publication language of the journal is English and continues publication since December 2017.

A Digital Object Identifier (DOI) number has been assigned for each article accepted to be published in JAEFS, starting from December 2017.

International Journal of Agriculture, Environment and Food Sciences are as of interest include, but are not limited to: Agricultural Engineering and Technology, Environmental Engineering and Technology, Food Engineering and Technology, Agriculture, Environment, Food, Agriculture History, Agricultural Economics, Agronomy, Animal Sciences, Aquaculture, Biochemistry, Biotechnology, Bioinformatics and Data Science, Crop Science, Dairy Science, Extension Science and Education, Entomology, Environmental Science, Fish and Fisheries, Food Processing, Food Chemistry, Food Culture, Food Health and Nutrition, Food History, Food Industry Development, Food Marketing, Food Policy and Practices, Food Safety, Forestry, Horticulture, Information Technologies and Systems, Irrigation, Molecular Biology, Organic Agriculture, Plant Physiology, Plant Breeding, Plant Protection, Plant Sciences, Precision Agriculture, Rural Development and Policy, Sustainable Agriculture, Soil Science, Plant Nutrition, Energy Crops, Veterinary, Water Resources.

Open Access Statement

International Journal of Agriculture, Environment and Food Sciences (JAEFS) publishes fully open access journal, which means that all articles are available on the internet to all users immediately upon publication.

All the original articles and review papers published in JAEFS journal are free to access immediately from the date of publication.

Price Policy

In order to start the evaluation process of the articles sent to the International Journal of Agriculture, Environment and Food Sciences, an Editorial Processing Charges (EPCs) will be requested.

This fee will be requested regardless of the acceptance/rejection condition of the article.

Authors pay a one-time Editorial Processing Charges (EPCs) to cover the costs of peer review administration and management, professional production of articles in PDF and other formats, and dissemination of published papers in various venues, in addition to other publishing functions. Please note that the option to process an advance payment remains but does not guarantee acceptance of manuscripts.

International Journal of Agriculture, Environment and Food Sciences (JAEFS) don't charge any fees for any reader to download articles and reviews for their own scholarly use.

Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. This is in accordance with the BOAI definition of open access.

International Journal of Agriculture, Environment and Food Sciences also operates under the Creative Commons Licence CC-BY-NC.

All authors publishing with the JAEFS accept these as the terms of publication.

Benefits of open access for Authors include:

Free access for all users worldwide

Authors retain copyright to their work

Increased visibility and readership

Rapid publication

No spatial constraints

Copyright Policy

Authors who publish with International Journal of Agriculture, Environment and Food Sciences (JAEFS) agree to the following terms:

Authors transfer the copyright to International Journal of Agriculture, Environment and Food Sciences (JAEFS) . Authors, however, upon the publication of their work, can republish it in an another journal or book provided that they obtain the permission of the Journal. Moreover, they are to include the note indicating that their work has first been published in International Journal of Agriculture, Environment and Food Sciences (JAEFS) and the link to their work in the journal.

Authors, upon the publication of their work, can publish it in online environments and/or social networks (personal webpages, course content websites, Facebook, etc.). However, they also provide the link to the work as published in the journal.

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

INSTRUCTIONS TO AUTHORS

MANUSCRIPT PREPARATION

Please read all of the following manuscript preparation instructions carefully and in their entirety. The manuscript must be in good scientific English; this is the author's responsibility.

All authors must have read and approved the most recent version of the manuscript.

Manuscripts can only be submitted through the International Journal of Agriculture, Environment and Food Sciences

online manuscript submission and evaluation system

Manuscripts submitted via any other medium will not be processed.

Manuscripts submitted to the International Journal of Agriculture, Environment and Food Sciences will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines.

Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

The journal management requires that all authors register to ORCID at http://orcid.org/register and get a free ORCID ID.

All submissions are screened by a similarity detection software.

We are using iThenticate plagiarism solution and our maximum allowed score, for the document in which the Materials and Methods and References sections truncated, is 20%. Higher scores are not allowed and the author will be given the plagiarism report and the manuscript will be returned to the authors.

You may download the International Journal of Agriculture, Environment and Food Sciences

Manuscript Template here.

MANUSCRIPT STRUCTURE

Font

Manuscripts must be prepared as a Microsoft Word file.
Word document, Times New Roman, 10 point, single line space.
Page margins are 2.5 for all sides.

Length

Maximum length for articles is 20 pages.
Articles over 20 pages in length can only be considered on an exceptional basis.

Title

A concise title of the paper.
Use bold 14-point Times Roman font.
Use title lowercase.
Make title in centered.

Name(s) and surname(s) of author(s)

Use centered, bold 10-point Times Roman font.

Affiliation(s) of author(s)

Use 8-point Times Roman font.
E-mail address of the corresponding author
Use 8-point Times Roman font.

ORCID(s) of author(s)

Use 8-point Times Roman font.

The journal management requires that all authors register to ORCID at https://orcid.org/register and get a free ORCID ID.

Abstract

Each article is to be preceded by a succinct abstract, of up to 200 words, that highlights the objectives, methods, results, and conclusions of the paper. The abstract should state briefly the purpose of the research, the principal results and major conclusions.

The abstract body is typed in Times New Roman, 10 pt.

Keywords

About four Keyword or phrases in order of importance, separated by commas and typed in Times New Roman, 10 pt.

Headings

Use bold, lowercase, 10 Times New Roman font for headings.

Introduction

This should define the problem and, if possible, the frame of existing knowledge.

Please ensure that people not working in that particular field will be able to understand the intention.

The word length of the introduction should be 150 to 300 words.

Materials and Methods

Materials and methods should be clearly presented to allow the reproduction of the experiments.

Results and Discussion

Results should be clear and concise and give the significance of the results of the work. Data must not be repeated in figures and tables.

Discussion should interpret the results in reference to the problem outlined in the introduction and of related observations by the author/s or others.

Implications for further studies or application may be discussed.

Conclusion

A conclusion should be added if results and discussion are combined.

Tables and Figures

Tables should have a short descriptive title.
The unit of measurement used in a table should be stated.
Tables should be numbered consecutively.
Figures should be prepared in GIF, TIFF, JPEG or PowerPoint.
Tables and Figures should be appropriately cited in the manuscript.

Acknowledgments

Acknowledgments of financial support, advice, or other kinds of assistance should be given at the end of the text under the heading "Acknowledgements". The names of funding organizations should be written in full.

References

They should be grouped at the end of the paper in surname order of appearance.

Use lowercase, 10 Times New Roman font for References.

The references must be written in alphabetical order by authors' names,

with the following elements:

Original Papers

Surname, N.N., Surname, N.N. (Year). The full title of the article. Journal Name, volume, and issue, first and last page. DOI number.

Book or Proceeding

Surname, N.N., Surname, N.N. (Year). Title of the contribution. In: Title of the book or proceeding. Volume (Publisher, City, first and last page

Thesis

Surname, N.N. (Year). Title of the thesis, University and Faculty, City. pages.

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

CONTENTS

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

CONTENTS

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 1-10
DOI: https://doi.org/10.31015/jaefs.2023.1.1

Evaluating the effect of cardamom on lipolysis in heat desiccated milk product (khoa)

Sagar M. Patel[1] • **Ravi P. Patel**[1] • **Bhavbhuti M. Mehta**[1] • **Amit Kumar Jain**[1]

[1] Dairy Chemistry Department, SMC College of Dairy Science, Kamdhenu University, Anand, Gujarat, India

Abstract

The present study was carried out in two phases. In phase one optimization for the stage, form and rate of the addition of cardamom to control lipolysis was carried out. The prepared khoa was packed in polypropylene (PP) containers and stored at 7±1°C and/or 15±1°C. During storage, khoa samples were evaluated at an interval of 48 hours for acceptability by sensory analysis and monitored for lipolytic changes in terms of free fatty acids (FFA) content. Based on the highest sensory acceptability and ability to control lipolysis, that stage, form and rate of addition of cardamom were selected. khoa prepared by adding fine particles of cardamom in milk at the rate of 0.3 per cent significantly control the lipolysis and maintain the flavour when stored at 7±1°C and/or 15±1°C in PP containers. In the phase two, the selected method of addition of cardamom has been used in the preparation of khoa and evaluated for chemical and sensory characteristics of khoa. The chemical characteristics, rheological properties as well as sensory evaluation of control and cardamom treated fresh samples of khoa were statistically non-significant.

Keywords: Heat desiccated milk product, Khoa, Cardamom, Lipolysis, Free fatty acid

Citation: Patel, S.M., Patel, R., Mehta, B.M., Jain, A.K. (2023). Evaluating the effect of cardamom on lipolysis in heat desiccated milk product (khoa). International Journal of Agriculture, Environment and Food Sciences, 7 (1), 1-10

Received: 22 August 2022
Revised: 09 October 2022
Accepted: 12 October 2022
Published Online: 07 January 2023

Correspondence: Bhavbhuti M. Mehta
E-mail: bhavbhuti5@yahoo.co.in

INTRODUCTION

India has emerged as the largest producer of milk in the world with over 187.7 million tonnes of production in 2018-19 (DAHD, 2019). According to Khatkar et al. (2017), there are good potential and availability of milk for the manufacture of milk products in India. Kumari et al. (2012) reported that 5.5% of the total milk produced is utilized for the production of khoa in India. Khoa is a heat desiccated dairy product. It is perishable product having lower shelf life. The higher nutritive value and water activity (a_w 0.96) of khoa are responsible for the growth of bacteria (Sawhney et al., 1994). Lipolysis as well as proteolysis shortens the shelf life of the khoa. Lipolysis produces free fatty acids that cause rancid, butyric, bitter, unclean, soapy or astringent flavour defects in dairy products (Deeth, 2011).

Various chemical preservatives are used to extend the shelf life of khoa such as potassium meta-bisulphites, propionic acid, sorbic acid, nisin, antioxidants, etc. Regular use of such food additives lead to hyperactivity, allergies, increase blood pressure and other health-related problems (Haas, 1999; Amchova et al., 2015). Therefore, there is a need for replacement of such synthetic food additives with natural origin. Cardamom is referred 'Queen of Spices' because of its very pleasant aroma and taste. It shows a health-promoting role against various conditi-

ons such as constipation, colic, diarrhoea, vomiting, headache, epilepsy, and cardiovascular diseases (Jadav and Mehta, 2018). Various researchers have used cardamom in different food products such as shrikhand, herbal beverages, basundi, kachchagola sandesh, pedha, sandesh, paneer to improve the quality and extend the shelf life (Sen and Rajorhia, 1996; Narwade, 2003; Eresam, 2009; Gupta et al., 2011; Chougule et al., 2014). However, direct studies on the effect of cardamom on lipolysis of khoa are limited. Therefore, the present study was contemplated with a view to evaluate the effect of cardamom on lipolysis in khoa.

MATERIALS AND METHODS

Material

For the preparation of khoa, standardized milk (4.5% Fat, 8.5% SNF) was collected from Amul parlour. Cardamom (*Elettaria cardamomum*) was obtained from the market. The outer husk of cardamom was removed manually. The coarse particles and fine particles of cardamom were prepared in our laboratory in using mixer grinder. The cardamom particles were passed from the different mesh size sieve to obtained coarse (8 to 15 mesh size) and fine (30 to 36 mesh size) particles. Polypropylene (PP) container was used for the packaging of samples.

Manufacturing of khoa

The samples of khoa (variety : pindi type) were prepared in the laboratory from standardized pasteurized milk using the method described by De (2004).

Incorporation of cardamom and storage study of khoa

The present study carried out in two phases (i) optimization for the rate of addition of cardamom to control lipolysis in khoa, (ii) analysis of khoa for chemical and sensory characteristics. In first phase the cardamom was added at different stages (the initial stage of milk at 40 °C and final stage of pat formation), in different forms (coarse and fine) and at different rates (0 (control), 0.1, 0.2, 0.3, 0.4 and 0.5 per cent). The subsequent steps in the preparation of khoa remained the same as described by De (2004). Each samples of khoa were packed into a PP container and stored at 7±1°C and/or 15±1°C. During storage, khoa samples were evaluated at an interval of 48 hours for acceptability by sensory analysis (flavour score) using a 9-point hedonic scale. Simultaneously, the khoa samples were also monitored for lipolytic changes. Three replications were taken. Based on the highest score in sensory evaluation and/or control the lipolysis, that stage, form and rate of the addition were selected for further study. In the phase two, the selected method of addition of cardamom were used in the preparation of khoa and evaluated for chemical, rheological and sensory characteristics of khoa.

Analysis of khoa for chemical characteristics

The free fatty acids content was determined by the extraction titration method as suggested by Deeth et al. (1975) with certain modifications. The acidity of the khoa sample was estimated according to BIS (1980) procedure specified for khoa.

Analysis of khoa for proximate composition

Moisture and ash content in khoa were determined according to BIS (1983) procedure specified for khoa. The fat content of khoa was estimated by the method described by Ladkani and Mulay (1974). Protein content was determined by the Kjeldahl method as described by Horwitz (1980). Carbohydrate (lactose) content in khoa was estimated by difference.

Determination of rheological properties of khoa

Rheological properties of khoa like chewiness, hardness, springiness, cohesiveness, gumminess and adhesiveness were analyzed by the Texture profile analyzer. Compression testing of khoa samples was done with Lloyd Instrument, Hampshire, UK (Model No. 01/2962).

Statistical analysis

The data obtained for each of the attributes under study were subjected to statistical analysis in a completely randomized design.

RESULTS AND DISCUSSION

Effect of stage of addition of cardamom on flavour score and lipolysis of khoa

Changes in flavour score of khoa revealed that there were a significant ($p<0.05$) difference between the flavour score of the khoa when cardamom was added at different stages. The data obtained for changes in flavour score of khoa during storage at 7±1°C are presented in Table 1 and the trend is depicted in Figure 1.

Table 1. Effect of stage of addition of cardamom on flavour score of khoa stored at 7±1°C

Storage periods (days)	Flavour score			
	Control	Milk	Pat formation	Mean (Days)
0	8.27	8.57	7.73	8.19
2	8.10	8.37	7.77	8.08
4	7.20	7.67	7.03	7.30
6	6.90	7.53	6.93	7.12
8	5.50	6.70	6.17	6.12
10	4.47	6.13	5.40	5.33
12	4.23	6.03	5.43	5.23
14	4.20	5.13	5.10	4.82
Mean	6.11	7.02	6.45	
Source of variation		Treatment (Stage)	Days	Treatment (Stage) x Days
SEm		0.125	0.204	0.353
CD (0.05)		0.36	0.58	NS
CV (%)		9.36		

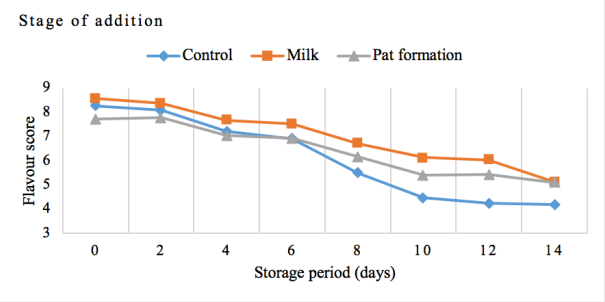


Figure 1. Effect of stage of addition of cardamom on flavour score of khoa stored at 7±1°C

The changes in flavour score revealed that both cardamom and storage period had significant ($p<0.05$) effect on the flavour score of the khoa. The flavour score decreased with the increasing storage period in all the khoa samples. Figure 1 revealed that extent of decrease in flavour score of khoa samples with cardamom added in milk was relatively less as compared to other samples upon storage. On the 8th day of storage, the flavour score was remained acceptable in all samples and maximum flavour score (6.70) was observed in a sample in with cardamom added in milk. The flavour score of all samples was unacceptable at the end of 14th days of storage when stored in PP containers at 7±1°C.

Desale and More (2017) observed that on the day of manufacture, highest flavour score (9) were obtained by cardamom treated basundi, followed by saffron treated basundi (8.4), while control samples and combination samples obtained minimum flavour score (8). Further, it was observed that the flavour scores of basundi decreased with increasing storage period (0-8 days). Prasad et al. (2017) worked on anti-oxidative, physico-chemical and sensory attributes of burfi affected by the incorporation of different herbs (clove, turmeric, basil leaves, curry leaf, ginger and cardamom) and its comparison with synthetic anti-oxidant (BHA). It was observed that the highest flavour (7.50) score was obtained by cardamom added burfi and the lowest flavour score of 5.20 was obtained for basil leaves incorporated burfi. Sensory evaluation revealed that among these herbs, cardamom added burfi was highly preferred followed by ginger, turmeric, clove, curry leaves and basil leaves.

The extent of lipolysis in khoa samples was measured using changes in free fatty acids (FFA) value. The changes in FFA value (per cent oleic acid) of khoa samples during storage at 7±1°C is presented in Table 2 and Figure 2.

A significant difference ($p<0.05$) was observed in the FFA content of cardamom treated and control samples of khoa. Though during storage FFA content was increased, the rate of increase in FFA of khoa samples with cardamom added in milk was relatively less as compared to other samples. The FFA content in the sample was 0.199 per cent oleic acid on 14th day of storage when khoa prepared from cardamom added in milk. This value of FFA was lowest among the all other samples indicating it was able to control the lipolysis in a sample during storage.

Table 2. Effect of stage of addition of cardamom on free fatty acids of khoa sored at 7±1°C

Storage periods (days)	FFA (% oleic acid)			
	Control	Milk	Pat formation	Mean (Days)
0	0.150	0.149	0.148	0.149
2	0.166	0.157	0.158	0.160
4	0.175	0.158	0.174	0.169
6	0.174	0.174	0.174	0.174
8	0.191	0.174	0.174	0.180
10	0.200	0.182	0.200	0.194
12	0.216	0.191	0.199	0.202
14	0.223	0.199	0.215	0.213
Mean	0.187	0.173	0.180	

Source of variation	Treatment (Stage)	Days	Treatment (Stage) x Days
SEm	0.002	0.003	0.005
CD (0.05)	0.005	0.009	NS
CV (%)	5.07		

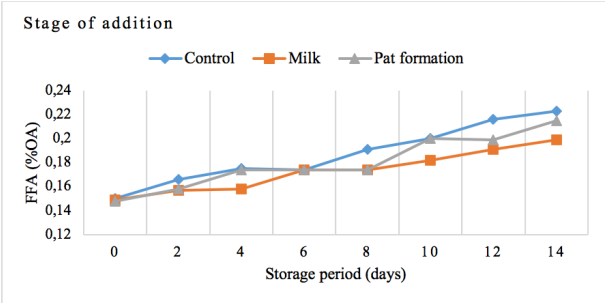


Figure 2. Effect of stage of addition of cardamom on FFA (% oleic acid) of khoa stored at 7±1°C

Gawde (2005) worked on enhancing the shelf life of kalakand using preservatives *viz*. cardamom, saffron and sorbic acid. It was observed that there was significant variation ($p<0.05$) in the free fatty acid content of kalakand due to different preservatives *viz*. saffron, cardamom and sorbic acid. The kalakand prepared with 0.15 per cent sorbic acid recorded minimum FFA of 0.23 per cent which was at par with 0.15 per cent cardamom (0.24 per cent) but different from the rest of the treatment. It could be seen that the storage period had significant ($p<0.05$) impact on the FFA content of kalakand. Ratiba et al. (2006) found that total volatile fatty acids (TVFA) of all cheese samples increased significantly ($p\leq0.05$) throughout the storage period when cardamom, thyme and clove powder were evaluated. There was some slight difference between the control and all other treatments in TVFA at the first stage of storage and the rate of increase in TVFA varied considerably among the treatments during storage. Cheese treated with 0.15 per cent thyme had the lowest value

throughout the storage period (45 days) while the control had the highest value of TVFA than other treatments. This suggested that added spices to the curd of cheese led to a slight decrease in TVFA because of inhibition of lipolytic enzymes.

Thus, the addition of cardamom at initially in milk having 40°C temperature was able to control both flavour as well as lipolysis in khoa during storage and hence this stage of the addition was selected for further study.

Effect of form of addition of cardamom on flavour score and lipolysis of khoa

Changes in flavour score of khoa revealed that there was a significant ($p<0.05$) difference between the flavour score of the khoa when cardamom was added in different forms. The data obtained for changes in flavour score of khoa during storage at 15±1°C are shown in Table 3 and the trends are depicted in Figure 3.

Table 3. Effect of form of addition of cardamom on flavour score of khoa stored at 15±1°C

Storage periods (days)	Flavour score			
	Control	Fine particles	Coarse particles	Mean (Days)
0	8.89	9.00	8.92	8.94
2	7.53	8.44	8.03	8.00
4	6.58	7.64	7.08	7.10
6	5.55	6.94	6.17	6.22
8	4.06	5.97	5.03	5.02
Mean	6.52	7.60	7.04	
Source of variation		**Treatment (Form)**	**Days**	**Treatment (Form) x Days**
SEm		0.078	0.100	0.173
CD (0.05)		0.23	0.29	0.50
CV (%)			4.25	

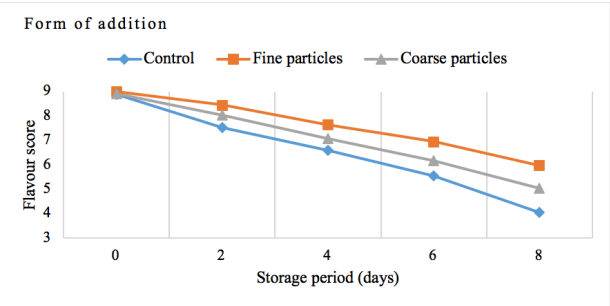


Figure 3. Effect of form of addition of cardamom on flavour score of khoa stored at 15±1°C

Figure 3 revealed that the addition of cardamom in different forms improved the flavour score of khoa samples compare to control sample during the storage period. The flavour scores of all samples were significantly ($p<0.05$) decreased during storage. Khoa samples with fine particles of cardamom possessed the characteristic flavour and secured significantly higher ($p<0.05$) average score. However, the flavour score of khoa with coarse particle decreases faster with storage periods than that of khoa with fine particles. The highest flavour score (5.97) was observed in khoa which was prepared using fine cardamom particles on the 8th day of storage. Visible mold growth was observed in all khoa samples after 8 days of storage at 15±1°C when stored in PP container.

Sen and Rajorhia (1996) studied the influence of powdered cardamom seeds as a natural preservative on the sandesh. Cardamom powder was incorporated at the rate of 0.05, 0.1 and 0.15 per cent by weight of chhana. The addition of 0.15 per cent cardamom (w/w) caused minimum chemical and microbiological changes during storage, but it imparted a strong aroma to the product. Sandesh samples treated with 0.10 per cent cardamom were found acceptable up to 24 days at 30°C and 85 days at 7°C as compared 4 and 47 days, respectively for control sandesh. This can be attributed to the presence of several antimicrobial phenolic compounds such as cineol, terpene, limonene, nerolidon, sabnine, pinene etc. Gaikwad and Hembade (2012) studied the physicochemical as well as the sensory quality of stored buffalo milk Ujani basundi, incorporated with or without potassium sorbate (0.1per cent w/w) and with or without cardamom (0.1 per cent w/w) at 30±1°C and 5±1°C over a period of 20 days. All the treatments secured nearly equal score on 0th day, except cardamom treated samples due to the pleasant flavour of cardamom. However, upto 5th days of storage, the flavour scores were decreased in all the samples. The flavour score of cardamom added samples found superior among all the treatment during storage. Patil (2018) studied the organoleptic and physico-chemical quality of Quarg type cheese on the addition of mango pulp and spices (cardamom and clove). The highest score of 47.85 was obtained by T_2 (72 per cent Quarg cheese + 27 per cent mango pulp + 0.6 per cent cardamom powder + 0.4 per cent clove powder) while lowest score of 47.00 was noticed for the T_5 (71.8 per cent Quarg cheese + 27 per cent mango pulp + 0.7 per cent cardamom powder + 0.5 per cent clove powder). Thus, T_2 was observed to be superior over other treatment for the flavour.

The changes in FFA value (per cent oleic acid) of khoa samples with fine particles and coarse particles of cardamom during storage at 15±1°C are shown in Table 4 and trends are depicted in Figure 4.

On addition of different forms of cardamom in khoa, there was a significant difference in the FFA value of the treated khoa compared to control khoa samples. It was observed that the FFA value of control khoa sample increases rapidly compare to cardamom treated khoa samples. The khoa with fine cardamom particles had the lowest FFA value compared to other khoa samples throughout the storage period. The lowest mean FFA value (0.166 % oleic acid) was of khoa with fine cardamom after 10 days of storage at 15±1°C.

Table 4. Effect of form of addition of cardamom on free fatty acids of khoa stored at 15±1°C

Storage periods (days)	FFA (% oleic acid)			
	Control	Fine particles	Coarse particles	Mean (Days)
0	0.150	0.149	0.149	0.149
2	0.158	0.157	0.158	0.158
4	0.175	0.158	0.166	0.166
6	0.183	0.166	0.175	0.175
8	0.200	0.175	0.199	0.191
10	0.208	0.192	0.200	0.200
Mean	0.179	0.166	0.175	
Source of variation		Treatment (Form)	Days	Treatment (Form) x Days
SEm		0.002	0.003	0.006
CD (0.05)		0.007	0.010	NS
CV (%)			6.09	

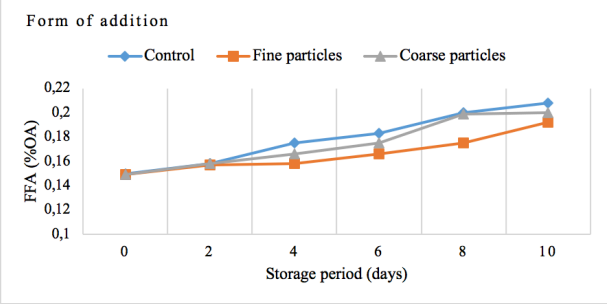


Figure 4. Effect of form of addition of cardamom on FFA (% oleic acid) of khoa stored at 15±1°C

Gaikwad and Hembade (2012) studied the physicochemical as well as the sensory quality of buffalo milk Ujani basundi. Free fatty acidity (per cent oleic acid) was measured to quantify hydrolytic changes during storage. The initial FFA found 0.54 per cent per cent oleic acid, these increased slowly during storage, however, the rate of increase of FFA in potassium sorbate (T_1) was very low as compared to control (T_0), and cardamom (T_2) treated samples. The sample (T_0) showed 0.66 per cent oleic acid whereas T_2 showed 0.64 per cent oleic acid FFA content on the 20th day of storage. The T_1 sample showed 0.60 per cent oleic acid on 20th day of storage. Therefore, it was reported that storage at low temperature with potassium sorbate and cardamom retards the increase in FFA. Jain et al. (2015) studied the influence of modified atmospheric packaging on microbial, textural, sensory and physico-chemical properties of Kalakand. The FFA content increased with increasing storage period. The values were found to be lowest for the samples stored at 10°C, which further increased in the case of samples stored at 25 and 37°C. The FFA content was higher in control samples during 30 days of storage as compared with MAP treated samples. It could be attributed to the absence of oxygen in the package of MAP treated samples.

Thus between the two forms of addition, the fine particles of cardamom was able to control both the flavour as well as lipolysis of khoa during storage at 15±1°C. Hence, the fine particles of cardamom was selected and carry forward in the next phase of the study.

Effect of rate of addition of cardamom on flavour score and lipolysis of khoa

Changes in flavour score of khoa revealed that there was a significant ($p<0.05$) difference between the flavour score of the khoa when cardamom was added at different rates. The data obtained for changes in flavour score of khoa during storage at 15±1°C are shown in Table 5 and the trend are depicted in Figure 5.

Table 5. Effect of rate of addition of cardamom on flavour score of khoa stored at 15±1°C

Storage periods (days)	Flavour score						
	0.0	0.1	0.2	0.3	0.4	0.5	Mean (Days)
0	8.78	8.36	8.83	8.95	8.58	8.22	8.62
2	8.06	7.97	8.61	8.78	8.00	7.36	8.13
4	7.22	7.25	7.86	8.14	7.11	6.81	7.40
6	6.03	6.39	7.00	7.42	6.14	5.92	6.48
8	4.67	5.17	5.80	6.42	5.72	5.61	5.56
Mean (Rate)	6.95	7.03	7.62	7.94	7.11	6.78	
Source of variation			Treatment (Rate)		Days		Treatment (Rate) x Days
SEm			0.053		0.048		0.118
CD (0.05)			0.15		0.14		0.33
CV (%)					2.83		

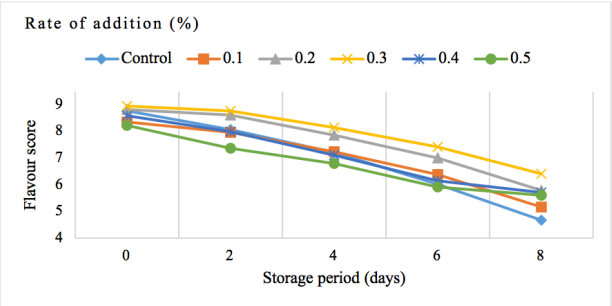


Figure 5. Effect of rate of addition of cardamom on flavour score of khoa stored at 15±1°C

The changes in flavour score revealed that both cardamom and storage period had significant ($p<0.05$) effect on the flavour score of the khoa. The flavour score of the khoa having 0.3 per cent cardamom was more or less similar to that of the control sample when it was fresh. However, higher than 0.3 per cent rate of addition of cardamom, the flavour score was decreased in the samples. Addition of higher rate of cardamom gave pronounced flavour to the khoa, which was not acceptable by the judges. The extent of decrease in flavour score of 0.3 per cent cardamom treated sample was relatively slow as compared to other samples upon storage. On the 6th days of storage, the flavour score was remained acceptable in all samples and maximum flavour score (7.42) was observed in 0.3 per cent cardamom treated sample. After the 8th day of storage, all the samples were unacceptable due to visible mold growth.

Kober et al. (2003) worked on the influence of cardamom powder on the shelf life of sandesh. Chhana sample was divided into four parts with the addition of cardamom powder at the rate of 0.05, 0.10 and 0.15 per cent with each part at the last stage of sandesh making. They observed that the sensory scores of all samples decreased gradually with increasing storage periods. It was suggested that the control samples were suitable for human consumption up to 12 days, but the addition of cardamom powder (0.05, 0.10 and 0.15 per cent) extended the storage life up to 20, 24 and 28 days at room temperature, respectively. Narwade (2003) reported that the use of different levels of crushed cardamom significantly affected the flavour score of pedha. Addition of 0.2 per cent cardamom had the highest mean flavour score (8.49) as compared to the addition of 0.4 per cent and 0.6 per cent. As the cardamom increased, the flavour became stronger which was not accepted by the judges. Chougule et al. (2014) prepared basundi from buffalo or cow milk. Efforts have been made to enhance the quality of basundi prepared by using cardamom and saffron. It was observed that up to 0.4 per cent level of cardamom, the mean flavour score was increased and later on, it was decreased in 0.6 per cent cardamom treated samples. This might be due to the higher concentration of cardamom flavour, which was unacceptable by the judges. The basundi treated with 0.4 per cent cardamom secured the highest flavour score (8.4) as compared to other treatments.

The changes in FFA value (per cent oleic acid) of khoa samples containing different rates of cardamom during storage at 15±1°C are shown in Table 6 and Figure 6.

The FFA content of the khoa samples varies from 0.149 to 0.150 per cent oleic acid on the first day. During storage, the free fatty acids content were increased and at the end of storage, the FFA content was varied from 0.210 to 0.241 per cent oleic acid in the samples. Though during storage FFA content was increased, the rate of increase in the 0.3 per cent added cardamom samples was relatively less as compared to other samples. The FFA content was 0.210 per cent oleic acid observed in 0.3 per cent cardamom treated sample on 10th day of storage, which was lowest among the all other samples indicating 0.3 per cent cardamom treated sample was able to control the extent of lipolysis in the sample during storage.

Table 6. Effect of rate of addition of cardamom on free fatty acids of khoa stored at 15±1°C

Storage periods (days)	FFA (% oleic acid)						
	0.0	0.1	0.2	0.3	0.4	0.5	Mean (Days)
0	0.149	0.150	0.150	0.149	0.150	0.149	0.150
2	0.175	0.173	0.157	0.153	0.160	0.175	0.166
4	0.191	0.177	0.166	0.163	0.175	0.197	0.178
6	0.216	0.200	0.174	0.169	0.183	0.207	0.191
8	0.225	0.225	0.199	0.186	0.199	0.224	0.210
10	0.241	0.233	0.224	0.210	0.228	0.236	0.229
Mean (Rate)	0.200	0.193	0.178	0.172	0.183	0.198	

Source of variation	Treatment (Rate)	Days	Treatment (Rate) x Days
SEm	0.002	0.002	0.004
CD (0.05)	0.004	0.004	0.011
CV (%)		3.45	

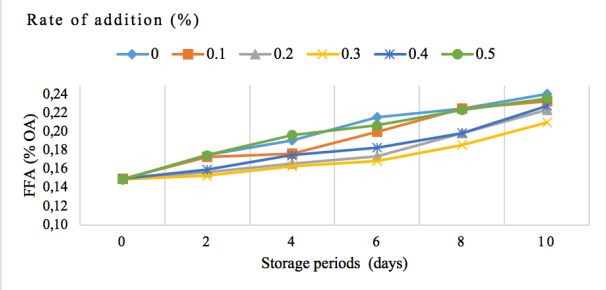


Figure 6. Effect of rate of addition of cardamom on FFA (% oleic acid) of khoa stored at 15±1°C

Sivakumar et al. (2014) studied the effect of betel leaves (Piper betel Linn) (0.5 per cent) extract on the different properties of cow milk khoa stored under room temperature. Lower rate of FFA production was observed in khoa samples treated with betel leaves extract than BHA treated samples during 9 days of storage. Hence, it was reported that BHA could be replaced by natural antioxidant like betel leaves extract. Bhatt (2012) observed that the addition of essential oils of spice had a significant effect on FFA content of paneer. The FFA content of control sample of paneer declined sharply from the beginning of the storage. Amongst the samples of paneer, the highest rate of increase in free fatty acid content was observed in case of essential oil of cinnamon and the lowest rate was observed in the case of cardamom. The FFA content in samples containing essential oil of cardamom remained constant during storage between 15 to 20 days. Thus, the

essential oil of cardamom proved to be the best spice extract to control the FFA content of paneer during storage. Eresam et al. (2015) tested the relative efficiency of black pepper, cardamom, cinnamon and clove in improving shelf life of paneer. The maximum rate of increase was observed in black pepper and the rate was minimum in cardamom. The FFA content in samples containing cardamom remained constant during storage between 21 to 28 days. Thus, cardamom proved to be the best spice to control the FFA content of paneer during storage.

Thus, 0.3 per cent fine particles of cardamom when added in milk for khoa making was able to control the flavour as well as lipolysis in khoa upon storage.

Chemical composition of fresh khoa

The fresh sample of control and cardamom (0.3 per cent) treated khoa were analyzed for per cent moisture, fat, protein, lactose (by difference) and ash content. The results obtained from this are shown in Table 7 and trend are depicted in Figure 7.

Table 7. Chemical compositions of fresh khoa

Sample of Khoa	Constituent (%)				
	Moisture	Fat	Protein	Lactose	Ash
Control	33.44	21.67	21.70	17.48	3.66
Cardamom treated (0.3%)	33.83	21.83	22.11	16.85	3.61
SEm	1.674	0.441	0.215	0.689	0.082
CD (0.05)	NS	NS	NS	NS	NS
CV (%)	8.62	3.51	1.70	6.96	3.89

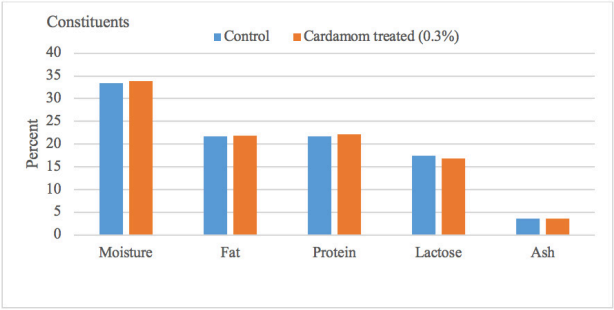


Figure 7. Chemical compositions of fresh khoa

The data obtained for the chemical composition of a control sample of fresh khoa revealed that the khoa contained 33.43 per cent moisture, 21.67 per cent fat, 21.70 per cent protein, 17.48 per cent lactose and 3.66 per cent ash while cardamom treated khoa contained 33.83 per cent moisture, 21.83 per cent fat, 22.11 per cent protein, 16.85 per cent lactose and 3.61 per cent ash. The per cent fat on dry matter basis of a control sample of khoa was 32.55 while cardamom added khoa was 32.99. Thus, data revealed that no significant difference ($p>0.05$) in the composition of control and cardamom treated khoa was observed.

The literature on the chemical composition of khoa indicates that the moisture, fat, protein, lactose and ash content of laboratory-made khoa samples vary from 23.8 to 35.00, 21.73 to 45.90, 16.30 to 25.80, 18.85 to 35.97 and 2.74 to 5.20 per cent respectively (Dastur and Lakhani, 1971; Kumar and Srinivasan, 1982; Gothwal and Bhavadasan, 1992; Kashyap, 2007; Kumar, 2013; Choudhary et al., 2019). Therefore, in the present study, the data obtained for the chemical composition of khoa was well within those reported in the literature. According to the Food Safety and Standards Authority of India (FSSAI, 2017), khoa should contain a minimum 30 per cent fat on dry matter basis and total solids should be minimum 55 per cent. The ash content should not exceed 6.0 per cent. Therefore, the samples of khoa prepared in the present study fulfilled the FSSAI requirements for the chemical composition.

Chemical characteristics of fresh khoa

The chemical characteristics like free fatty acids and acidity of control and cardamom (0.3 per cent) treated fresh khoa were analysed. The results obtained from this are shown in Table 8.

The data obtained for chemical characteristics of a control sample of fresh khoa revealed that the khoa have FFA content: 0.150 per cent oleic acid and acidity: 0.54 per cent lactic acid while cardamom treated khoa sample contained 0.149 per cent oleic acid FFA and 0.53 per cent lactic acid. It was observed that the chemical characteristics of the samples were statistically non-significant ($p>0.05$).

The literature on the chemical composition of khoa indicates that the FFA content of fresh khoa ranged from 0.065-0.332 per cent oleic acid (Rudreshappa and De, 1971; Sai, 1981; Sharma, 1999; Choudhary et al., 2019). The acidity of the fresh sample of khoa ranged from 0.54-0.59 per cent lactic acid (Rudreshappa and De, 1971; Sharma, 1999; Kashyap, 2007; Chaudhary et al., 2019). Therefore, in the present study, the data obtained for FFA content and acidity of khoa was well within those reported in the literature.

Rheological Properties of Fresh Khoa

The fresh sample of control and cardamom (0.3 per cent) treated khoa were analyzed for textural analysis by texture profile analyzer. The results obtained are shown in Table 9 and trend are depicted in Figure 8.

The data obtained for the rheological properties of a control sample of fresh khoa revealed that the khoa had 37.51 N hardness, 0.13 cohesiveness, 2.99 mm springiness, 4.25 N gumminess, 15.20 Nmm chewiness and 0.15 Nmm adhesiveness while cardamom treated khoa had 36.90 N hardness, 0.16 cohesiveness, 2.81 mm springiness, 5.45 N gumminess, 17.71 Nmm chewiness and 0.13 Nmm adhesiveness. Thus, statistical data show that rheological properties of fresh control and cardamom (0.3 per

Table 8. Chemical characteristics of fresh khoa

Sample of Khoa	Chemical characteristics			
	Free fatty acids (% oleic acid)	Acidity (% LA)	Peroxide value (millimolecules of oxygen per kg of fat)	Thiobarbituric acid value (OD at 532 nm)
Control	0.150	0.54	0	0
Cardamom treated (0.3%)	0.149	0.53	0	0
SEm	0.001	0.026	0	0
CD (0.05)	NS	NS	NS	NS
CV (%)	1.06	8.41	0	0

Table 9. Rheological properties of fresh khoa

Sample of Khoa	Rheological properties					
	Hardness (N)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (Nmm)	Adhesiveness (Nmm)
Control	37.51	0.13	2.99	4.25	15.20	0.15
Cardamom treated (0.3 %)	36.90	0.16	2.81	5.45	17.71	0.13
SEm	3.365	0.008	0.218	0.506	1.799	0.007
CD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	15.67	10.04	12.99	18.06	18.93	9.33

cent) treated khoa was not statistically different ($p>0.05$) from each other.

Patil et al. (1990) reported that the khoa market sample has a hardness (N) 46.1, cohesiveness 0.43, adhesiveness 17.3, springiness (mm) 14.8, gumminess (mN) 19.7, chewiness (mN.mm) 291.8. Puranik et al. (1998) studied the textural analysis of recombined milk and cow milk khoa and reported hardness (N) 1.23, cohesiveness 0.26, adhesiveness 1.30, springiness (mm) 5.00, gumminess (mN) 0.32, chewiness (mN.mm) 1.60. Dodeja and Deep (2012) reported that danader khoa market sample has a hardness (N) 33.553, cohesiveness 0.222, adhesiveness -0.311, springiness (mm) 0.349, gumminess (mN) 7.448, chewiness (mN.mm) 2.59. Badola et al. (2018) reported no significant effect ($p>0.05$) on the instrumental textural attributes of burfi by incorporation of herbal essential oil at any level of incorporation. This may be attributed to the negligible amount of essential oil present in the burfi. Prasad et al. (2018) evaluated effect of incorporation of essential oils of ginger, turmeric and cardamom on the instrumental texture parameters. Hardness values in the burfi samples ranged from 39.59 N to 41.58 N, adhesiveness from (-) 2.64 Ns to (-) 2.93 Ns, springiness (mm) 0.14 to 0.20, cohesiveness from 0.12 to 0.15, gumminess from 5.35 to 6.02 and chewiness from 0.95 to 1.17. It was observed that essential oil or synthetic antioxidant incorporation did not result in significant ($p>0.05$) modification of the textural attributes of burfi, compared with the control burfi.

Sensory evaluation of fresh khoa

The fresh khoa were analyzed for sensory evaluation by 9-point hedonic scale. The colour, flavour, body & texture and overall acceptability score of control and khoa treated with cardamom (0.3 per cent) are shown in Table 10 and trend were depicted in Figure 9.

Table 10. Sensory evaluation of fresh khoa

Sample of Khoa	Sensory Score			
	Colour	Flavour	Body & texture	Overall acceptability
Control	8.33	8.67	8.50	8.57
Cardamom treated (0.3%)	8.60	8.83	8.67	8.73
SEm	0.189	0.167	0.312	0.113
CD (0.05)	NS	NS	NS	NS
CV (%)	3.86	3.30	6.29	2.26

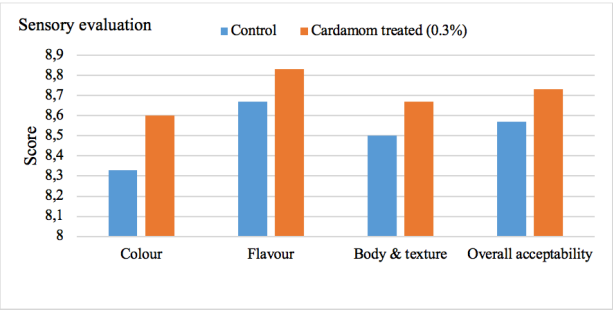


Figure 9. Sensory evaluation of fresh khoa

From the Table 10, it was observed that the colour, flavour, body & texture and overall acceptability scores of a control sample of fresh khoa were 8.33, 8.67, 8.50 and 8.57 respectively while for cardamom added fresh khoa

was 8.60, 8.83, 8.67 and 8.73 respectively. The sensory evaluation data of control and cardamom treated khoa were not statistically different (p>0.05) from each other.

Sivakumar et al. (2014) observed that the there was no statistical difference between the scores for colour and appearance, flavour, body and texture and overall acceptability between control and betel leaves (0.5 per cent) extract-treated khoa which indicates that the addition of aqueous extract of betel leaves did not influence the sensory quality of khoa. Anurag et al. (2017) studied the shelf life of bottle gourd burfi. For fresh bottle gourd burfi, the flavour, colour & appearance, texture and overall acceptability scores were 8.40, 8.43, 8.14, and 8.26, respectively. Prasad et al. (2017) worked on anti-oxidative, physicochemical and sensorial properties of burfi affected by the incorporation of different herbs (clove, turmeric, basil leaves, curry leaf, ginger and cardamom) and its comparison with BHA. It was observed that the lowest flavour score of 5.20 was obtained for basil leaves added burfi, while highest score was obtained by cardamom incorporated burfi (7.50), which was not significantly different (p>0.05) from the control sample. Colour and appearance scores of the herbs added burfi ranged from 5.10 for clove added burfi to 7.30 for ginger incorporated burfi. Texture scores of herbs added burfi ranged from 7.26 for cardamom added burfi to 7.33 for turmeric burfi. No significant (p>0.05) difference was observed in the textural scores of burfi upon the incorporation of different herbs. It was observed that overall acceptability scores for the herbal burfi ranged from 5.27 for basil leaves added burfi to 7.39 for cardamom-incorporated burfi. Thus, data shows that sensory evaluation of fresh control and cardamom (0.3 per cent) treated khoa was not statistically different (p>0.05) from each other.

CONCLUSION

This study, therefore, entails to conclude that the addition of cardamom helps to control the lipolysis in khoa during storage. Addition of cardamom initially into milk was more effective than pat formation stage for khoa making. Khoa samples prepared using fine particles of cardamom were effective in controlling the FFA during storage. Among the various rate of additions, the 0.3 per cent fine particle of cardamom in milk for khoa making was found best in controlling lipolysis of khoa during storage. Addition of cardamom did not affect the rheological properties of khoa samples and it also fulfilled the legal requirements for quality standards prescribed under FSSAI.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

References

Amchova, P., Kotolova, H., & Ruda-Kucerova, J. (2015). Health safety issues of synthetic food colorants. Regulatory Toxicology and Pharmacology, 73(3), 914-922. [URL]

Anurag, C. R., Khatkar, S. K., & Bhaskar, D. (2017). Effect of temperature on physico-chemical and sensory attributes of bottle gourd burfi. Indian Journal of Dairy Science, 70(4), 391-397. [URL]

Badola, R., Panjagari, N. R., Singh, R. R. B., Singh, A. K., & Prasad, W. G. (2018). Effect of clove bud and curry leaf essential oils on the anti-oxidative and anti-microbial activity of burfi, a milk-based confection. Journal of Food Science and Technology, 55(12), 4802-4810. [URL]

Bhatt, A. R. (2012). Evaluation of extracts from selected spices to extend shelf life of paneer. Master Thesis, Anand Agricultural University, Anand.

BIS (1980). Indian standard specification for khoa. Bureau of Indian Standards, New Delhi.

BIS (1983). Hand book of Food Analysis (Part XI-1981). Dairy products. Bureau of Indian Standards, New Delhi.

Choudhary, S., Arora, S., Kumari, A., Narwal, V., & Singh, A. K. (2019). Effect of quality of milk on physico-chemical characteristics of buffalo milk concentrate (khoa) during storage. Journal of Food Science and Technology, 56(3), 1302-1315. [URL]

Chougule, V. M., Pawar, B. K., & Choudhari, D. M. (2014). Sensory quality of Basundi prepared by using cardamom and saffron. Research Journal of Animal Husbandry and Dairy Science, 5(1), 14-16. [URL]

DAHD (2019). Milk Production in India. [URL]

Dastur, N. N., & Lakhani, A. G. (1971). Chemical composition of khoa. Indian Journal of Dairy Science, 24(4), 223-224. [Google Scholar]

De, S. (2004). Outlines of Dairy Technology. 19th ed., Oxford publishing company, New Delhi.

Deeth, H. C. (2011). Lipolysis and Hydrolytic Rancidity. Encyclopedia of Dairy Sciences, 2, 721-726. [Google Scholar]

Deeth, H. C., Fitz-Gerald, C. H., & Wood, A. F. (1975). A convenient method for determining the extent of lipolysis. Australian Journal of Dairy Technology, 30, 109-111. [URL]

Desale, R. J., & More K. D. (2017). Shelf life studies of basundi prepared by using herbal preservatives. Trends in Biosciences, 10(48), 9628-9632. [URL]

Dodeja, A. K., & Deep, A. (2012). Mechanized manufacture of danedar khoa using three stage SSHE. Indian Journal of Dairy Science, 65(4), 274-278. [URL]

Eresam, K. K., Pinto, S., & Aparnathi, K. D. (2015). Concise and in-

formative title: evaluation of selected spices in extending shelf life of paneer. Journal of Food Science and Technology, 52(4), 2043-2052. [URL]

Eresam, K. K. (2009). Evaluation of the selected spices for extending the shelf life of paneer. Master Thesis, Anand Agricultural University, Anand.

FSSAI (2017). Food Safety and Standards Authority of India. Ministry of health and family welfare. Government of India.

Gaikwad, S. M., & Hembade, A. S. (2012). Effect of storage temperature on microbiological quality of standardized cow milk Ujani basundi. International Journal of Dairy Science, 7(2), 34-42. [Google Scholar]

Gawde, A. M. (2005). Shelf life studies of Kalakand. Doctoral Thesis, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri.

Gothwal, P. P., & Bhavadasan, M. K. (1992). Studies on the browning characteristics in dairy products. Indian Journal of Dairy Science, 45(3), 146-151. URL Link:

Gupta, R., Upadhyay, P. K., & Singh, V. (2011). Effect of sugar and cardamom on the quality and shelf-life of Kachchagola sandesh. Research Journal of Animal Husbandry and Dairy Science, 2(1&2), 43-46. [Google Scholar]

Haas, E. M. (1999). Feed your family safely. In: Staying healthy shopper's guide. Celestial Arts, Berkeley.

Horwitz, W. (1980). Dairy products. In Official Methods of Analysis. 13th ed., Benjamin Franklin Station, Washington, D.C, 186-219.

Jadav, K. D., & Mehta, B. M. (2018). Cardamom: Chemistry, medicinal properties, applications in dairy and food industry: A review. Research & Reviews: Journal of Dairy Science and Technology, 7(3), 9-19. [URL]

Jain, V., Rasane, P., Jha, A., Sharma, N., & Gautam, A. (2015). Effect of modified atmospheric packaging on the shelf life of Kalakand and its influence on microbial, textural, sensory and physico-chemical properties. Journal of Food Science and Technology, 52(7), 4090-4101. [URL]

Kashyap, N. (2007). Shelf life extension of khoa at village level. Doctoral Thesis, Indira Gandhi Krishi Vishwavidyalaya, Raipur.

Khatkar, A. B., Ray, A., & Kaur, A. (2017). Studies on shelf life extension of paneer with the addition of plant essential oil and different packaging materials. International Journal of Current Microbiology Application Science, 6(9), 376-389. [URL]

Kober, A. K. M. H., Islam, M. N., & Amin, M. R. (2003). Effects of cardamom on shelf life of sandesh (milk sweet) at room temperature. Bangladesh Veterinarian, 20(1), 64-71. [URL] Link:

Kumar, G., & Srinivasan, M. R. (1982). Effect of packaging and storage on the sensory characteristics of khoa samples. Indian Journal of Dairy Science, 35(2), 132-137. [Google Scholar]

Kumar, K. S. (2013). Utilisation of concentrated and lactose hydrolysed whey in the preparation of khoa. Doctoral Thesis, National Dairy Research Institute, Karnal.

Kumari, R., Shrivastava, S. L., & Mishra, H. N. (2012). Optimization of khoa and tikhur mix preparation of khoa-jalebi sweet. Open Access Scientific Reports, 1(10), 1-4.

Ladkani, B. G., & Mulay, C. A. (1974). Feasibility of using Gerber fat test for the rapid estimation of fat in khoa. Journal of Food Science and Technology, 11, 29-31. [Google Scholar]

Narwade, S. G. (2003). Effect of processing and compositional variables on the quality of pedha. Doctoral Thesis, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Patil, G. R., Patel, A. A., Garg, F. C., Rajorhia, G. S., & Gupta, S. K. (1990). Interrelationship between sensory and instrumental data on texture of khoa. Journal of Food Science and Technology, 27(3), 167-170. [Google Scholar]

Patil, V. P. (2018). Preparation of mango herbal quarg type cheese. Master Thesis, Mahatma Phule Krishi Vidaypeeth, Rahuri.

Prasad, W., Khamrui, K., Mandal, S., & Badola, R. (2017). Anti-oxidative, physico-chemical and sensory attributes of burfi affected by incorporation of different herbs and its comparison with synthetic anti-oxidant (BHA). Journal of Food Science and Technology, 54(12), 3802-3809. [URL]

Prasad, W., Khamrui, K., Mandal, S., & Badola, R. (2018). Effect of combination of essential oils on physicochemical and sensorial attributes of burfi in comparison with individual essential oil and BHA. International Journal of Dairy Technology, 71(3), 810-819. [URL]

Ratiba, B. A., Wedad, A. M., Mohamed, O., & El-Aziz, H. A. (2006). Effect of cardamom, thyme and clove powder on the composition and quality of white soft cheese made from goat's milk. Assiut Journal of Agricultural Science, 37(4), 139-157. [Google Scholar]

Rudreshappa, K. G., & De, S. (1971). Studies on preservation of khoa. Journal of Food Science & Technology, 8, 50-52. [Google Scholar]

Sai, P. B. (1981). Physico-chemical changes in the major milk constituents during manufacture of khoa from lactose hydrolysed buffalo milk. Master Thesis, Anand Agricultural University, Anand.

Sawhney, I. K., Patil, G. R., Kumar, B., & Rao, K. J. (1994). Evaluation of glycerol as a water activity modifier in Khoa. Journal of Food Science and Technology, 31(3), 252-254.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 11-20
DOI: https://doi.org/10.31015/jaefs.2023.1.2

Refinery technology of used cooking oil by utilizing coffee dregs and sugar cane bagasse as raw materials for making antiseptic transparent soap of guava leaf extract

Muh. Agus Ferdian[1] • **Randhiki Gusti Perdana**[1] • **Poegoeh Prasetyo Rahardjo**[2]

[1] Institut Pertanian Malang, Faculty of Agricultural Technology, Agro-Industrial Technology Department, Malang, Indonesia
[2] Fakultas Kehutanan, Institut Pertanian Malang, Malang, Indonesia

Citation: Ferdian, M.A., Perdana, R.G., Rahardjo, P.P. (2023). Refinery technology of used cooking oil by utilizing coffee dregs and sugar cane bagasse as raw materials for making antiseptic transparent soap of guava leaf extract. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 11-20

Received: 26 April 2022
Revised: 13 October 2022
Accepted: 17 October 2022
Published Online: 09 January 2023

Correspondence: Muh. Agus Ferdian
E-mail: ferdian1608@gmail.com

Abstract

Cooking oil is an important basic ingredient in the frying process with the main function as a medium for conducting heat, adding savory taste, in-creasing nutritional value and heating food ingredients. The massive use of cooking oil also has an impact on the waste produced, namely used cooking oil (UCO), which is more increasing. The sustainable use of used cooking oil can damage health. The aim of this study was to obtain purification of used cooking oil with coffee dregs and bagasse as raw materials for making antiseptic transparent soap with guava leaf extract. Preparation of raw materials, extraction of guava leaves, oil refining process, production of antiseptic transparent soap with guava leaf extract, and sample quality analysis are the stages of research. The data from the analysis was then tested with ANOVA. Purification of used cooking oil using bagasse adsorbent produced oil with a pH value of 6.43-6.86, moisture content of 0.67-0-769%, free fatty acids of 0.301-1.982% and acid value of 2.3-3.490%, while for the type of coffee dregs adsorbent produced oil with a pH value of 6.60-6.83, moisture content of 0.068-0.549%, free fatty acids of 0.292-0.921 and acid value of 1.120-5.850. The antiseptic transparent soap formulation results obtained a pH value of 11.427-13,687, moisture content of 27.78-36.505, foam height of 19-21.5 mm and antiseptic power of 2-12 colonies.

Keywords: Bagasse, Coffee dregs, Purification, Transparent soap, Used cooking oil

INTRODUCTION

Indonesia is world's fourth most populous nation after Republic of China, India and the United States of America. The population of Indonesia reached around 267 million people in 2019 (Bappenas 2018). It makes the basic need for food sources increased. One of them is the need for cooking oil. Cooking oil is an important basic ingredient in the frying process with the main function as a medium for conducting heat, adding savory taste, increasing nutritional value, and heating food ingredients (Ketaren 2005b). The data of Global Agricultural Information Network USDA 2019 shows that Indonesia's consumption of cooking oil reached 13,110 thousand metric tons at most.

The widespread use of cooking oil has an effects on waste production, including the increasing of used cooking oil. Used cooking oil (UCO) is the repeated use of cooking oil result. Consuming used cooking oil is extremely hazardous to one's health. The use of cooking oil regularly and continuously in the frying process causes a degradation response, lowering the quality of the cooking oil (Nasrun et

al. 2017). The sustainable use of used cooking oil might be harmful to one's health (Ningrum and Kusuma 2013). Used cooking oil can deposit fat in blood vessels, and liver cancer (Guenther 1987). However, if the used cooking oil is disposed of, it pollutes the environment.

There are three approaches that may be employed to improve the environment concerning used items, they are Reduce, Reuse, and Recycle. The Recycle technique can be utilized to make efforts relating to used cooking oil. Recycling used cooking oil may be accomplished by reprocessing discarded cooking oil into commodities that still have economic worth (Susilawaty et al. 2017).

Since it still contains free fatty acids and a high peroxide value, as well as other impurities, used cooking oil cannot be directly utilized as raw material. Therefore, it is necessary to do treatment to minimize some of these substances by utilizing absorbents. Some materials that can be used as adsorbents are bentonite (Rahayu and Purnavita 2014), zeolite (Alamsyah et al. 2017), activated carbon (Riyanta and Nurniswati, 2016; Rosita and Widasari, 2009; Yusriana et al. 2014), Coffee dregs (Hayati et al. 2012), bagasse (Hajar et al. 2016; Trisnaliani et al. 2019; Wannahari and Nordin 2012) and many others.

Transparent soap is a type of soap that is used for the face and body which can produce a softer foam on skin and has a shinier appearance compared to other types of soap (Hambali et al. 2005). Transparent soap is made by melting the fat phase and preparing water to dissolve sucrose, glycerin, and preservatives. Both of these phases were reacted by an alcoholic solution of caustic soda under controlled heating. After the reaction is complete, the soap is ready to be colored, fragranced, or treated with an active anti-bacterial component. The soap is then poured into separated molds or glasses and allowed to set before being packaged (Butler 2001).

The addition of antibacterial chemicals or components to soap can improve its ability and use, allowing it to be used as an antiseptic soap. One of the anti-bacterial components that can be combined with transparent soap to produce antiseptic soap is an ingredient derived from guava leaves (*Psidium guajava* Linn). Guava leaves contain 9-12% tannin compounds, essential oils, fatty oils and malic acid (Yuliani et al. 2003). The aim of this study was to obtain the purification of used cooking oil with coffee dregs and bagasse as raw materials for making antiseptic transparent soap with guava leaf extract.

MATERIALS AND METHODS

Materials and Equipment

The equipment used was a titration tool, hot plate, stirrer, Erlenmeyer flask, glass beaker, dropper, thermometer, oven, filter paper, funnel, VCO bottle, analytical balance, blender, sieve, measuring cup, pH meter, petri dish and spatula. Meanwhile, the ingredients used include used cooking oil derived from Palm Oil, Sugarcane Bagasse, Coffee Dregs, NaOH, Citric Acid, Stearic Acid, PP Indicator, Glycerin, Ethanol, Aquadest, Sugar and Fragrance.

Raw Material Preparatiaon

a. Bagasse Processing

Bagasse was obtained from sugar cane juice sellers around the city of Malang. The residue from the sugarcane juice mill was washed thoroughly and then dried in the blazing sun. Furthermore, the dried bagasse was ground by grinding and sifting to obtain bagasse powder (Ramdja et al. 2010).

b. Coffee Dregs Processing

Coffee dregs were collected from coffee shops across Malang as a result of filtering coffee beverages. The coffee dregs were washed and dried in the sun's heat. The dry coffee dregs should then be ground to produce softer, more homogeneous pulp particles (Baryatik et al. 2019). The selection of coffee grounds from the same sort of coffee is vitally necessary in order to generate the same coffee powder.

Guava Leaf Extraction

Extraction of guava leaves using a modified maceration method (Ningsih et al. 2014). Extraction was carried out by maceration with 70% ethanol as solvent. A total of approximately 200 grams of guava leaves which had been ground were soaked in 650 ml of 70% ethanol, closed and then stirred using a stirrer with a rotation speed of 120 rpm for 1 and 2 hours.

Oil Refining Process

Sample as much as 100 ml of used cooking oil obtained from street vendors and put it in an Erlenmeyer. Then the oil was heated at temperatures of 50 °, 60 ° and 70° C and absorbent was added to the oil, then the sample solution was stirred with rotational speeds of 50, 100 and 150 rpm. The results of the purification process were then filtered to separate it from the absorbent material and clear oil. Then the analysis was carried out and the best purified oil was selected based on the determined parameters.

Making Antiseptic Transparent Soap of Guava Leaf Extract

The addition of guava leaf extract was carried out with variations in concentrations of 1, 2 and 3%. The available dough is then stirred at a constant speed at a temperature of 70-80° C until all ingredients were perfectly mixed and appear transparent.

Sample Quality Analysis

pH

The pH value is also known as the degree of acidity. pH is used to assess the level of acidity or alkalinity of a material.

Moisture Content

Determination of water content is done by gravimetric method. The gravimetric procedure begins with weighing 5 grams of the sample in a petri dish whose weight is known, then the sample is heated in a drying cabinet at a temperature of 105°C for 2 hours until the weight remains constant.

FFA Content

Free fatty acids are determined as the fatty acid content mostly found in a particular oil.

Acid value

The sum of the acid numbers in the oil is the total fatty acids, either sodium-bound fatty acids or free fatty acids plus neutral fatty acids (triglycerides or unsaponifiable fat).

Saponification value

The saponification value shows the relative size of the fatty acid molecules contained in the glycerides. The saponification rate is expressed as the number of mg of KOH required to completely saponify the oil from 1 gram of the oil.

Organoleptic analysis

Sensory or organoleptic analysis in this study used a hedonic scoring test on 6 formulated products. Tests were carried out on the attributes of taste, aroma, texture, color, and overall. The score scale used was a five-point category scale, in which 1 = strongly dislike to 5 = strongly like. The panelists used were 30 untrained panelists, who were students of the Malang Agricultural Institute

Antiseptic power test

Antiseptic power testing was carried out using a modified replica method and was carried out at the Biomedical Laboratory, Faculty of Medicine, Muhammadiyah University, Malang. The steps taken are by washing hands with water and followed by soap. Respondents wash their hands properly using water for 60 seconds, then dry them by shaking them for 75 seconds. Fingers were attached to nutritional medium to form a zigzag line in a petri dish. Furthermore, responders followed the test stage by first waiting for 60 seconds to see the percentage of bacterial reduction. Respondents placed another finger on the soap surface according to the type of formula on different fingers

Data Analysis

The data from the analysis were then tested with ANOVA, further tests were carried out using the DMRT (Duncan Multiple Rang Test) test at a level of 0.05.

RESULTS AND DISCUSSION

Purification of used cooking oil with adsorbent

The main objective of this study was to obtain used cooking oil as a result of purification using bagasse and coffee dregs as raw materials for making antiseptic transparent soap with guava leaf extract. Purification of used cooking oil was carried out as an effort to eliminate or reduce the content that interferes with the next goal process. The main process in soap making is saponification. The saponification process is the reaction of triglyceride fatty acids with alkali and producing glycerol as a by-product, while the neutralization process does not produce glycerol. The use of cooking oil increases the amount of free fatty acids in the oil, which might hinder the saponification process (Hambali et al. 2005). Free fatty acids are the most common characteristics used as oil quality control because these fatty acids affect the physical, chemical, and stability of the oil during the frying process (Patty et al. 2017). The high content of free fatty acids is caused by the repeated use of cooking oil at high temperatures. In addition, the changes that occur include physicochemical properties (oil damage) such as color, aroma and increasing peroxide value (Ketaren 2005a). Such cooking oil is no longer suitable for use, especially for consumption because it can cause diseases such as cancer, narrowing of blood vessels and itchy throat. As a result, refining used cooking oil should be sought with the goal of saving money while not harming health and being simple to perform. Efforts to process spent cooking oil may be accomplished in a variety of ways, one of which is by adsorption. Adsorption was chosen since it is simple to execute and cost-effective (Oko et al. 2020).

Bagasse is efficient and effective in the process of used cooking oil adsorption (Hajar and Mufidah, 2016; Trisnaliani et al. 2019). The use of bagasse as an absorbent which is able to bind impurities in used cooking oil is like other types of absorbents used in oil refining such as bleaching earth and bleaching carbon. (Ramdja et al. 2010). The use of bagasse in the refining of used cooking oil produced a chemical quality in the form of a significant decrease in the value of free fatty acids, the peroxide value and moisture content that were in accordance with the quality standards of cooking oil chemically were 0.3 of the maximum free fatty acid content, 1 for the maximum peroxide value, 0.3 for the maximum moisture content (Sulung et al. 2019).

Another type of absorbent which could be used for oil refining was coffee dregs. The results showed that the purification of used cooking oil with coffee dregs as an adsorbent for a soap product had an average moisture content of 13.82%, pH 9, foam height 3.5 cm, fatty acids of 46.82%, and free alkali of 0.09% (Riyanta and Nurniswati, 2016).

In this study, used cooking oil was purified by using bagasse and coffee dregs as absorbents through the variations of temperature treatment (50, 60, 70 °C) and rotational speed (50, 100, 150 rpm) in the absorption process. The analysis was carried out on the purified oil samples. The analysis parameters are pH, moisture content, free

fatty acids and acid value. The main result of this stage is that the most effective treatment in refining used cooking oil is purification using bagasse with a temperature and rotation speed of 70° C and 150 rpm. The data from the analysis of the used cooking oil purification samples are presented in Table 1.

of 5 (Misrawati et al. 2015). The requirement for the quality of palm cooking oil pH according to SNI are 6.5-8 (IS: 6357-1971), so that the used cooking oil purified using bagasse in this study is included in the SNI quality category.

The analysis of variance showed that the use of the absorbent type with the treatment of temperature factors and stirring rotation speed resulted in P values = 0.614 and 0.520 (P>0.005), meaning that the temperature factor and stirring rotation speed had no significant effect on the pH parameters produced at the 5% level.

Table 1. Data analysis results of used cooking oil purification samples

No	Adsorbent Type	Temperature	Rotation Speed	Parameter			
				pH	Moisture Content	Free Fatty Acids	Acid Value
1	Sugar cane Bagasse	50	50	6.800a	0.703cb	1.982cc	2.335ac
2	Sugar cane Bagasse	50	100	6.775a	0.769cc	1.556cb	2.300aa
3	Sugar cane Bagasse	50	150	6.590a	0.276ca	0.397ca	2.300ab
4	Sugar cane Bagasse	60	50	6.710a	0.243bb	0.905bc	2.310bc
5	Sugar cane Bagasse	60	100	6.520a	0.357bc	0.652bb	2.310ba
6	Sugar cane Bagasse	60	150	6.430a	0.067ba	0.360ba	2.335bb
7	Sugar cane Bagasse	70	50	6.860a	0.438ab	0.668ac	3.490cc
8	Sugar cane Bagasse	70	100	6.620a	0.152ac	0.567ab	2.335ca
9	Sugar cane Bagasse	70	150	6.610a	0.247aa	0.301aa	2.300cb
10	Coffee Dregs	50	50	6.830a	0.101cb	0.921cc	1.120ac
11	Coffee Dregs	50	100	6.630a	0.458cc	0.830cb	2.335aa
12	Coffee Dregs	50	150	6.740a	0.071ca	0.745ca	2.310ab
13	Coffee Dregs	60	50	6.650a	0.083bb	0.878bc	3.515bc
14	Coffee Dregs	60	100	6.660a	0.100bc	1.007bb	2.335ba
15	Coffee Dregs	60	150	6.710a	0.549ba	0.505ba	3.490bb
16	Coffee Dregs	70	50	6.650a	0.099ab	0.544ac	5.850cc
17	Coffee Dregs	70	100	6.600a	0.278ac	0.398ab	2.300ca
18	Coffee Dregs	70	150	6.730a	0.068aa	0.292aa	2.310cb
19	Control			6.560	0.791	2.214	3.490

Note: Different letter notations show a significantly different effect at the 5% level

The pH value is also called the degree of acidity. pH is used to assess the level of acidity or alkalinity of a material. pH is related to the concentration of hydrogen ions as part of the acidity component and the concentration of hydroxyl ions as part of the basic component (Rondinini et al. 2001). The analysis results of oil samples that had been purified using sugarcane bagasse against pH parameters were in the range of 6.43 – 6.86, while used cooking oil purification using coffee dregs results were in the range of 6.60 -6.83 which means that the average sample had a weak acidic pH. The results of the pH parameter analysis of used cooking oil samples are presented in Figure 1.

Based on the analysis results above, it is known that the pH value of the oil purified with bagasse and coffee dregs shows a weak acid category. This is showed by the pH value which leads to an acid value that tends to approach neutral value. These results are in line with a study conducted by [28] that the used cooking oil as a result of the purification which has a pH value in the range of 4.95-6.55 is classified as the weak acid. A similar study conducted a study on a refining used cooking oil using noni fruit to produce used cooking oil with a pH value

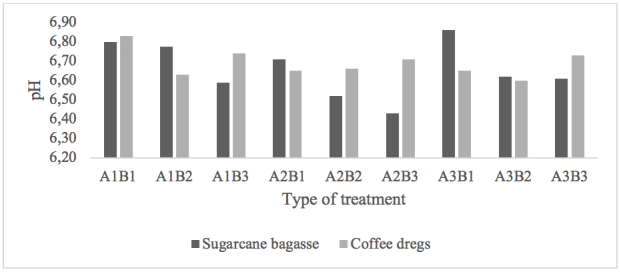


Figure 1. The analysis results of the pH parameters of the used cooking oil purification sample

Determination of moisture content was performed by gravimetric method. This gravimetric process involved weighing 5 grams of the sample in a known-weight porcelain dish, then heating the sample in a drying cabinet or oven at 105°C for 3 hours until the weight remains constant. The moisture content in the oil determined the

oil quality. The amount of water contained in the high oil had an impact on the emergence of a hydrolysis reaction, so that the quality of the oil decreases (Sumarna 2014). Analysis of moisture content used the oven method with gravimetric principles. The value of moisture content purified by the type of sugarcane bagasse absorbent had a moisture content value range between 0.666 - 0.7686%, while the absorbent type of coffee dregs had a moisture content value range between 0.0679 - 0.5487%. The parameter analysis results of the moisture content of used cooking oil samples are presented in Figure 2.

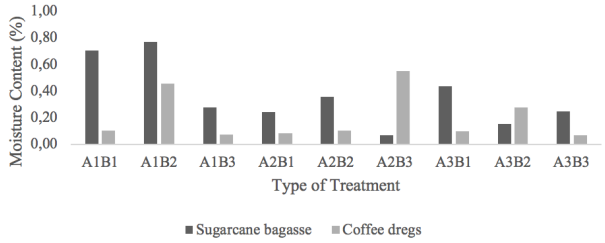


Figure 2. The analysis results of moisture content of the used cooking oil purification sample

Based on Table 1 and Fig. 2 above, it is known that the overall sample treatment had a lower moisture content value compared to the control. This means that there was a change in the moisture content after the purification process. This is influenced by the temperature treatment given, causing the moisture content in the sample to evaporate. Observations of temperature differences also affected the moisture content. The higher the temperature was, the more the water in the material evaporated. Thus, the moisture content in the material decreased (Leviana and Paramita 2017), notwithstanding the fact that it had not been completely proved in observation. In addition, the decrease in moisture content could be caused by the speed of stirring and absorption of the absorbent material, the stirring speed affected the possibility of absorbent colliding with the used cooking oil sample, because the faster the rotation was led to the larger coils or eddies would be (Ferdian et al. 2016), The results of another study stated that soaking using bagasse was able to absorb the moisture content of used cooking oil up to 0.27% (Hajar et al. 2016).

In this study, the lowest moisture content was 0.0666% with the type of bagasse absorbent treatment using a temperature of 60 ^{O}C and a rotation speed of 100 rpm. Meanwhile, SNI 01-3741-2002 concerning Quality Standards for Cooking Oil stipulated the moisture content in cooking oil, which is a maximum of 0.3%. Therefore, the results of the moisture content test in the sample oil fulfilled the SNI requirement.

The analysis results of variance showed that the use of the absorbent type with the treatment of temperature factors and stirring rotation speed resulted in a P value = 0.00 (P <0.005), meaning that the temperature factor and stirring rotation speed had no significant effect on the moisture content parameter produced at the 5% level.

Free fatty acids are fatty acids that are not esterified with glycerol (Trisnaliani et al. 2019). The results showed that the lowest ALB which was produced by the treatment of the absorbent type of coffee dregs with 70 ^{O}C of the temperature and 150 rpm of rotation speed was 0.2924%, while for the highest ALB treatment the absorbent type of bagasse which was produced with a temperature and rotation speed of 50 ^{O}C and 50 rpm was 1.9818%. The analysis results of the free fatty acid parameters of used cooking oil purification samples are presented in Figure 3.

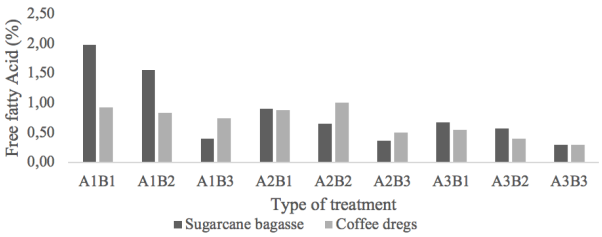


Figure 3. The analysis results of the free fatty acid parameters of the used cooking oil purification sample

Free fatty acids appeared due to the hydrolysis reaction of triglycerides (oil). Free fatty acid oxidation caused unpleasant odor and flavor. Thus, the value of free fatty acids in oil was often used as a damage parameter of used cooking oil (Kusumastuti 2004). Bagasse contained 32.1 percent of cellulose and 25.1 percent of lignin components, respectively. It is known that bagasse was effectively used as an adsorbent because of the role of -OH group bound to the cellulose and lignin (Tomi 2010). The purification principle of used cooking oil using sugarcane bagasse or coffee dregs was when triglyceride compounds reacted to water, ALB was formed, the ALB is written as RCOOH. RCOOH was the formula for a carboxylic acid compound. If the RCOOH was reacted with the -OH group contained in the bagasse, the H atoms of the compound reacted to produce RCOO and H2O compounds (water). Hence, when testing the ALB levels of used cooking oil which was purified with bagasse would be able to reduce the ALB value. This strengthens the previous research regarding to the use of bagasse to reduce ALB levels (Ratno et al. 2013). Therefore, the lower the value of free fatty acids produced by used cooking oil purification sample showed that the purification carried out was getting better. According to the comparison of the value of SNI 06-3532-1994, which indicates that the minimum ALB oil content is 2.5 percent (Hajar et al. 2016), so that the results of refining used cooking oil using coffee dregs and sugarcane bagasse fulfilled the applicable SNI criteria.

The analysis results of variance showed that the use of the absorbent type with the treatment of temperature factors and stirring rotation speed resulted in a P value = 0.00 (P <0.005), meaning that the temperature factor

and stirring rotation speed had no significant effect on the free fatty acid parameters at the 5% level.

The amount of acid value in the oil is the total fatty acids, either sodium-bound fatty acids or free fatty acids plus neutral fatty acids (triglycerides or unsaponifiable fat). The principle of determining the amount of acid value was to separate the amount of fatty acids from the sodium soap bond with the addition of strong acid, then extract it with a cake containing a mixture of solid paraffin, free fatty acids, neutral fats and mineral oils which might be exist (Riyanta and Nurniswati 2016). The analysis results of the acid value parameters of used cooking oil purification samples are presented in Figure 4.

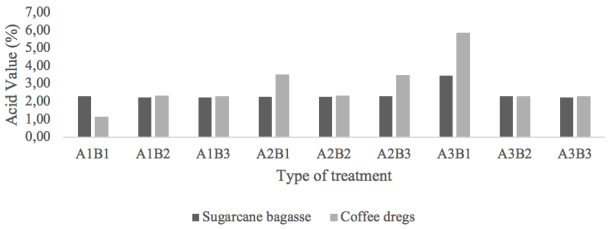


Figure 4. The analysis results of the Acid value parameters of the used cooking oil purification sample

Based on Fig. 4, it shows that the total acid content contained in the purified oil using the type of sugarcane bagasse absorbent was in the range of 2.24 – 3.44, while for the type of coffee dregs absorbent it was in the range of 1.12–5.85. The decrease in the acid value parameter for used cooking oil which was purified using bagasse was 39% with the acid value in the control was 3.49. The acid value was relatively low compared to previous studies which had a decrease in acid value of 49.39%. This was due to the difference in time and temperature used, the time taken was 20-40 minutes with a temperature of 100 ^{O}C, while in this study it was only 15 minutes and 70 ^{O}C. The longer the time that lasts for the absorption process, the more effective it was in reducing the acid value to the maximum time (saturation), besides the temperature used could stimulate the absorption reaction to be more effective (Rahayu et al. 2014).

According to the research results on the acid value parameter, it was not included in the SNI category because the standard of the maximum value required by SNI (3774 2013) is 0.6 mg KOH/g. This condition was possible because the adsorption process required the initial treatment which helped to maximize the purification process in the form of sample neutralization (Oko et al. 2020).

The variance analysis results showed that the use of the absorbent type with the treatment of temperature and stirring rotation speed factors resulted in a P value = 0.00 (P <0.005), meaning that the temperature and stirring rotation speed factors did not significantly affect the acid value parameter at 5% level.

Antiseptic transparent soap formulation from used cooking oil

The second stage of this research was to produce an antiseptic transparent soap formulation from the used cooking oil purification by adding guava leaf extract. The main ingredient for making transparent soap was used cooking oil (UCO) which was purified by the type of coffee dregs absorbent treatment, at a rotation speed of 150 rpm and a temperature of 70 ^{O}C. The antiseptic substance used in transparent soap products was the result of refining used cooking oil using guava leaves. Guava leaf extract has the ability to prevent the growth of Staphylococcus aureus bacteria which can cause disease (Nuryani et al. 2017). The implementation of the research at this stage was the variations treatment in the concentration of guava leaf extract by 1%, 2% and 3%. The results of the antiseptic soap formulation were then analyzed chemically (pH, moisture content), physically (high foam) and microbiologically (antiseptic power). The chemical analysis results of antiseptic transparent soap samples are presented in Table 2.

Table 2. Data analysis results of antiseptic transparent soap formulation

No	Treatment	Parameter			
		pH	Moisture Content	Foam Height	Antiseptic Power
1	Control	11.427a	36.505d	19a	14a
2	1%	13.06b	27.78a	21.5a	12a
3	2%	13.687c	30.67b	19a	8a
4	3%	13.629c	34.095c	20a	2a

Note: Different letter notations show a significant different effect at the 5% level

The pH value in the observation of antiseptic transparent soap samples from used cooking oil purification was in the pH range of 13.06 – 13.687. T his indicates that the sample had an alkaline pH category. The analysis results of the pH parameters of the antiseptic transparent soap sample are presented in Figure 5.

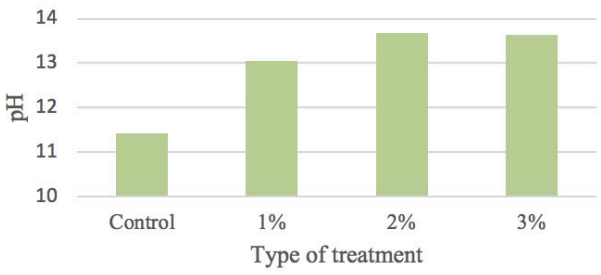


Figure 5. The analysis results of pH parameters of the antiseptic transparent soap sample

The pH value of cosmetic products, particularly soap, has an essential physical feature. pH is one of the parameters for soap quality that customers use in accordance with

Indonesian National Standards. (SNI 3532:2016). The pH value is an essential factor in evaluating the quality of bath soap; a very high or extremely low pH value can boost the absorption capability of the skin, allowing irritated skin to absorb more (Wasitaatmadja 2007). The soap pH produced in this study was classified as alkaline. The alkaline feature of the transparent soap was obtained from the use of NaOH solution which has alkaline properties as the main ingredient for the process of saponification reactions. Standard pH for bath soap ranges from 9-11 (Hernani et al. 2010). Therefore, the samples of antiseptic transparent soap products from used cooking oil fulfilled the predetermined soap quality criteria.

The variance analysis results showed that the addition of guava leaf extract in antiseptic transparent soap made from used cooking oil had a P value of 0.00 (P <0.05), meaning that the addition of guava leaf extract had a significant effect on the pH parameters at 5% level.

The value of moisture content in the observation of antiseptic transparent soap samples of used cooking oil purification was in the range of 27.78 – 36.505%. The value of the moisture content in the sample shows a relatively high moisture content. The parameter analysis results of the moisture content of the antiseptic transparent soap sample are presented in Figure 6.

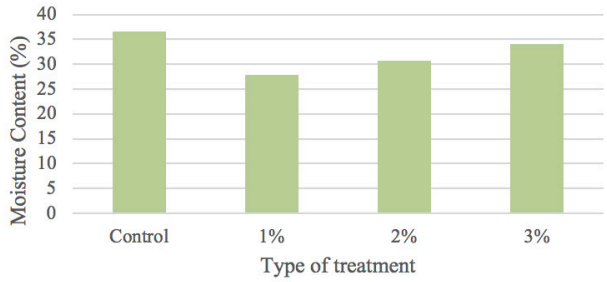


Figure 6. The analysis results of moisture content parameters of the antiseptic transparent soap sample

The moisture content of transparent soap in this study was quite high. It was influenced by the addition of water to several materials which functioned as the materials solvents, such as in dissolving NaOH (30%) and other materials (DEA and Sucrose). This is in line with a previous research which states that the value of the moisture content of transparent soap was influenced by the amount of water used (Afrozi et al. 2021). Based on SNI (3235-2016), the results of this research were still far from the criteria set, which is a maximum of 15%. The variance analysis result showed that the addition of guava leaf extract in antiseptic transparent soap from used cooking oil had a P value of 0.00 (P <0.05), meaning that the addition of guava leaf extract had a significant effect on the moisture content parameter at 5% level.

The foam height test was carried out to see the foam-power of antiseptic transparent soap made from used cooking oil purification which was made according to the high standard of soap foam set by the Indonesian National Standard (SNI) which is 13-220 mm. The high value of foam on the observation of antiseptic transparent soap samples made from used cooking oil purification was in the range of 19 – 21.5 mm. The parameter analysis results of the foam height of antiseptic transparent soap sample are presented in Figure 7.

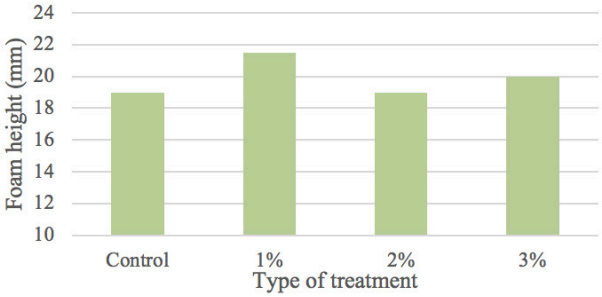


Figure 7. The analysis results of foam height parameters of the antiseptic transparent soap sample

The test results on the foam height of soap formula with a concentration of 1% guava leaf extract had the highest value of 21.5 mm, followed by a concentration of 3% and the lowest concentration of 2% and the control which had the same value of 19 mm. The results of the foam-height test of the soap showed that this value still tended to be low. Foam is a colloidal system in which the dispersed phase is a gas and the dispersing phase is a liquid (Fauziyah et al. 2019).

The presence of surfactant components in soap has a role in the cleaning process. The primary function of soap as a dirt cleanser is unaffected by the amount of foam produced; nevertheless, the more foam produced has an effect on skin sensitivity, which can lead to dry skin. The foam height is determined by the foam's stability, which is achieved by the foaming agent acting to maintain the foam contained in a thin layer that covers the gas molecules scattered in the liquid. If the active substance is mixed with water, it will work in solution (Fauziyah et al. 2019)

Antiseptic-power testing was carried out with a modified replica method (Sari and Dewi 2006). Soap samples consisted of 3 types (1%, 2% and 3%) and one sample without treatment. The next step was in accordance with the steps above. The analysis results of the antiseptic power of the antiseptic transparent soap sample are presented in Figure 8.

The percentage reduction, i.e. how much the soap can lower the amount of bacteria on hands, indicates the antiseptic activity of hand soap. According to Fig. 8, the percentage of inhibition appears to rise (the number of colonies decreases) when the concentration of guava leaf extract increases. The percentage values for the control soap formula, 1%, 2%, and 3% were 14, 12, 8, and 2, res-

pectively. Guava leaf extract has been shown to prevent the growth of Staphylococcus aureus bacteria (Nuryani et al. 2017) and *Escherichia Coli* bacteria (Qonita et al. 2019).

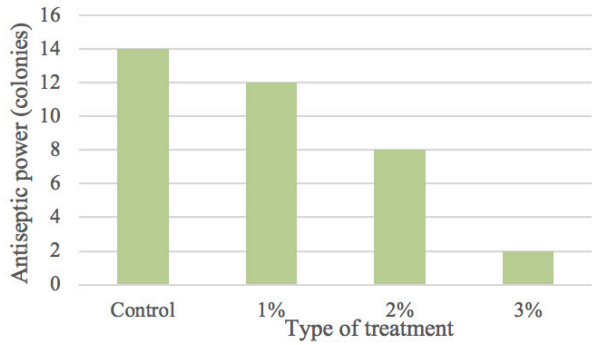


Figure 8. The analysis results of the antiseptic power of the antiseptic transparent soap sample

The ability of guava leaf extract is influenced by the content of tannin compounds. Tannin compounds have the ability to deactivate the adhesion of bacteria, inhibit the work of enzymes and inhibit the transport of proteins contained in cell membranes. The process of destroying bacterial cell membranes and the formation of metal ion complex bonds from tannins has a role in the toxicity of tannins (Fratiwi 2015).

Based on the test results on the antiseptic power of transparent soap, it was found that the most effective transparent soap was transparent soap with the addition of 3 percent guava leaf extract since it had the least number of colonies. The variance analysis results showed that the use of guava leaf extract concentration factor in the antiseptic transparent soap formula which made from used cooking oil did not significantly affect the antiseptic power parameter produced at 5% level. The results of the antiseptic power test are presented in Figure 9.

CONCLUSION

Purification of used cooking oil using the type of sugarcane bagasse absorbent produced oil with a pH value of 6.43-6.86, moisture content 0.67-0-769%, free fatty acids of 0.301-1.982% and acid value of 2.3-3.490%. Meanwhile, for the type of coffee dregs absorbent, it produced oil with a pH value of 6.60-6.83, moisture content of 0.068-0.549%, free fatty acids of 0.292-0.921 and acid value of 1.120-5.850. The results of variance analysis showed that there was a significant effect on the type of absorbent treatment, temperature and stirring speed on the parameters of moisture content, free fatty acid and acid value, while it was not significantly different on the pH. The results of the antiseptic transparent soap formulation obtained a pH value of 11.427-13,687, moisture content of 27.78-36.505, foam height of 19-21.5 mm and antiseptic power of 2-12 colonies. The findings of the variance analysis revealed that the concentration of guava leaf extract had a significant effect on the pH and moisture content parameters, but not on the foam height or antimicrobial power.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

There are no conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

This article is the result of a Novice Lecturers Research Program (PDP) in 2021 funded by the Directorate of Research and Community Service, the Directorate General of Research Strengthening and Development of the Ministry of Research, Technology and the National Innovation Research Agency.

REFERENCES

Afrozi, A. S., Safitri, N., & Nurhasanah, S. (2021). Pembuatan dan uji kualitas sabun transparan dengan variasi minyak kelapa murni atau Virgin Coconut Oil (VCO) dan minyak kelapa sawit. *Jurnal Ilmiah Teknik Kimia*, *5*(1), 31–45. [Google Scholar]

Alamsyah, M., Kalla, R., & Ifa, L. (2017). Pemurnian minyak jelantah dengan proses adsorbsi. *Journal Of Chemical Process Engineering*, *2*(2), 22–26. [CrossRef]

Bappenas. (2018). *Demografi pembangunan*.

Baryatik, P., Moelyaningrum, A. D., Asihta, U., Nurcahyaningsih, W., Baroroh, A., & Riskianto, H. (2019). Pemanfaatan arang aktif ampas kopi sebagai adsorben kadmium pada air sumur. *Jurnal Teknologi Lingkungan Lahan Basah*, *02*(1), 11–19. [CrossRef]

Butler. (2001). *Poucher's Perfumes, Cosmetics and Soap*. Kluwer Academic Publisher.

Fauziyah, I. R., Riyanta, A. B., & Sari, M. P. (2019). Formulasi dan uji sifat fisik sabun padat ekstrak maserasi daun sambiloto (Andrographis Paniculata Ness) dengan penambahan susu sebagai surfaktan. *Karya Tulis Ilmiah*, 1–9.

Ferdian, M. A., Hambali, E., & Rahayuningsih, M. (2016). Studi perbandingan produk insektisida formulasi EC dengan penambahan surfaktan DEA menggunakan vortex, mixer dan homogenizer. *Jurnal Teknologi Industri Pertanian*, *26*(1), 60–67.

Fratiwi, Y. (2015). The potential guava leaf (Psidium guajava L.)

for diarhe. *Jurnal Majority*, *4*, 113–118. [Google Scholar]

Guenther. (1987). *Minyak Atsiri. diterjemahkan oleh Ketaren dan Mulyono*. UI Press.

Hajar, E. W. I., & Mufidah, S. (2016). Penurunan Asam Lemak Bebas Pada Minyak Goreng Bekas Menggunakan Ampas Tebu Untuk Pembuatan Sabun. *Jurnal Integrasi Proses*, *6*(1), 22–27. [Google Scholar]

Hajar, E. W. I., Purba, A. F. W., Handayani, P., & Mardiah. (2016). Proses pemurnian minyak jelantah menggunakan ampas tebu untuk pembuatan sabun padat. *Jurnal Integrasi Proses*, *6*(2), 57–63. [Google Scholar]

Hambali, E., Bunasor, T. K., Suryani, A., & Giri, A. K. (2005). Aplikasi dietanolamida dari asam laurat minyak inti sawit pada pembuatan sabun transparan. *Jurnal Teknologi Industri Pertanian*, *15*(2), 46–53. [Google Scholar]

Hayati, R., Marliah, A., & Rosita, F. (2012). Sifat kimia dan evaluasi sensori bubuk kopi arabika. *J. Floratek*, *7*, 66–75.

Hernani, Bunasor, T. K., & Fitriati. (2010). *Formula Sabun Transparan Antijamur Dengan Bahan Aktif Ekstrak Lengkuas (Alpinia galangan L. Swartz)*. Fakultas Teknologi Pertanian Institut Pertanian Bogor.

Ketaren, S. (2005a). *Pengantar Teknologi Minyak Dan Lemak Pangan*. Universitas Indonesia.

Ketaren, S. (2005b). *Teknologi minyak dan lemak pangan*. Universitas Indonesia.

Kusumastuti. (2004). Kinerja zeolit dalam memperbaiki mutu minyak goreng bekas. *Jurnal Teknologi Dan Industri Pangan Universitas Negeri Semarang*, *15*(2).

Leviana, W., & Paramita, V. (2017). Pengaruh Suhu Terhadap Kadar Air Dan Aktivitas Air Dalam Bahan Pada Kunyit (Curcuma Longa) Dengan Alat Pengering Electrical Oven. *Metana*, *13*(2), 37. [CrossRef]

Misrawati, Retnawaty, S. F., & Fitri, Y. (2015). Uji karakteristik fisis, pH dan organoleptik pada minyak jelantah dengan penambahan buah mengkudu (Morinda citrifolia). *Photon: Jurnal Sain Dan Kesehatan*, *5*(2), 21–28. [CrossRef]

Nasrun, D., Samangun, T., Iskandar, T., & Mas'um, Z. (2017). Pemurnian minyak jelantah menggunakan arang aktif dari sekam padi. *Jurnal Penelitian Teknik Sipil Dan Teknik Kimia*, *1*(2), 1–7. [Google Scholar]

Ningrum, N. ., & Kusuma, M. A. . (2013). Pemanfaatan minyak goreng bekas dan abu kulit buah randu (Soda qie) sebagai bahan pembuatan sabun mandi organik berbasis teknologi ramah lingkungan. *Jurnal Teknologi Kimia Dan Industri*, *2*(2), 275–285. [Google Scholar]

Ningsih, D. R., Zusfahair, & Purwati. (2014). Potensi ekstrak daun kamboja (Plumeria alba L.) sebagai antibakteri dan identifikasi golongan senyawa bioaktifnya. *Molekul*, *9*(2), 101–109. [Google Scholar]

Nuryani, S., Putro, R. F. S., & Darwani. (2017). Pemanfaatan ekstrak daun jambu biji (Psidium guajava Linn) sebagai antibakteri dan antifungi. *Jurnal Teknologi Laboratorium*, *6*(2), 41–45. [CrossRef]

Oko, S., Mustafa, M., Kurniawan, A., & Muslimin, N. A. (2020). Pemurnian minyak jelantah dengan metode adsorbsi menggunakan arang aktif dari serbuk gergaji kayu ulin (Eusideroxylon zwageri). *Jurnal Riset Teknologi Industri*, *14*(2), 124–132. [CrossRef]

Patty, D. N., Papilaya, P. M., & Karuwal, R. L. (2017). Penentuan kadar asam lemak bebas pada minyak jelantah dengan penambahan antioksidan alami kulit pisang raja (*Musa sapientum*). *BIOPENDIX: Jurnal Biologi, Pendidikan Dan Terapan*, *3*(2), 124–128. [CrossRef]

Qonita, N., Susilowati, S. S., & Riyandini, D. (2019). Uji aktivitas antibakteri ekstrak daun jambu biji (*Psidium guajava* L.) terhadap bakteri escherichia coli dan Vibrio cholerae. *Acta Pharm Indo*, *7*(2), 51–57. [Google Scholar]

Rahayu, L. H., & Purnavita, S. (2014). Pengaruh suhu dan waktu adsorpsi terhadap sifat kimia-fisika minyak goreng bekas hasil pemurnian menggunakan adsorben ampas pati aren dan bentonit. *Momentum*, *10*(2), 35–41. [Google Scholar]

Rahayu, L., Purnavita, S., & Sriyana, H. (2014). Potensi sabut dan tempurung kelapa sebagai adsorben untuk meregenerasi minyak jelantah. *Jurnal Momentum UNWAHAS*, *10*(1), 138279. [Google Scholar]

Ramdja, A. F., Febrina, L., & Krisdianto, D. (2010). Pemurnian minyak jelantah menggunakan ampas tebu sebagai adsorben. *Jurnal Teknik Kimia*, *17*(1), 7–14. [Google Scholar]

Ratno, Mawarani, L. J., & Zulkifli. (2013). Pengaruh waktu perendaman ampas tebu sebagai biomaterial adsorbent pada proses pretreatment terhadap karakteristik biodiesel minyak jelantah. *Jurnal Teknik POMITS*, *2*(2), B262–B267. [Google Scholar]

Riyanta, A. B., & Nurniswati. (2016). Adsorpsi minyak jelantah menggunakan karbon aktif dan serbuk kopi pada pembuatan sabun padat ramah lingkungan. *SENIT 2016*, 118–123. [Google Scholar]

Rondinini, S., Buck, R. P., & Covington, A. K. (2001). *The Measurement of pH Definition, Standards and Producers*. IUPAC Provisional Recommendations.

Rosita, A. F., & Widasari, W. A. (2009). *Peningkatan Kualitas Minyak Goreng Bekas dari KFC dengan menggunakan Adsorben Karbon Aktif*. Universitas Diponegoro.

Sari, R., & Dewi, I. (2006). Studi Efektivitas Sediaan Gel Antiseptik Tangan Ekstrak Daun Sirih (Piper betle Linn.). *Majalah Farmasi Indonesia*, *17*(4), 163-9. [Google Scholar]

Sugiharta, S., Yuniarsih, N., & Ridwanuloh, D. (2021). Evaluasi pemurnian minyak jelantah menggunakan carbon active resin coated powder berdasarkan kadar asam lemak bebas. *Jurnal Buana Farma: Jurnal Ilmiah Farmasi*, *1*(2), 15–22. [Google Scholar]

Sulung, N., Chandra, A., & Fatmi, D. (2019). Efektivitas ampas tebu sebagai adsorben untuk pemurnian minyak jelatah produk Sanjai. *Jurnal Katalisator*, *4*(2), 125–132. [Google Scholar]

Sumarna, D. (2014). Studi metode pengolahan minyak kelapa sawit (Red Palm Oil) dari Crude Palm Oil. *Jurnal Jurusan Teknologi Hasil Pertanian Fakultas Pertanian Universitas Mulawarman*. [Google Scholar]

Susilawaty, A., Ibrahim, H., & Ugi, N. T. (2017). Pemanfaatan minyak jelantah dengan tambahan ekstrak daun cengkeh (Zyzygium aromaticum) sebagai sabun antiseptik dalam menurukan jumlah kuman pada telapak tangan. *Higiene*, *3*(1), 15–21. [Google Scholar]

Tomi, Z. B. (2010). *Analisis Senyawa Selulosa dan Licnin dalam Ampas Tebu*. Kimia FMIPA - Universitas Andalas.

Trisnaliani, L., Jaksen, Ridwan, K. A., & Fitriyanti, D. (2019). Pretreatment minyak jelantah dengan menggunakan adsorben sebagai bahan baku biodiesel. *Jurnal Kinetika*, *10*(03), 19–24.

Wannahari, R., & Nordin, M. F. . (2012). Reduction of peroxide value in used palm cooking oil using bagasse adsorbent. *American International Journal of Contemporary Research*, *2*(1), 185–191. [Google Scholar]

Wasitaatmadja, S. M. (2007). *Ilmu Penyakit Kulit dan Kelamin, Edisi kelima, cetakan kedua*. Fakultas Kedokteran Universitas Indonesia.

Yuliani, S., Udarno, L., & Hayani, E. (2003). Kadar tanin dan quersetin tiga tipe daun jambu biji (Psidium guajava). *Buletin Tanaman Rempah Dan Obat*, *14*(1), 17–24. [Google Scholar]

Yusriana, C. S., Budi, C. S., & Dewi, T. (2014). Uji Infusa daun nangka (Artocarpus heterophyllus) terhadap pertumbuhan bakteri (Staphylococcus aureus). *Jurnal Permata Indonesia* ., *5*(2).

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 21-28
DOI: https://doi.org/10.31015/jaefs.2023.1.3

Analysis of housewives' knowledge levels and behaviors toward food waste and sustainable nutrition

Melisa Türk[1] • **Neda Saleki**[2]

[1] Nutrition and Dietetics, Health Sciences Faculty, Medipol University, Istanbul, Türkiye
[2] Nutrition and Dietetics, Health Sciences Faculty, Medipol University, Istanbul, Türkiye

Abstract

This article aims to reveal the knowledge, attitudes and behaviors of housewives living in Turkiye about food waste and sustainable nutrition. This study was conducted with 90 housewives between the ages of 25-65 from Turkey using the survey method. According to the research results; 24% of the participants stated that they waste food, while 53% stated that they do it sometimes. A significant positive correlation was found between education level and red meat and meat products, milk and dairy products and egg waste ($p<0.05$). Sustainable nutrition knowledge scores of the participants were found to be insufficient, with an average of 22.54 ± 3.80. A positive correlation was found between the amount of protein and zinc intake and the level of sustainable nutrition knowledge ($p=0,027$, $r=0,233$ and $p=0,033$, $r=0,225$). A significant and positive relationship was found between the scores of the participants in the scale of sustainable nutrition and healthy eating behaviors and their age ($p=0,040$, $r=0,220$). It has been determined that the knowledge levels of housewives about sustainable nutrition are deficient and insufficient. The issues of food waste and sustainable nutrition gain importance in terms of raising awareness and taking precautions for today and future generations, both economically and socioculturally.

Keywords: Level of knowledge, Housewife, Food waste, Sustainable nutrition, Turkiye

Citation: Turk, M., Saleki, N. (2023). Analysis of housewives' knowledge levels and behaviors toward food waste and sustainable nutrition. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 21-28

Received: 27 September 2022
Revised: 25 Novemberr 2022
Accepted: 28 November 2022
Published Online: 22 January 2023

Correspondence: Neda Saleki
E-mail: nyousefirad@medipol.edu.tr

INTRODUCTION

Food waste refers to discarded, leftover, and decaying human food regardless of any cause at the consumer level, leading to potentially serious environmental and socioeconomic consequences (Oral, 2015). According to Food Waste Index Report 2021; food waste occurs at the rate of 39% among manufacturers, 5% among retailers, 14% in the catering sector, and 42% at homes. Considering that housewives often spend time at home, where the majority of food waste occurs, the demonstration of awareness, attitudes, and behaviors toward food waste and sustainable nutrition among housewives shall be important for the reduction and prevention of food wastage. A total of 931 million tons of food are wasted worldwide annually. Globally, 17% of ready-to-eat foods go directly to waste at retail outlets, homes, and restaurants. While 40% of food waste occurs during post-harvest handling and processing in developing countries, more than 40% of the loss occurs at the retail and consumer levels in industrialized countries. Consumer food waste is reported as 95-115 kg/year in Europe and North America, and 6-11 kg/year in Sub-Saharan Africa and South/Southeast Asia.

The United Nations' 2021 report on Food Waste states that 931 million tons of food are wasted globally every year. Food waste in Turkey is 93 kg per capita per year, bringing the country to the top tiers of the list among others with the highest wastage in the world. Various causative factors such as improper processing of food, poor storage conditions, poor planning, failure to pay attention to recommended shelf lives, uneaten cooked food, and environmental factors are major contributors to food waste (UNEP Food Waste Inde Report, 2021).

Sustainable nutrition refers to those diets with low environmental impacts, contributing to food and nutrition security and healthy lives of current and future generations (FAO/WHO, 2019). Such diets are mindful of biodiversity and the ecosystem, helping preserve environmental resources. Sustainable nutrition should ensure healthy diets, which are acceptable culturally, accessible, economically affordable, nutritionally sufficient, and safe. The term sustainable nutrition was first used by Gussow and Clancy in 1986 (FAO, 2012). Adoption of a sustainable nutrition model with consequent reductions in food wastage is necessary to ensure food security and promote nutrition for the future, and improve the livability of the world for future generations. Sustainable diets are essential for the sustainability of the Earth. Sustainable nutrition aims to ensure optimal growth and development for all individuals globally, enhance the physical, mental, and social well-being and promote the functionality of the current and future generations, prevent any kind of malnutrition, reduce the risk of nutritionally-related non-communicable diseases, and contribute to the protection of the Earth's biodiversity and sustainability (FAO/WHO, 2019).

Our study aimed to examine the knowledge levels and behaviors of housewives toward food waste and sustainable nutrition. Because housewives usually spend most of their time at home, where the majority of food waste occurs, the study shall provide important results for reducing and avoiding food waste by demonstrating the awareness, attitudes, and behaviors of housewives toward food waste and sustainable nutrition. Because only a few studies on this subject matter are available in the literature, we think that the results of our study will be important for paving the way for future studies.

MATERIAL AND METHOD

Study Sample and Data Collection Tools

The research was carried out between January 2022 and June 2022**.** The sample of study consisted of 90 housewives aged 25-65 years, who lived in the following cities of Turkey, including Istanbul, Şanlıurfa, Aydın, İzmir, Kars, Kırklareli, Sivas, and Gaziantep. The majority of participants were from Istanbul. Employed women were excluded but women, who spent most of their time at home, were included in the study. The minimum number of participants required to be included in the study was calculated using G-POWER to achieve a 5% margin of error, 95% power, and moderate effect size. Accordingly, the minimum number of participants to be included in the study was calculated to be 84. Because the number of subjects, who voluntarily agreed to participate in the study was 90, it can be argued that the sample size was sufficient for the generalizability of the results. A questionnaire form was used as a data collection tool. The questionnaire was administered to participants during face-to-face interviews. The approval to conduct the study was obtained from the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (Decision No.03/02/2022 /127). After informed consent forms were obtained, subjects were sequentially interviewed on scheduled dates. The study complies with the provisions of the Declaration of Helsinki.

Demographic Characteristics and Anthropometric Measurements

The first part of the questionnaire consisted of questions about demographic information including age, educational status, marital status, city of residence, monthly income, and anthropometric measurements (height, weight, and Body Mass Index -BMI-). The body weight and the height of the subjects were recorded based on their statements. BMI was calculated by the researcher dietitian. The BMI value was obtained by dividing the body weight (kg) by the square of the height (m^2) and was evaluated according to the World Health Organization (WHO) classification. According to the WHO classification, subjects with a BMI of <18,5 kg/m^2 were categorized as underweight, those with a BMI of 18,5-24,9 kg/m^2 were categorized as having a normal body weight, those with a BMI of 25,0-29,9 kg/m^2 were categorized as overweight, and those with a BMI of >30,0 kg/m^2 were categorized as obese (WHO. WHO STEPS Surveillance)

Evaluation of the Nutritional Status

As the second part of the questionnaire, a 24-hour retrospective food intake form was used. To ensure the correct reporting of the quantities of food intake by participants, the visuals and measures in the "Food and Nutrition Image Catalogue-Measures and Quantities" book were utilized (Rakıcıoğlu et al., 2017). Collected data were analyzed using the Nutrition Information System Software (BeBis 8.1) and the intakes of energy, macronutrients, and micronutrients were calculated. Participants' food intake records were evaluated according to Turkey Dietary Guidelines 2015 (TÜBER, 2015).

Knowledge Levels and Attitude toward Food Waste and Sustainable Nutrition

The third part of the questionnaire consisted of questions about food waste specific to each study participant

(causes of food waste, quantities and frequencies of buying and wasting foods according to food groups, and practices toward non-consumable foods). The last part of the questionnaire included questions about participants' approaches toward the concept of sustainable nutrition and their knowledge levels of this subject matter. For the assessment of sustainable and healthy eating behaviors in the study, the Turkish version of the Sustainable and Healthy Eating Behaviors Scale was used. The scale was developed based on sustainable nutrition principles, the LiveWell Approach®, and Food and Agricultural Organization's (FAO) definition of the concept of sustainable nutrition as described by Zakowska-Biemans et al. (Zakowska-Biemans et al. 2016). The validity and reliability analysis of the Turkish version of the scale was conducted by Koksal et al. (Koksal et al. 2022). The Sustainable and Healthy Eating Behaviors Scale consists of a total of 8 factors and 34 items. The 8 factors of the scale are listed as Healthy and Balanced Diet, Quality Labels (Regional and Organic), Reducing Meat Consumption, Local Food, Low Fat, Avoiding Food Waste, Animal Welfare, and Seasonal Food. Participants were asked to respond to each item by marking one of the following options, including "never", "very rarely", "rarely", "sometimes", "often", "very often", and "always". Measurements were performed on a 7-point scale, where "never" was scored as 1 point and "always" was scored as 7 points.

The Sustainable Diet Index was used to measure participants' knowledge levels of sustainable nutrition. There were 15 different expressions in this index and participants were asked to score their behavior on a 5-point Likert-type scale, concerning the expressions. The expression, which did not suit the sustainable nutrition behavior of the participant, was scored 0 points. The expressions, which were found appropriate by participants, were scored on a scale from 1 to 4 points. Because the index consisted of 15 questions, the highest score that could be obtained was 60. Participants with a score of ≤ 30, which was 50% of the highest score that could be obtained, were considered to have inadequate knowledge levels of sustainable nutrition. Participants with scores of > 31 were considered to have adequate knowledge levels.

Statistical Analysis

The statistical analysis of the study data was performed using the IBM SPSS 26.0 software. The conformity of the numerical variables to the normal distribution was examined using the Shapiro-Wilk normality test. As descriptive statistics for numerical variables, mean and standard deviation were used for normally distributed data, and median, minimum, and maximum were used for variables, which did not conform to a normal distribution. Categorical variables were presented as numbers and percentages. Correlations between numerical variables were examined by Pearson's or Spearman's correlation coefficients, depending on the conformity of variables to the normality assumption. The relationship between categorical and numerical variables was examined using the Chi-square test.

RESULTS

The study included 90 housewives from several cities in Turkey. The majority of participants (75,3%, n:70) were from Istanbul. Demographic and socioeconomic information about participants is presented in Table 1. Table 1 shows that participants were 25-68-year-old housewives. Of the participants; 30% (n:27) were 35-44 years old; 34,4% (n:31) were 45-54 years old. The examination of the educational status of participants revealed that 2,2% (n:2) were illiterate. Of the participants; 30% (n:27) were graduates of high school, and 29.8% (n:27) were graduates of a university. Of the participants, 81,1% (n: 73) were married and 7,8% (n:7) were single. Subjects with 4 or more household members and subjects with 1-3 household members accounted for 46,7% (n:42) and 53,3% (n:48) of participants, respectively. The average household income was ≤3000 TL in 6,7% (n:6) of participants, 3001-4000 TL in 13,3% (n:12), 4001-5000 TL in 17,8% (n:16), 5001-6000 TL in 18,9% (n:17), and ≥6001 TL in 43.3% (n:39) of participants.

Regarding the question of whether they had ever heard of the term "sustainable nutrition", 32,6% (n:29) and 67,4% (n:60) of participants answered "yes" and "no", respectively. Figure 1 shows how subjects, who answered "yes" to the question, first encountered the term, sustainable nutrition. Accordingly, 37% (n:11) of housewives first heard of the term sustainable nutrition on social media, 30% from television and radio, and 23% first heard of the term from healthcare professionals including physicians, dietitians, etc.

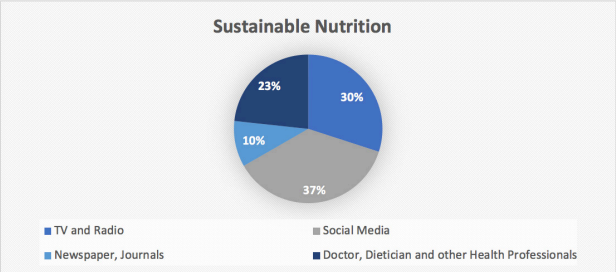


Figure 1. Source/Person/Place Where They Heard The Concept of Sustainable Nutrition

To the question "Do you waste food unintentionally?", 53% (n:48) of participants answered "sometimes", 24% (n:22) answered "yes", and 22% (n:20) answered "no". Participants, who reported wasting food, were asked about their causes of food wastage. The results are presented in Figure 2. Figure 2 shows that 20,4% (n:19) of the participants reported wasting food because of the expiration of shelf lives, and 31% (n:22) reported wastage because of storing foods for too long.

Table 1. Sociodemographic Characteristics of the Participants

Features		Number	Percentage (%)
Age	25-34	15	16,7
	35-44	27	30
	45-54	31	34,4
	55-64	15	16,7
	>65	2	2,2
Education	No read-write	2	2,2
	Primary School	17	18,9
	Middle School	17	18,9
	High School	27	30
	Bachelor	25	27,8
	Master	2	2
Marital Status	Single	7	7,8
	Married	73	81,1
	Divorced	5	5,6
	Widow	5	5,6
Number of Persons in Household	1	2	2,2
	2	21	233
	3	25	27,8
	≥ 4	42	46,7
City	İstanbul	70	75,3
	Other cities (Adana, Aydın, Gaziantep, Mersin. Izmir, Diyarbakır, Sivas, Şanlıurfa, Kars)	20	24,7
Average Income	≤3000 TL	6	6,7
	3001-4000 TL	12	13,3
	4001-5000 TL	16	17,8
	5001- 6000 TL	17	18,9
	≥6000 TL	39	43,3

Figure 2. Reasons for Wasting Food

The chi-square test was used to examine the relationsh ip between the educational status of housewives and their levels of food waste. The analysis results are presented in Table 2. Educational status was positively correlated with the waste of red meat and meat products, milk and dairy products, eggs, poultry meat and products, bread and bakery productsand pulses (p<0,05). With higher levels of educational status, the level of waste of red meat and meat products, milk and dairy products, eggs, poultry meat and products, bread and bakery products, and pulses increased.

Table 2. The relationship between education level and food waste level

Educational Level	Food Waste							
	Red Meat and Meat Products					Chi-Square		
	1	2	3	4	5	Value	sd	*p
No read-write	1	0	0	0	1	46,054	10	0,000*
Primary school	17	0	0	0	0			
Middle School	16	1	0	0	0			
High school	25	2	0	0	0			
Bachelor's Degree	24	1	0	0	0			
Master	2	0	0	0	0			
	Milk and Dairy Products					Chi-Square		
	1	2	3	4	5	Value	sd	*p
No read-write	1	0	0	0	1	33,577	20	0,029*
Primary school	13	4	0	0	0			
Middle School	12	1	0	0	0			
High school	17	9	0	0	1			
Bachelor's Degree	14	6	2	1	0			
Master	1	1	0	0	0			
	Poultry and Products					Chi-Square		
	1	2	3	4	5	Value	sd	*p
No read-write	1	0	0	1	0	31,670	15	0,007*
Primary school	16	0	0	1	0			
Middle School	14	2	1	0	0			
High school	22	4	0	0	0			
Bachelor's Degree	23	1	0	0	0			
Master	2	0	0	0	0			
	Fish Products					Chi-Square		
	1	2	3	4	5	Value	sd	*p
No read-write	2	0	0	0	0	7,724	10	0,656
Primary school	16	1	0	0	0			
Middle School	14	3	0	0	0			
High school	24	3	0	0	0			
Bachelor's Degree	24	0	0	1	0			
Master	2	0	0	0	0			
	Cooked Food					Chi-Square		
	1	2	3	4	5	Value	sd	*p
No read-write	1	1	0	0	0	9,524	20	0,976
Primary school	9	7	0	1	0			
Middle School	9	5	2	1	0			
High school	14	8	2	2	0			
Bachelor's Degree	12	10	1	0	1			
Master	0	1	0	0	0			
	Packaged Food					Chi-Square		
	1	2	3	4	5	Value	sd	*p
No read-write	1	1	0	0	0	6,456	15	0,971
Primary school	10	5	1	0	0			
Middle School	11	6	0	0	0			
High school	13	12	2	0	0			
Bachelor's Degree	14	7	2	1	0			
Master	1	0	0	0	0			
	Bread and Bakery Products					Chi-Square		
	1	2	3	4	5	Value	sd	*p
No read-write	1	0	0	0	1	25,569	15	0,043*
Primary school	7	9	1	0	0			
Middle School	9	8	0	0	0			
High school	13	12	2	0	0			
Bachelor's Degree	10	13	1	0	1			
Master	1	1	0	0	0			
	Egg					Chi-Square		
	1	2	3	4	5	Value	sd	*p
No read-write	1	0	0	1	0	43,278	10	0,000*
Primary school	16	1	0	0	0			
Middle School	16	1	0	0	0			
High school	25	1	0	0	0			
Bachelor's Degree	22	1	0	0	0			
Master	2	0	0	0	0			

*Chi-square test was applied. (p<0.05: Significant)
(1: None, 2: Less than 10%, 3: 11-25%, 4: 26-50%, 5: More than 50%)

The mean scores of participants from the Sustainable and Healthy Eating Behaviors Scale ranged from 1,22 to 6,34 with an overall mean score of 4.34 ± 1,12. The mean scores by the factors in the scale are presented in Table 3 showing that the highest mean score (4.84 ±1,44) was from the healthy and balanced diet factor, and the lowest mean score was from the local food factor (2,95±1,16).

Table 3. Average Scores Received from the Sustainable Nutrition and Healthy Eating Behaviors Scale by Factors

Factors	Mean ($\bar{X}$± SS)
Quality Marks (regional and organic)	4,40 ±1,25
Seasonal Foods and Avoiding Food Waste	4,59±1,37
Healthy and Balanced Nutrition	4,84 ±1,44
Local Food	2,95±1,16
Reducing Meat Consumption	3,81 ±1,42
Animal Health	4,45±1,72
Low Fat	4,12 ±1,52

The scores of the Sustainable Diet Index ranged from 14 to 29, with a mean score of 22,54, which was considered inadequate. Spearman's correlation analysis revealed a positive and significant relationship between age and the scores of the Sustainable and Healthy Eating Behaviors Scale. With the increased age of participants, the scores obtained from the Sustainable and Healthy Eating Behaviors Scale increased. No significant correlations were found between the Sustainable Diet Index and age (Table 3).

Table 4. Age relationship with the scores obtained from the scales

Spearman's correlation test was used to examine the relationship between selected nutrients and participants' knowledge levels of sustainable nutrition (Table 5). The analysis revealed that the quantities of protein and zinc intake and the knowledge level on sustainable nutrition were positively correlated (p<0,05).

Sustainable Nutrition and Healthy Eating Behavior Scale							
Scores	Age					Correlation	
	25-34	35-44	45-54	55-64	65+	p	r
1,00-2,99	4	5	5	0	0		
3,00-4,99	7	13	16	11	0	0,040*	0,220
5,00-6,99	1	9	10	4	2		
Scores	Age					Correlation	
	25-34	35-44	45-54	55-64	65+	p	r
14,00-19,99	6	6	10	4	0		
20,00-24,99	6	15	12	7	0	0,104	0,173
25,00-29,99	3	6	9	4	2		

*Spearman correlation test was applied. (p<0,05: Significance)

Table 5. The Relationship Between Sustainable Nutrition Scale Scores and Nutrient Intake Amounts

Variables	p	r
Energy	0,335	0,103
Carbohydrate (g)	0,191	0,139
Protein (g)	**0,027***	**0,233**
Fat (g)	0,484	0,075
Cholosterol	0,857	0,019
Fiber	0,327	0,104
Saturated- fatty acids	0,098	0,176
Vitamin A	0,635	0,051
Folat	0,939	0,008
Vitamin B12	0,137	0,158
Vitamin C	0,847	0,021
Vitamin E	0,865	0,018
Zinc	**0,033***	**0,225**
Iron	0,074	0,189
Calcium	0,291	0,112
Sodium	0,761	0,032
Potassium	0,562	0,062

*Spearman correlation test was applied. (p<0,05: Significance)

DISCUSSION

Today, excess food consumption is a major problem affecting all people, especially when population increases are considered. This brings the question "what will people eat?" to minds. The prevention of food waste is of great importance in the fight against hunger in the world. Food waste occurs more commonly in developing and developed countries compared to less developed countries (Gustavsson et al., 2011)Various measures should be implemented and changes to current practices should be introduced in order to reduce wastage and achieve improvements in freedom from hunger. However, striking changes have been observed in diet patterns. Compositions of diets have changed with increasing intakes of energy. Increasing levels of income along with urbanization and globalization have increased the demand for different types of food. These all emphasize the importance of the term sustainable nutrition more than before, which has recently become widely heard. Food wastage is reported to be 72% at home and during processing. Of the 64 million tons of total food waste from households and processing stages, 47 million tons of food are wasted by households. (Tekiner et al., 2021.) Therefore, in this study, we surveyed food waste among 90 housewives, who were actively involved in kitchen management at home.

Of the study participants, 30% (n:27) were in the 35-44 age group and 34,4% (n:31) were in the 45-54 age group. Thirty percent (n:27) of participants were high school graduates, 29,8% (n:27) were university graduates, and 81,1% (n:73) were married. To the question "Do you

waste food unintentionally?", 53% (n:48) of participants answered "sometimes", 24% (n:22) answered "yes", and 22% (n:20) reported that they did not waste food at all. The examination of the relationship between the educational level and food waste revealed that, as the educational level increased, the waste of red meat, poultry meat, milk and dairy products, eggs, bread and bakery products, and pulses increased ($p<0,05$). In a study conducted by Aydın and Yıldız in 2011 with 400 randomly selected consumers from different socio-economic levels in the province of Sivas, it was observed that bread wastage increased with increased levels of education. In that study, 31% (n:22) of the participants reported that they wasted food because of storing them for too long.(Aydın &Yıldız, 2011) Because the socioeconomic income level may decrease with decreasing levels of education, the purchasing power of persons with low income levels would be less, resulting in lower amounts of food waste compared to individuals from higher income levels. On the other hand, people with a high income would not be cautious about wastage and cause high amounts of food waste because of the high purchasing power. Another study on household members included 203 people, and 30,1% (n:126) of the participants in that study reported that they wasted food because of the expiration of shelf life. This result is in line with the results of our study (Demir, 2020). Similarly, in a study conducted with 150 consumers, who were responsible for kitchen management and living in Izmir, it was found that 68% of the participants wasted food and the most common cause of food wastage was the expiration of shelf life (Daysal and Demirbaş, 2020).

The term "sustainable nutrition" has been borrowed from the term "sustainable agriculture" and aims to minimize the wastage of natural resources and ensure natural food production for seasonal consumption. There are only a few studies on sustainability and sustainable nutrition in the scientific literature from Turkey (Burlingame Dernini, 2011). In this study, we measured participants' knowledge levels of sustainable nutrition. The results show that subjects' knowledge levels of the definition of sustainable nutrition are variable ($p<0.005$). Participants were asked whether they had ever heard of the term "sustainable nutrition". Of the participants, 32,6% (n:29) answered "yes" and 67,4% (n:60) answered "no" to this question. A study reported that 24,3% of individuals, who were aged 20 and over and who were not students, had heard of the definition of sustainable nutrition before (Gülsöz,2017). In a study conducted with dietitians and dietitian candidates, it was found that subjects heard of the definition of sustainable nutrition most commonly -corresponding to a rate of 33.76%- during academic and scientific activities such as lectures and conferences during their undergraduate education (Özen, 2019) It could be considered likely that people, who are involved in the science of nutrition, would have heard of the definition of sustainable nutrition previously. We may suggest that housewives' knowledge level on this subject matter is low because they do not have that many opportunities to participate in scientific events such as conferences and congresses.

In our study, we used the Sustainable Diet Index to measure participants' knowledge levels of sustainable nutrition. The sustainable nutrition knowledge scores of the participants were evaluated over 30 points. The mean score from the scale was 22,54 + 3,80 and inadequate. In a study participants' sustainable nutrition knowledge scores were higher compared to the scores in our study (Özen, 2019) Participants were dietitians in that study and this difference between the samples of the two studies may explain different results. The study by Gülsöz et al. reported increasing knowledge levels of sustainable nutrition with increasing age (Gülsöz, 2017). We suggest that the lack of any increase in sustainable nutrition knowledge scores with increased age in our study may have resulted from differences in educational status across our study participants.

Of the subjects, who have heard of the term sustainable nutrition before, 37% reported that they heard of this term on social media. Therefore, several types of posts should be promoted through several social media tools used by people from different age groups in order to increase public awareness of sustainable nutrition. A significant correlation was found between the increase in participants' sustainable nutrition knowledge scores and the daily intake of zinc and protein based on food intake records ($p<0,05$). In the abovementioned study with dietitians and dietitian candidates, the comparison of energy and nutrient intake with the sustainable nutrition knowledge levels of participants revealed differences in the amounts of intake of carbohydrates, fiber, non-essential amino acids, and iron by sustainable nutrition knowledge levels, with high levels of intake among participants with adequate knowledge (Özen, 2019). Pelletier et al. (2013) found high levels of vegetable and fruit intake among subjects, who considered the sustainable, local, and organic alternative food production systems important, compared to subjects with moderate or low levels of keenness toward that subject matter. Furthermore, the intake of added sugar and fat was lower and the dietary fiber intake was higher in the former group compared to the latter in that study (Pelletier, 2013). In our study, the knowledge levels were inadequate among housewives and this led to different results compared to those reported in the literature. However, the intake of zinc and protein was high in our study. We think that the difference in the results may be associated with inadequate knowledge levels of sustainable nutrition, dietary habits, and high levels of consumption of animal-sourced foods.

In this study, the sustainable and healthy eating attitudes of subjects were evaluated with the 'Sustainable and Healthy Eating Behaviors Scale'. The mean scores of the

Sustainable and Healthy Eating Behaviors Scale were found to be in the range of 1.22 - 6.34 with an overall mean score of 4,34 ± 1,12. A significant correlation was found between the scale scores and age. The highest mean score (4,84 ±1,44) was obtained from the 10-item 'Healthy and Balanced Diet' factor, which included avoiding sugary drinks, limiting salt intake, and preferring additive-free and natural foods and foods with high nutritional value, containing vitamins and minerals. We suggest that this result may be explained by the adoption of healthy eating behaviors because of the health problems occurring with increased age. The lowest mean score was obtained from the local food factor (2,95±1,16). We suggest that the low score was associated with limited access of our study participants to local foods because a majority of them lived in urban areas. Similar results were obtained in the aforementioned study conducted on nutrition and dietetics students and it was reported that participants obtained the highest score from the healthy and balanced diet factor (Kıyan et al., 2020)

Sustainable nutrition refers to a concept with changes in dietary preferences to reduce excess consumption and promote the adoption of nutritious diets with lower environmental impact, reducing losses and waste in food systems (Alsaffar, 2016). Ensuring adequate nutrition through sustainable nutrition systems is critical globally (Stock et al., 2018). With increasing independence during the transition from adolescence to young adulthood, young adults experience difficulties to select healthy foods. It has been reported that old individuals are more keen and dependent on sustainable nutrition than younger individuals, males, individuals with low income or education levels, and individuals relegated to the fringe of society (Gilg et al., 2005). A study on young subjects with a median age of 21 years reported low levels of knowledge of sustainable nutrition and a serious need for training about the subject matter in this group of individuals (Yolcuoglu et. al., 2021). In our study, consistent with the results reported in the literature, the scores from the sustainable and healthy eating behaviors scale increased with the increased age of participants ($p<0,05$). We think that, with increasing age, awareness of and keenness toward social issues increase along with the increasing need for the adoption of healthy diets for the alleviation of chronic diseases, resulting in modifications in the behaviors of individuals.

Nutrition is one of the most basic needs, yet, it is inadequate or irregular for most people, presenting as a multidimensional global problem. In this study in association with this subject matter, we have observed that housewives do not adequately understand the terms food waste and sustainable nutrition, and have not adopted relevant practices yet. However, through the reports by participants during the administration of the questionnaires in this study, we have also observed that the keenness on food waste and loss of food has begun to increase due to economic concerns in the presence of increasing economic challenges.

CONCLUSION

In this study, it was found that the term sustainable nutrition is not adequately known among housewives. It was found that the majority of participants wasted food. When people, who reported wasting food, were asked about the causes of wastage, the majority reported that they wasted foods because of storing them for too long. A positive correlation was found between the educational level of participants and the wastage of red meat and meat products, milk and dairy products, eggs, poultry meat and products, bread and bakery products, and pulses ($p<0,05$). The overall mean score obtained by participants from the sustainable and healthy eating behaviors scale was low. The mean score from the sustainable diet index was inadequate. A positive and significant correlation was found between the sustainable and healthy eating behaviors scale scores and age. The limitation of the study is that the study included exclusively women, who were not employed and who spent most of the time at home.

Although the term sustainable nutrition is not a newly introduced concept, its importance has just begun to be understood. It is known that there are developments in the subject matters of sustainable nutrition, solid waste disposal, and food waste in Turkey but they are inadequate. It is of great importance for future generations to increase society's level of knowledge and awareness of food waste and sustainable nutrition. Improvements in housewives' knowledge levels of such subject matters shall be especially important for the benefit of society because housewives are a group of individuals, who allocate the majority of time to household work. For this purpose, in addition to the importance of the concept of adequate and balanced nutrition, the importance of sustainable nutrition should also be communicated. Sustainable nutrition should be included in national nutritional guidelines of countries. Articles about food waste and sustainable nutrition should be published in newspapers, magazines, and journals, and relevant advertisements and visual aids should be prepared and delivered. Courses relevant to sustainable nutrition and food waste should be included in the curricula of universities. In order to increase housewives' attentiveness and awareness of sustainable nutrition and food waste, scheduled home visiting courses of training need to be developed. Cooking courses aiming to ensure sustainable nutrition and reduce food waste should be developed and included in the list of free courses provided by municipalities in Turkey.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (Decision No. 03/02/2022 /127).

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

I would like to thank all voluenteers that take a part in this study.

REFERENCES

Alsaffar, A. A. (2016). Sustainable diets: The interaction between food industry, nutrition, health and the environment. Food science and technology international, 22(2), 102-111. https://doi.org/10.1177/108201321557202

Aydin, F., Yildiz, S. (2011). Determination of Bread Consumption Habits and Consumer Dynamics in Sivas Province. Journal of Atatürk University Faculty of Agriculture, 42(2), 165-180.

Burlingame B, Dernini S. Sustainable diets: the Mediterranean diet as an example. Public Health Nutrition, 2011; 14 (12A): 2285–2287. https://doi.org/10.1017/S1368980011002527

Daysal, H., Demirbaş, N. (2020). Causes of household food waste and suggestions to reduce it: The case of İzmir province. Balkan and Near East Social Sciences Journal. 06 (03): 40-47.

Demir, Y. (2020). A Study to Evaluate Individuals' Perceptions of Household Food Waste. Black Sea International Scientific Journal, 1 (48), 10-26. https://doi.org/10.17498/ksea.750092

FAO/WHO. Sustainable healthy diets-Guiding principles. Rome; 2019.

FAO. (2012). Food and Agriculture Organization of the United Nations. Sustainable diets and biodiversity. Rome.

Gilg, A., Barr, S., & Ford, N. (2005). Green consumption or sustainable lifestyles? Identifying the sustainable consumer. Futures, 37(6), 481-504. https://doi.org/10.1016/j.futures.2004.10.016

Gülsöz S. (2017). Evaluation of the knowledge and practices of individuals aged twenty and over on sustainable nutrition: Başkent University Institute of Health Sciences. https://doi.org/10.31015/jaefs.2021.2.3

Gustavsson J., Cederberg J., Sonesson U. (2011). Global Food Losses and Food Waste, Save Food Congress, 16 May, Düsseldorf.

Kıyan, E. K., & İkizoğlu, B. (2020). Types of waste in the context of waste management and general overview of waste disposal in Turkey. International Journal of Agriculture Environment and Food Sciences, 4(4), 520-527. https://doi.org/10.31015/jaefs.2020.4.17

Koksal, Eda & Bilici, Saniye & Dazıroğlu, Merve & Erdoğan Gövez, Nazlıcan. (2022). Validity and Reliability of the Turkish Version of the Sustainable and Healthy Eating Behaviors Scale. British Journal of Nutrition. 1-20. https://doi.org/10.1017/S0007114522002525

Oral, Z. (2015). Practices for Food Waste and Prevention in the World and in Turkey, Ministry of Food, Agriculture and Livestock, General Directorate of European Union and Foreign Relations, EU Specialization Thesis, Ankara.

Ozen G.U. (2019). Evaluation of Knowledge and Attitudes of Dietitians and Dietetic Candidates on Sustainable Nutrition, Hacettepe University Institute of Health Sciences, Ankara.

Pelletier, J.E., Laska, M.N., Neumark-Sztainer, D., Story, M. (2013). Positive attitudes toward organic, local, and sustainable foods are associated with higher dietary quality among young adults. Journal of the Academy of Nutrition and Dietetics, 113(1):127-32. https://doi.org/10.1016/j.jand.2012.08.021

Pieniak, Z., Żakowska-Biemans, S., Kostyra, E., Raats, M. (2016). Sustainable healthy eating behaviour of young adults: towards a novel methodological approach. BMC Public Health, 16(1):577. https://doi.org/10.1186/s12889-016-3260-1

Rakıcıoğlu, N., Acar Tek, N., Ayaz, A., Pekcan, G. (2017) Food and Nutrition Photo Catalogue.Ankara.

Stok, F. M., Renner, B., Clarys, P., Lien, N., Lakerveld, J., & Deliens, T. (2018). Understanding eating behavior during the transition from adolescence to young adulthood: A literature review and perspective on future research directions. Nutrients, 10(6), 667. https://doi.org/10.3390/nu10060667

Tekiner, İ. H. , Mercan, N. N. , Kahraman, A. & Özel, M. (2021). An overview of food waste and loss in the world and in Turkey. Journal of Istanbul Sabahattin Zaim University Institute of Science and Technology, 3 (2), 123-128. https://doi.org/10.477769/izufbed.884219

TÜBER, (2015). Turkey-Specific Food and Nutrition Guide, Hacettepe University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Revised 1st Edition, Ankara.

UNEP, (2021). United Nations Environment Programme Food Waste Index Report 2021. Nairobi, Retrieved from https://www.unep.org/resources/report/unep-food-waste-index-report-2021. (Accessed 05.11.2022).

WHO, (2022). WHO STEPS Surveillance PART 3: Data Collection 3-5-1 Section 5: Collecting Step 2 data: Physical Measurements, Retrieved from https://cdn.who.int/media/docs/default-source/ncds/ncd-surveillance/steps/part3-section5.pdf?sfvrsn=a46653c7_2 (Accessed 05.11.2022).

Yolcuoğlu, İ.Z., Kızıltan, G. (2021). Effect of Nutrition Education on Diet Quality, Sustainable Nutrition and Eating Behaviors among University Students. J Am Coll Nutr. Jul 30: 1-7. https://doi.org/10.1080/07315724.2021.1955420

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 29-40
DOI: https://doi.org/10.31015/jaefs.2023.1.4

Socio-economics characteristics, income inequality, and poverty status of female headed cassava (*Manihot esculenta Crantz*) farming households in federal capital territory, Nigeria

Funso Omolayo Alabuja[1] • **Luka Anthony**[1] • **Elizabeth Samuel Ebukiba**[1]

[1] Department of Agricultural Economics, University of Abuja, PMB 117, Gwagwalada, Federal Capital Territory, Nigeria

Citation: Omolayo Alabuja, F., Anthony, L., Samuel Ebukiba, E. (2023). Socio-economics characteristics, income inequality, and poverty status of female headed cassava (*Manihot esculenta Crantz*) farming households in federal capital territory, Nigeria International Journal of Agriculture, Environment and Food Sciences, 7 (1), 29-40

Received: 16 July 2022
Revised: 25 October 2022
Accepted: 29 October 2022
Published Online: 30 January 2023

Correspondence: Luka Anthony
E-mail: gqluka11@gmail.com

Abstract

The study evaluated the socio-economic characteristics, income inequality and poverty status of female headed cassava farming households in Federal Capital Territory, Nigeria. Primary data were used for the study. A multi-stage sampling technique was used to select a total sample size of three hundred and three (303) households from the two area councils. The data were analyze using descriptive statistics, Foster-Greer-Thorbecke (FGT) poverty index, Gini coefficients, Probit model analysis, and principal components analysis (Factor Analysis). From the results about 59.73% of the female headed cassava farming households were less than 50 years old. 31.35% of the female headed cassava farming household were married. The mean household size was about12.00 persons. The mean annual income was 374, 868 Naira. About 56.77% of the female headed cassava farming household were poor given a poverty line ₦9, 009.37. In addition, 76% of female headed cassava farming households fell into annual income of below ₦500, 000 and they control 40% of the market share. The Gini coefficient was calculated to be 0.62. Maximum Likelihood Estimates (MLE) of the Probit Model shows that the coefficients of marital status ($P<0.01$), educational level ($P<0.05$), household size ($P<0.01$), income ($P<0.1$), and sources of livelihood ($P<0.1$) were the statistically and significant factors influencing poverty status among the female headed farming households in the study area. The results of the multinomial Logit model analysis show that the factors that statistically and significantly influencing the income inequality of female headed farming households in the study area, were coefficient of marital status ($P<0.05$), educational level ($P<0.10$), access to credit($P<0.05$), and sources of livelihood ($P<0.05$) for low income earners. Educational level ($P<0.01$), access to credit ($P<0.10$) and farm size ($P<0.01$), were statistically and significant factors influencing income inequality or income distribution among high income earners among female headed farming households. Trading enterprise, cassava flour/garri processing, and palm/ groundnut oil pressing were major coping strategies employed by the female headed households to against poverty and income inequality. Based on the findings it was concluded that there was high income gap or income inequality among female headed farming households and they were poor. It was recommended that policies that will help create more credit access/programs in terms of loan at low interest rates for women should be implemented at all tiers of government to help mitigate and reduce the poverty among female headed household.

Keywords: Poverty, Income, inequality, Cassava, female farming households, Nigeria

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is mostly considered and regarded as a 21st century staple crop for majority of the smallholder farmers across the globe especially for farmers in Africa. The cassava crop is also recognized as the root crop that is most widely cultivated and equally treated as food security crop that is consumed in tropical region (Otekunrin, *et al.*, 2022). Cassava root in Nigeria is considered as one of the top most important crop by production and regarded as second most important crop consumed as asserted by (Otekunrin & Sawicka, 2019; Sahel, 2016). The importance of cassava is on the increase on daily basis among the crops grown in Nigeria. It is not only connected to its increasing demand as food but also as food security and industrial purposes (FAO, 2018). An enhanced and improved cassava value chain expansion could lead to increase in the income of the female headed cassava farming household while reducing their poverty index and also generate a total export value of about 2.98 billion dollars to the Nigerian economy as a foreign exchange. This can be achieved by adding value to the commodity in order to produce derivatives and by-products such as sweeteners, ethanol, cassava starch etc., through local manufacturing and processing to meet local industries need and direct consumption is strategically important to the growth of the agriculture sector and the overall economy (PWC, 2020).

Poverty and inequality has remained for a long time a subject of major concern of many governmental and non-governmental organizations in both the developed and developing nations. For example, eradication of extreme poverty and hunger is the first goal of the United Nations Eight Millennium Development Goals (UN, 2000). According to World Bank (2011), "poverty is the economic condition in which people lack sufficient income to obtain certain minimal levels of health services, food, housing, clothing and education which are necessities for standard of living". Poverty is a situation when the resources of individuals or families are inadequate to provide a socially acceptable standard of living (Agwu & Kadiri, 2014). It is seeing as a state of involuntary deprivation, lack of capabilities to carry out certain activities and lack of adequate basic necessities of life (Odusola & Ogwumike, 2001). However, a person's perception of poverty is a function of his present experience, condition of his environment, the aim of such definition, his vocation and his definition of the good life (Fasoranti, 2010). Poverty is defined as a 'pronounced deprivation in well-being'. It can be defined narrowly or more broadly, depending on how well-being is understood (Haughton & Khandker, 2009). Poverty is a scourge that continues to have adverse effects and severe hardship on millions of people all over the world. More than three billion people equivalent to almost half of the world's population live on less than $2.50 a day and over 80 percent of the world's population live in countries where income gaps are increasing with the poorest 40% of the world's population accounting for 5% of global income, while the richest 20% accounts for three quarters of world income (Human Development Report, HDR 2007).The World-Bank report (2012) reveals that an estimated 1.29 billion people in 2008 lived below $1.25 a day in developing countries. Majority of those who live in extreme poverty reside in developing worlds of Africa, Asia, and Latin-America (Gbosi and Omoke, 2004). Nigeria is a country with the largest population on the African continent, of this magnitude, 49% are female, while outstanding 51% are male. It is among the thirty most unequal countries in the world with respect to income distribution, while the poorest half of the population holds only 10% of national income (British Council, 2012; Idowu *et al*., 2011). More disturbing is the fact that 54% of Nigerians still live in poverty and the proportion has doubled since 1980 (when about 28% were classified as poor). Human development indicators are also worse than those of comparable lower middle-income countries; 42% of Nigerian children are malnourished. The averages hide a context that is worse for women and girls. Nearly six million young women and men enter the labour market each year but only 10% are able to secure a job in the formal sector, and just one third of these are women (British Council, 2012). The average poverty incidence in Nigeria increased from 0.28 to 0.42 between 1980 and 1992 respectively and by 1996, the situation worsened to an average of 0.66. By implication, out of every 100 Nigerians, 66 were dwelling below the poverty line with great difficulties (NAPEP, 2006, Nwachukwu and Ezeh, 2007). Several socio-economic problems such hunger, infant immortality, sicknesses and disease outbreak continue to plague many in developing nations due to extreme level of poverty, sadly this deplorable situation is preventable if properly managed. Sub-Saharan Africa as a continent has a tragic record of highest incidence of poverty with about 47 percent of its population reported being poor (World Bank 2012).Many have attributed different reasons for the slow improvement in poverty alleviation in Africa, factors such as labour market shortages, macro-economic shocks and failures, poor governance, corruption, low economic growth, huge debt burden , environmental degradation, migration, unemployment and underdevelopment , crime and violence (Ajakaiye and Adeyeye, 2002) .

Inequality is a challenge to the eradication of extreme poverty and tends to reduce the pace and durability of growth (UNICEF et al., 2014; Ostry et al., 2014). Inequalities have also been found to hinder social cohesion and increase the risk of violent conflict (UNDP, 2013; Stewart, 2010). Inequality undermines social justice and human rights. Inequalities have resulted in the poorest people–including many women, young and older people, persons with disabilities, indigenous peoples, and rural populations–making less progress

towards development goals (Kabeer, 2010; World Bank, 2013). Economic, political, and social inequalities tend to reproduce themselves over time and across generations (World Bank, 2006). There is some overlap between those affected by poverty and those negatively affected by inequality, although it is important to note that certain groups and individuals are disproportionally affected. Deprivation or inequality in one dimension can influence other dimensions: for example, social inequality can lead to economic inequality (Sumner, 2013; Kabeer, 2010). Households refer to group of people who live together under a roof and accept the headship of a particular person. Due to modern living conditions whereby two or more families who are unrelated by blood or family ties reside under a roof (house). Beaman and Dillon (2009) defined households as groups of people living under the same dwelling place who eats meals together and acknowledge the authority of a man or woman who is the head of household. Household headship is usually attributed to an adult male (especially in rural settings) in the household who is most often than not, the husband (father). However, headship can be transferred due to death of previous household head, divorce, migration as well as serious illnesses. In any of these situations, headship is usually transferred to the oldest person who can either be a male or female. In cases of deaths of husbands whereby the children are still minors, headship is handed over to the wife (especially in a monogamous family). Irrespective of who takes up the headship of households, the situation of the households in all ramifications is most likely going to experience some changes. Although most poor women can also be found in households headed by a man, the poorest women are in female-headed households (UNFPA, 2002). For instance, it is of common knowledge that majority of women in the world especially in developing countries live in poverty. As reported by Quisumbing *et al*. (2001), 70% of the world's poverty stricken populations were women. The incidence of female headship of households is becoming increasingly popular in both developing and developed countries (Chant, 2007). Due to gender inequality in terms of access to productive resources, female-headed farming households have been found to be more vulnerable to poverty and its negative consequences such as food insecurity, malnutrition among children, drop-out of children from school, etc. than male-headed households. The issue of whether or not a female headed farming household is poor is widely recognized as an important, indicator of a female headed farming household's wellbeing. This is reflected in the central role the concept of poverty plays in analysis of social protection policy. In recent years, however, the term vulnerability has come to be widely used alongside poverty in discussions of poverty alleviation and social protection strategies (Oni and Yusuf, 2006). In Nigeria, the problem of poverty has, for a fairly long time, been a cause of concern to the government (Nwaobi, 2003). As a result, the government's efforts at combating the menace actually started immediately after the attainment of independence in 1960 (Ovwasa, 2000; Omotola, 2008). Nwaobi (2003) observed that the initial attention was focused on rural development and country planning as a practical means of dealing with the problem. He further noted that the failure to adequately implement these programs can be seen as the precursor to most of the present causes of poverty in Nigeria. However not every developing nations has witnessed steady reduction in poverty. Since 1980 the poverty incidence in Nigeria has been escalating (UNDP, 2005). Recent statistics from Nigeria are shocking and distressing. The National Bureau of Statistics (NBS) 2011 of the nation reported that in spite of the rapid economic growth of the Nigerian economy, 60.9% of Nigerians in 2010 were living in absolute poverty, as compared to 54.7% in 2004. This 60.9% absolute poverty shows that more than 100 million people out of the 204,381,889 million Nigerians (population estimate Feb, 2020, United Nations) were extremely poor. Previous study done by Federal Office of Statistics (FOS) (2000) indicated that poverty incidence increased from 26.1 to 46.3% between 1980 and 1985 and 42.7 to 65.6% between 1992 and 1996, respectively. The report also revealed that poverty incidence is highest in the rural communities and women are the most affected. This is not surprising as many rural people lack capabilities in terms of employment opportunities as they mostly rely on subsistence agriculture as primary means of livelihood and also lack access to infrastructural development that can improve their wellbeing. Moreover, the Human Development Index (HDI) report (2011) of the United Nations Development Programme UNDP ranked Nigeria 156 out of 186 countries with the HDI 0.453 which is below Sub-Saharan Africa's average of 0.463, clearly suggesting that Nigeria is still one of the 40 poorest nations in the world. Several factors were attributed to the worsening case of poverty in Nigeria; changing socioeconomic, political, environmental conditions as rural inhabitants (Olutayo, 2009). Well as unstable and decreasing income, low rate of capital accumulation and declining agricultural output due to the rapidly changing climatic conditions in Nigeria have continued to exacerbate the living conditions of several households especially those of rural inhabitants (Olutayo, 2009). Besides, huge income inequality between the poor and the rich, bad governance, corruption, high unemployment rate, rapidly growing population and poor infrastructural developments also contribute to the escalation of poverty. Despite the fact that the past and present Nigerian governments have initiated and implemented numerous policies and poverty alleviation programmes to tackle the scourge, their efforts have yielded little or no result as the situation of the poor continue to worsen day by day. Extremely high level of poverty can have grave consequences on individuals and the nation at large, it is dehumanizing and detrimental

to economic growth, it can ignite and incite the impoverished population to various forms of social vices, crisis and crimes as means of survival. In view of above to achieve this goal, a comprehensive knowledge of the poverty profile and its determinants both at household and regional level are imperative because characteristics such as age and gender of households, educational status of household head and other socio economic factors are fundamental factors that could have an impact of poverty status of Female headed farming households in Nigeria (Osinubi, 2003). This is the central focus of this research work, the result will help in making an informed decision by policy makers on specific factors and regions to focus on in order to achieve rapid improvement in reduction of extreme poverty.

Research Questions

This study intends to provide answers to the following research questions:

- What is the poverty status among female headed cassava farming households in Federal Capital Territory, Nigeria?
- What are the income distributions and inequalities among female headed cassava farming households in the study area?
- What are factors influencing poverty status of female headed cassava farming households in the study area
- What are the coping strategies against poverty and income inequality of female headed cassava farming households in the study area?

Objectives of the Study

The broad objective is to evaluate poverty status, income inequality and socio-economic characteristic of female headed farming households in Federal Capital Territory, Nigeria. The specific objectives were to:

- determine the poverty status among female headed cassava farming households,
- determine the income distributions and inequalities among female headed cassava farming households,
- evaluate factors influencing poverty status of female headed cassava farming households,
- identify the coping strategies against poverty and income inequality of female headed cassava farming households in the study area.

MATERIALS AND METHODS

The Study Area

The study was carried out in Federal Capital Territory, Nigeria. It was carved out in 1976 from parts of Nasarawa, Niger, and Kogi States in the central parts of Nigeria. The territory is located just off the confluence of the River Niger and Benue River. It is bounded by the state of Niger to the west and north, Kaduna to the northeast, Nasarawa to the east and south, and Kogi to the southwest (Dawan, 2000). There are six Area Councils in Abuja namely: Abaji, Bwari, Gwagwalada, Kuje, Kwali and Abuja Municipal Area Councils. Federal Capital Territory has total land area of about 8,000 Sq. Kilometers with a total population of 776,298 people at the 2006 census (NPC, 2006). It is located at the extreme South west near the flood plain of River Gurara which transverses the territory from North to South at an elevation of 70m above sea level. The area lies between Latitudes $07^0.57^{'}$N and Longitudes $07^0.7^{'}$E. The vegetation combines the best features of the southern tropical rain forest and guinea savanna of the North. This reflects the full transitional nature of the area as between the Southern forest and Northern grassland which have the woods and shrubs respectively. The soil is reddish with isolated hills filled by plains and well drained sandy clay loams which supports farming of the major crops such as sorghum, millet, melon, yam, soybean, benniseed, cassava and rice cultivation (Abuja ADP, 2004). The duration of sunshine ranges from 8 to 10 hours per day. The average rainfall per annum is 163.2mm. The original settlers are Gwari, Koro, Bassa, Gade and the Hausa Fulani as well as immigrants' population of other Nigerians and expatriates. It is the industrial zone of the Federal Capital Territory and over 26 headquarters of Federal agencies are situated in the Area Council including the University of Abuja and a Specialist hospital.

Sampling Technique and Sampling Size

This study employed purposive sampling method to select Federal Capital Territory, Nigeria. First, because of the proximity of the area to the base of the researcher. Secondly, the female headed farming households are many in the area. This followed a preliminary survey carried out in the area. Multi-stage sampling method was used to select the target respondents (household-head). In the first stage, two (2) area Councils were randomly selected using ballot –box method, they were Kwali and Gwagwalada. In the second stage, six (6) wards were randomly selected using ballot-box, they were: Tunga-maje, Kutunku, Gwako, Ashara, Kilankwa, and Kwali. In the third stage, twenty-one (21) villages were randomly selected using ballot-box method. From equation (3.1) a proportionate-random sampling method was used to select a sample size of three hundred and three (303) households from the sample frame of one thousand, two hundred and forty-three (1243) household heads in the two area councils.

Yamane (1967) will be used to select the sample size:

$$n = \frac{N}{1 + N(e^2)} \ldots\ldots\ldots\ldots (1)$$

Where,

n = Sample Size (Units)

N= Sample Frame (Units)

e=Level of Precision (5%)

Method of Data Collection

Primary data were used. Data were collected with the use of questionnaire, interview schedule, and Focus Group Discussion (FGD). Enumerators were recruited and trained on the contents of the questionnaire and interviewing process. Thereafter, primary data were collected through the administration of well-structured questionnaire by the team of trained enumerators. Information collected include; age, sex, marital status, household head, income, household size sources of livelihood etc.

Method of Data Analysis

The following analytical tools will be used to achieve stated objectives

- Descriptive Statistics,
- Foster-Greer-Thorbecke (FGT) Poverty index,
- Gini Coefficients,
- Probit Model Analysis
- Principal Components Analysis (Factor Analysis)

Descriptive Statistics

Descriptive statistics such as frequency distribution, mean, standard deviation, percentages, graphs and tables was used to describe the variables and socioeconomic characteristics of the respondents. It was used to achieve part of specific objectives (i) & (v).

Foster-Greer-Thorbeck (FGT) Poverty Index

The most widely used poverty indices are measures proposed by Foster, Greer-Thorbecke (1984) as used by (Duniya and Sanni, 2015). These three poverty indices measures are: the poverty headcount ratio, the poverty gap and squared poverty gap. These poverty indices measure the basic desirable property of poverty. The FGT model is given as;

$$P_{\alpha i} = \frac{1}{n}\sum_{i-1}^{q}\left(\frac{z-y_i}{z}\right)^{\alpha} \quad \text{.....(2)}$$

Where:

Pα = is the FGT poverty index for the i^{th} sub-groups,

N= The total number of female headed households in the population,

Yi= The per capital expenditure of i^{th} households,

Z = The poverty line,

q = The number of the sampled household population below the poverty line and,

α = The degree of aversion and take on the value of 0,1,2

Poverty Head Count Ratio

The headcount ratio measures the incidence of poverty and it is obtained as:

$$p_0 = \frac{1}{n}\sum_{i-1}^{q}\left(\frac{z-y_i}{z}\right)^0 = \frac{q}{n} \quad \text{.....(3)}$$

when (α = 0)

Pα= p_0 n= poverty incidence or head count ratio

Where

q = The number of individuals below poverty line

n = The number of individuals in reference population.

Poverty Gap

When α is equal to 1, it shows uniform concern and equation becomes

$$p_1 = \frac{1}{n}\sum_{i-1}^{q}\left(\frac{z-y_i}{z}\right)^1 \quad \text{.....(4)}$$

This measure the depth of poverty (the proportion of expenditure shortfall from the poverty line) according to Hall and Patrinos (2005), it is otherwise called the poverty gap or expenditure gap- the average difference between the income and the poverty line. The poverty gap index p_1 was used to measure the depth of poverty of the female headed cassava farming households in the study area.

Square Poverty Gap

When α is equal to 2 distinctions is made between the poor and the poorest, that is, the severity of poverty (Foster, Greer and Thorbecke, 1984) and (Assadzadeh and Paul, 2003). The equation becomes.

$$p_2 = \frac{1}{n}\sum_{i-1}^{q}\left(\frac{z-y_i}{z}\right)^2 \quad \text{..... (5)}$$

The equation gives a distribution sensitive FGT index of the distribution of expenditure among the poor. This measure takes account of the incidence of poverty, depth of poverty and the inequality amongst the poor. 2/3 of mean per capital household expenditure MPCHE was used as the poverty line, the extreme poor (those spending <1/3 of MPCHE), moderately poor (those spending <2/3 of MPCHE and the non-poor (those spending >2/3 of MPCHE).

This was used to achieve part of specific objective (i)

Gini Coefficient

To determine the income distribution and income inequalities among cassava farming female headed households, the Gini coefficient was applied. The Gini Coefficient formula is in line with Wilson *et al* (2010);

Madu (2006); Damgaard and Weiner (2000). Income inequality will be measured using the Gini-coefficient. Following Morduch and Sicular (2000).

The Gini- Coefficient is stated thus:

$$G.C = 1 - \sum_{i=1}^{k} X_i Y_i \dots\dots\dots\dots (6)$$

Where,

G.C = Gini-Coefficient (Units)

X_i = Proportions of Non-Poor Female Headed *i*th Class of Cassava Farming Households, (Units)

Y_i = Cumulative Proportion of the Income of Non-Poor Female headed in the *i*th Class of Cassava Farming Households (Naira)

$\sum$= Summation Sign

k = Observed values

Gini Coefficient (GC) Varies from Zero (0) to 1.

$$0 \leq GC \leq 1$$

Where,

0 = Implies Perfect Equality in the Distribution of Income

1 = Represent Perfect Inequality in the Distribution of Income

This will be used to achieve specific objective (ii)

Probit Model Analysis

The Probit Model is stated thus:

$Y = f(X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, U_i)$ ..... (3.6)

$$Y_i = b_0 + \sum_{i=1}^{10} b_i X_i + e_i \dots\dots\dots\dots (7)$$

The explicit function is stated thus:

$Y_{ij} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + X_6 + b_7X_7 + b_8X_8 + b_9X_9 + b_{10}X_{10} + e_i$ ..... (9)

Where,

Y_{ij} =Poverty Status (1, Poor; 0, Otherwise)

X_1 = Age of Household Head (Years)

X_2 = Gender Dummy (1, Female; 0, Otherwise)

X_3 = Educational Level (0, Non-Formal; 1, Primary; 2, Secondary; 3, Tertiary)

X_4 = Household Size (Number of Persons)

X_5= Household Income (Naira)

X_6 = Marital Status (1, Married; 0, Otherwise)

X_7 = Farm Size (Hectares)

X_8 =Access Credit Dummy (1, Yes; 0, Otherwise)

X_9 = Extension Services Dummy (Number of Extension Contact in a Month)

X_{10} = Source of Livelihood (1, Farming;2, Business; 3, Employed)

b_0 = Constant Term

b_1- b_{10} = Regression Coefficients

e_i = Error Term

This was used to achieve specific objective (iii)

Principal Component Analysis

Constraints faced by female headed Cassava farming households was subjected to Principal Component Analysis or Factor Analysis. The principal Component Analysis is stated thus:

$$x = (x_1, x_2, x_3, \dots, x_p \dots\dots\dots\dots\dots\dots (10)$$
$$\alpha_K = (\alpha_{1k}, \alpha_{2k}, \alpha_{3k}, \dots \alpha_{pk}) \dots\dots\dots\dots (11)$$
$$\alpha_k^T X = \sum_{j=1}^{p} \alpha_{kj} X_j \dots\dots\dots\dots\dots\dots\dots (12)$$
$$Var = [\alpha_k^T X] \; is \; Maximum \dots\dots\dots (13)$$

Subject to:

$$\alpha_k \alpha_K = 1 \dots\dots\dots\dots\dots\dots (14)$$

and

$$cov\,[\alpha_1^T X - \alpha_2^T X] = 0 \dots\dots\dots\dots (15)$$

The variance of each of the principal components are:

$$Var[\alpha_k^T X] = \lambda_k \dots\dots\dots\dots (16)$$
$$S = \frac{1}{n-1}(X - X)(X_i - \overline{X}_i)^{\intercal} \text{.....(17)}$$
$$S = \frac{1}{n-1}\sum_{i=1}^{n}(X_i - \overline{X_i}\,(X_i - \overline{X}_i)^{\intercal} \text{.....(18)}$$

Where,

X= Vector of p Random Variables

α_k = Vector p Components

λ_κ = Eigen Value

T = Transpose

S = Covariance Matrix

This was used to achieve specific objective (iv)

RESULTS AND DISCUSSION

Table1 shows the result of the socio-economic characteristics of the female headed cassava farming households in the study area. From the result, about 59.73% of the female headed cassava farming households

were between the ages of 31 and 50 years. The mean value was 49 years. This implies that most of the female headed cassava farming households were energetic, resourceful, in their youthful age. This implies that the household head may be more industrious and capable of undertaking several livelihood strategies. This result is in line with the findings of Odusina (2014). Also, 31.35% of the female headed cassava farming households were married. About 13.20% of them were single. The findings with Igbalajobi (2013). About 9.90% of the female headed cassava farming households in the study area had tertiary education, 13.20% had secondary education, and 86.8% had primary education. This implies that majority of the female headed cassava farming households in the study area had completed the minimum 6 years of schooling. Hence had formal education and may be able to read and write in English language fluently. Education is good for adoption of innovations (improved technologies and research findings) by the female headed cassava farming household because it may be relatively easier to introduce a new technology to the female headed cassava farming households in the study area. The findings agree with the findings of Sallawu *et al* (2016). The mean household size was about 11.61 persons. This depict that on average the household sizes of female headed farming households in the study area was about 12 persons. The size of the household might be because of the labour needed for cultural practices and livelihood activities in the study area. Furthermore, larger household sizes can serve as source of family and increase food insecurity. The results agree Adebayo (2012) who reported that the larger the family size the lesser the food availability to each person within the household. The average farm size was 2.42 hectares. This shows that the farming households are subsistence in nature. Farm size is a reflection of own-food production ability and source of incomes for the family. It is believed that increase in farm size will result in increased food production which ultimately, increased likelihood of household food security. About 71.29% of the sampled female headed farming households had access to credit in the study area. This result indicates that agricultural loans were relatively accessible to households in the study area. The study reveals it a ratio 3:1 in terms of access to credit. This agrees with the findings of Akpan *et. al*., (2013). Furthermore, majority (79.54%) of the female headed farming households in the study area had contact with an extension agent. This shows that the female headed farming households had information on improved technologies, innovation, and research findings. According to Adeniyi *et al* (2015), extension services are very essential to the improvement of farm productivity and efficiency among household. Also, 66.01% of the female headed farming households were members of cooperative society, while 39.99% of the female headed farming households did not belong to any cooperative society. This may greatly aid their ability to pull their resources together for agricultural production. Membership of clubs, association or cooperative societies help household with opportunity to obtain bulk purchases credit, receive inputs at subsidized or at cheaper rates; and to obtain important and recent information concerning their farming activities. The mean years of female headed farming households experience was about 19.71 years, that is, on the average a female headed cassava farming household' in the study has about 20 years of experience in farming. This means that the female headed cassava farming household in the study area may be able to make sound decisions as regards resource allocations and management of their farms. This result is in line with the report of Sallawu *et al* (2016) that classified households with 14 years of farming experience are regarded as "experienced household". The mean annual income was ₦374, 868. This implies that which means on average the female headed farming households earns on average ₦374, 868 from female headed farming activity per annum.

Poverty Status of Female Headed Cassava Farming Households in the Study Area

The poverty line used for this study was collected from monthly maximum and minimum per capital expenditure (MPCE) of the sampled female headed cassava farming households as shown table 2. Two third (₦9, 009.37) of the monthly per capita expenditure of the sampled female headed farming households was used as poverty line in the same method as the index developed by Foster *et al* (1984) and used by Omonona *et al* (2007). This is the minimum cost of eliminating poverty (poverty line), this shows the amount that could be transferred to the poor to bring their expenditure up to the poverty line. The poverty of the female headed farming households which included poverty head count or incidence ($_0$), poverty gap or depth ($_1$), and squared poverty severity ($_2$) were analyzed using Foster, Greer and Thorbecke (FGT) index ($0 \leq P \leq 1$). The ($_0$) for the entire households was 0.666. This means that 66.6% of the female headed cassava farming households in the study area were poor. Which means that's about 172 households were poor and 131% households were non-poor. The poverty gap index (P_1) usually referred to as the depth of an average poor person from the poverty line was 0.366 which means 36.6% of the female headed farming households in the study area were poor, the poverty severity (P_2) which measures the distance of each poor person to one another was found to be 0.247. This means that among the poor households, 24.7% were severely poor. The average per capital expenditure (PCE) was (₦13, 514.06) for the sampled female headed farming households. The poverty line obtained is above ₦7, 599.26 as reported by Folorunso (2018) in Plateau State Nigeria, and also above (389.2 Ethiopian Birr) (₦ 4,737.23) Afar Regional State, Ethiopia as reported by Araya and Gabriel (2014), and above ₦6,224.96 reported by Adekoya (2014) in Ogun State. Furthermore, 56.77%

Table 1. Socio-Economic Profiles or Characteristics of the Female Headed Cassava Farming Households in the Study Area

Variables	Frequency	Percentage	Mean (Std. Dev)
Age (Years)			
< 30	13	4.29	
31–40	48	15.84	**49.06 (10.79)**
41–50	133	43.89	
> 50	109	35.97	
Marital Status			
Single	40	13.20	
Married	95	31.35	
Widow	112	36.96	
Divorced	56	18.48	
Educational Status			
Primary	147	86.80	
Secondary	40	13.20	
Tertiary	30	9.90	
No Formal Education	86	28.38	
Household Size (Units)			
≤ 5	27	8.91	
6–10	105	34.65	
11–15	110	36.30	
≥ 16	61	20.13	**11.61 (5.23)**
Farm Size (Hectares)			
≤ 1.9	41	13.53	
2.00 – 2.90	144	47.52	**2.42 (0.84)**
3.0 – 5.00	118	38.94	
Access to Credit			
Yes	216	71.29	
No	87	28.71	
Extension Contact			
Yes	241	79.54	
No	62	20.46	
Cooperative Membership			
No	103	39.99	
Yes	200	66.01	
Years of Experience			
< 10	37	12.21	**19.71 (9.64)**
11≤20	120	39.60	
21–30	106	34.98	
≥ 31	40	13.20	
Total Annual Income (N)			
≤ 500, 000	231	76.00	
500, 001 ≤ 1, 000, 000	54	18.00	**374, 868**
1, 000, 001 ≤ 1, 500, 000	11	4.00	
1, 500, 001 ≤ 2, 000, 000	7	2.00	
Total	**303**	**100.0**	

Source: Field Survey (2022) Computed Using STATA 14

of the female headed farming household were poor this means that the incidence of poverty was more among the female headed farming household in the study area.

Income Inequality/Income Distributions Among Female Headed Cassava Farming Households

Table 3 summarized the total annual sales made by female headed cassava farming households and was categorized with an interval of ₦500, 000. The results show that 76% of female headed farming households who fell into annual income below 500, 000 Naira actually control 40% of the market share. 18% of female headed farming households who are in the category of 500, 001 – 1, 000, 000 Naira annual incomes control 36% of the

market share. Furthermore, 4% of the female headed farming households who are in the category of 1, 000, 001– 1, 500, 000 Naira annual income controls 2% of the market share. The Gini coefficient was 0.62 and shows that inequality and distribution of income among female headed cassava farming households. Gini coefficient value of 0.62 which is closer to 1 shows income gap and unequal income distributions among the female headed cassava farming households in the study area. This result is line with Anthony et al, (2021) who reported that the value of G.C greater than 0.35 is high signifying that there is inequality in the distribution of income.

Table 2. Poverty Status of Female Headed Farming Households in the Study Area

Indicators	FGT
Poverty Incidence (P_0)	0.666
Poverty Depth (P_1)	0.366
Poverty Severity (P_2)	0.247
Mean Expenditure (Naira)	13, 514.06
Minimum Expenditure (Naira)	555.556
Maximum Expenditure (Naira)	250, 000.00

Source: Field Survey (2022) Computed Using STATA 14

Maximum Likelihood Estimate shows that the Log Likelihood was -142.95, while Chi-Square value was 128.56 and were significant at 1% levels of probability. This implies that the overall effect of the explanatory variables included in the model were statistically significant and responsible for the variation in the poverty status among the female headed farming households. The coefficient of determinations (Pseudo R Square) was 0.3102 (31.02%). This indicates that 31.02% of the variations in the poverty status (i.e. dependent variable) was explained by the explanatory variables included in the regression model. However, as noted in Gujarati and Porter (2009), in models with binary dependent variables, goodness of fit is of secondary importance. What matters are the signs of the regression coefficients of the explanatory variables and their statistical and/ or practical significance? Marital status had negative coefficient and was significant at 1% probability level. This implies that a unit change in marital status will result to about 0.001% marginal decrease in the poverty status among the female headed farming households. This may be so because if the female headed farming were married it is expected that they were better informed about poverty coping strategies and also the presence

Table 3. Income Inequality/Income Distributions Among Female Headed Cassava Farming Households

Income (Naira)	Frequency	Proportion (Xi)	Relative Income	Proportion (Yi)	XiYi
Below 500, 000	231.00	0.76	538, 500.00	0.40	0.30
501, 000 – 1, 000, 000	54.00	0.18	4, 100, 000.00	0.36	0.06
1, 000, 001 – 1, 500, 000	11.00	0.04	1, 470, 000.00	0.13	0.00
1, 500, 001 – 2, 000, 000	7.00	0.02	1, 250, 000.00	0.11	0.00
Sum	**303.00**	**1.00**	**11, 358, 500.00**	**1.00**	**0.38**
Gini Coefficient = 0.62					

Source: Field Survey (2022) Computed Using STATA 14

Factors Influencing Poverty Status Among Female Headed Cassava Farming Household in the Study Area

Maximum Likelihood Estimates (MLE) of the Probit Model presented in Table 4 shows that out of the nine (9) explanatory variables included in the Probit model, the coefficients of marital status (P<0.01), educational level (P<0.05), household size (P<0.01), income (P<0.10), and source of livelihood (P<0.10) were statistically and significant factors determining poverty status among the female headed farming households in the study area. Positive sign on a parameter indicates their direct relationships, hence higher values of the variable tends to increase the likelihood of poverty status. Similarly, a negative sign of coefficient implies an inverse relationship, hence higher or additional value of the variable tend to decrease the likelihood or probability of poverty status among female headed farming households.

male counterpart will have a great contribution to the household's income. The coefficient of educational level of the female headed farming households was negative and statistically significant at 5% level of probability. The result means that educational level was a significant factor in determining the poverty status of the female headed farming households in the study area. The marginal effect was 0.145 which is about 14.5%. This implies as 1% increase in access to education or acquire more educational qualifications would lead to a 14.5% decrease in poverty among female headed farming tends to acquire more educational qualification. The coefficient of household sizes of the female headed farming households measured was positive and statistically significant at 1% level of probability. The result implies that household sizes was a significant factor in determining the poverty status of the female headed farming households in the study area. Household size increases the probability of being

Table 4. Results of the Maximum Likelihood Estimate (MLE) of the Probit Model

Variables	Coefficient	Standard Error	t-Score	Marginal Effect
Marital Status ($_1$)	-0.546	0.110	-4.990***	-0.001
Age ($_2$)	0.004	0.013	0.330	-0.073
Farm Size ($_3$)	-0.020	0.125	-0.160	0.062
Access to Credit ($_4$)	0.070	0.218	0.320	0.000
Education Level ($_5$)	-0.276	0.117	-2.370**	-0.145
Household Size ($_6$)	0.232	0.033	6.920***	0.005
Income ($_7$)	0.000	0.000	-1.830*	-0.019
Extension Visit ($_8$)	-0.375	0.261	-1.440	-0.100
Source of Livelihood ($_9$)	0.403	0.236	1.700*	0.107
Constant	-0.240	0.584	-0.410	

Significant at P≤0.05, *** - Significant at P≤0.01; * - Significant at P≤0.10; Log-Likelihood = -142.95***; Pseudo=0.3102, Chi-Square = 128.56

Source: Field Survey (2022) Computed Using STATA 14

poor and this could be because an increase in household size directly or indirectly reduces income per-head (per-capita income) as well as reduce the standard of living of the households. The marginal effect was 0.005 which depict a 0.5% increase in poverty status as household size increases by 1%. The coefficient of income was negative and was statistically significant at 10% level of probability in determining poverty status of female headed farming household in the study area. The marginal effect was 0.019. Sources of livelihood had positive coefficient and was significant at 10% level of probability in determining poverty status of female headed farming households in the study area. The marginal effect was 0.107.

data set for factor analysis. This result is in line with Noor *et al.* (2015). Table 5 further revealed the results of the perceived coping strategies employed by female headed households in mitigating the effects poverty in the study area. The coping strategies were ranked in the order of magnitude according to the eigen-value. The study shows that of all the strategies employed trading enterprise was ranked 1st as the coping strategy with the highest eigen-value; cassava flour/garri processing was ranked 2nd, and palm/ groundnut oil pressing was ranked 3rd respectively. These findings were in consonance with empirical studies carried out by Seinfeld and Polsky (2006).

Table 5. Results of Principal Component Analysis of Poverty and Income Inequality Coping Strategies Used by Female Headed Cassava Farming Households in the Study Area

Component Mean (Std Dev)	Eigen-Value	Difference	Proportion	Cumulative
Trading Enterprise	5.69778	3.53333	0.3799	0.3799
Cassava Flour/Garri Processing	2.16446	0.938924	0.1443	0.5241
Palm/ Groundnut Oil Pressing	1.22553	0.231055	0.0817	0.6059
Bartlett Test of Sphericity				
Chi-Square = 1854.745***				
Rho = 1.0000				
KMO = 0.839				

Source: Field Survey (2022) Computed Using STATA 14

Coping Strategies Against Poverty and Income Inequality of Female Headed Cassava Farming Households in the Study Area

From the result presented on Table 5 the number of principal components retained using the Kaiser criterion, is three (3) that is where the Eigen Value is 1 and above. At this component 60.59% of the variations has been explained by the components captured in the model. The Kaiser-Meyer-Olkin measures of sampling adequacy (KMO) of 0.839 and Bartlett test of sphericity of 1854.745 was statistically significant at 1% level of probability and demonstrated the feasibility of employing the

CONCLUSION

Majority of the female headed cassava farming households were poor with a given poverty line 9, 009.37 Naira. About 56.77% of the female headed farming households were poor. The Gini coefficient was calculated to be 0.62. There are high income gap or income inequality among female headed cassava farming households. Marital status, educational level, household size, income, and sources of livelihood were the significant factors influencing poverty status among the female headed farming households in the study area. Trading enterprises, cassava flour/garri processing, and

palm/ groundnut oil pressing were the major coping strategies employed by the female headed households against poverty and income inequality.

Recommendations

The following policy recommendations were made from this study:

(i) Policies that will help create more credit access/ Programs in terms of loan at low interest rates for women should be implemented at all tiers of government to help mitigate and reduce the poverty among female headed Cassava farming household. Female farmers that are heading families should be given priority being that the household depends on their succecces.

(ii) Programs should be put in place to help counsel women generally on the benefits of family planning. This will help reduce the large household sizes common among female headed farming households and rural farmers in general.

(iii) Access to educational for women, adult-education, and girl-child education should be encouraged and implemented.

(iv) Women should also be encouraged to diversify their sources of livelihood this will help them to have a relative equality or balance in their income levels all year round.

(v) Trading enterprise, cassava flour/garri processing, and palm/ groundnut oil pressing sub-

sectors should be encouraged by governments.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Adebayo, P.F. (2012). Food Security in Nigeria: An Overview. European Journal of Sustainable Development, 1, 199-222.

Adekoya, O. A. (2014). The Patterns and Determinants Of Agricultural Credit Use Among Farm Households In Oyo State, Nigeria. Asian Economic and Financial Review, 2014, 4(10): 1290-1297

Adeniyi, R. T. and Yekinni, O. T. (2015). Arable crop farmers' characteristics affecting the utilization of information and communication technology for agricultural marketing information in Oyo State, Nigerian. Journal of Rural Sociology, 15(2), 23-29.

Agwu, M. E. & Kadiri, K. I. (2014). Analysis of critical strategic factors for successful implementation of poverty alleviation programmes in Nigeria. International Journal of Computational Engineering & Management, 17(1), 1-9.

Ajakaiye, D. O. and Adeyeye, V. A. (2002). Concepts, measurement and causes of poverty. CBN Economic & Financial Review, Vol. 39 N0. 4

Akpan, B. Udoh, E. J. Patrick, I. V. (2013). Sustaining Small Scale Farming: Evidence of Poverty and income Disparity among Rural Farming Households in South-South Region of Nigeria Sunday 2, № 9(14)

Anthony, L., Alabi, O.O., Ebukiba E.S. , Gamba. V. (2021). Factors influencing output of rice produced and choice of marketing outlets among smallholder farming households, Abuja, Nigeria. Sarhad Journal of Agriculture, 37(1): 262-277.

Beaman, L. and Dillo, A. (2009). Do household definitions matter in survey design? Results from a randomized survey experiment in Mali.

British Council (2012). Gender in Nigeria Report: Improving the Lives of Girls and Women in Nigeria, 2nd Edition, British Council, Nigeria. Retrieved from http://www.britishcouncil.org.ng/gender2012

Damgaard. C., And Weiner, J. (2000). Describing Inequality in Plant Size or Fecundity. Ecology, 81(4), 2000, pp. 1139–1142.

Duniya, K. P., and Rekwot, G. Z. (2015). Determinants of Poverty among Groundnut Farming Households in Jigawa State, Nigeria. Asian Journal of Agricultural Extension, Economics & Sociology, 4(3): 224-230

FAO, (2018). Food Outlook-Biannual Report on Global Food Markets – November 2018. Rome, p.104.License:CCBY-NC-SA3.0IGO. Retrieved from http://www.fao.org/3/ca2320en/CA2 320EN.pdf

Fasoranti, M. M. (2010). The Influence of Micro-credit on Poverty Aleviation Among Rural Dwellers: A Case Study of Akoko North West Local Government Area of Ondo State. *African Journal of Business Management*, 4(8): 1438-1446

Gbosi, A. and Omoke, P. C. (2004): The Nigerian Economy and Current Problems. Pack Publishers, Abakaliki, Ebonyi state.

Hall, G. and Patrinos, H. (2005) Indigenous peoples, poverty and human development in Latin America: 1994-2004. Retrieved from http://go.worldbank.org/TXWP6Y78N0

Haughton, J., & Khandker, S.R. (2009). Handbook on Poverty and Inequality. Washington, DC: World Bank. Retrieved from http://go.worldbank.org/4WJH9JQ350

Human Development Index Report (2011): Nigeria Country Profile: Human Indicators. Available at http://hdr.undp.org, Accessed on 05/06/2012

Human Development Report (HDR) (2007). United Nations Development Program, November 27, 2007, p.25.

Idowu, A.O., T.T. Awoyemi, B.T. Omonona & A.O. Falusi (2011). Non-farm Income Diversification and Poverty Among Rural Farm Households in Southwest Nigeria, *European Journal of Social Sciences*, 21(1): 163 – 176.

Igbalajobi, O., Fatuase, A.I. and Ajibefun, I. (2013) Determinants of Poverty Incidence among Rural Farmers in Ondo State,

Nigeria. American Journal of Rural Development, 1, 131-137

Ignatius, A. M., (2006). The Underlying Factors of Rural Development Patterns in the Nsukka Region of Southeastern Nigeria. Journal of Rural and Community Development www.jrcd.ca Case Study 2: 110-122.

Morduch, J. and Sicular, T. (2000). Rethinking Inequality Decomposition, With Evidence from Rural China the Economic Journal, 112 (January), 93-106

James Foster, Joel Greer and Erik Thorbecke Source: Econometrica, Vol. 52, No. 3 (May, 1984), pp. 761-766

Kabeer, N. (2010). Can the MDGs Provide a Pathway to Social Justice? The Challenge of Intersecting Inequalities. Brighton: Institute of Development Studies. Retrieved from https://www.ids.ac.uk/download.php?file=files/dmfile/MDGreportwebsiteu2WC.pdf

Kerrie, A., Wilson, Budiharta, S., Elizabeth A., Law, A., Poh, T.M., Ancrenaz, M., Struebig, M.J., Meijaar, E. (2019). Does oil palm agriculture help alleviate poverty? A multidimensional counterfactual assessment of oil palm development in Indonesia World Development 120 103-117

National Bureau of Statistics (NBS), (2011). Nigeria Poverty Profile.

National Poverty Eradication Programme (NAPEP) (2006). Retrieved from https://www.napep.org/ Assessed 20/1/06.

Nwachukwu, I.N. and Eze, C.I. (2007). Impact of Selected Rural Development Programmes on Alleviation I Ikwuano LGA, Abia State, Nigeria vol 7 No 5 1-18

Nwaobi, G.C. (2003). "Solving the Poverty Crisis in Nigeria: An Applied General Equilibrium Approach". December 17, 2012. Retrieved from http://ideas.repec.org/p/wpa/wuwpco/0312003.pdf

Odusina Olaniyi, A. (2014). Assessment of Households' Food Access and Food Insecurity in Urban Nigeria: A Case Study of Lagos Metropolis. Global Journal of Human-Social Science: Economics Volume 14 Issue 1 1-30

Omonona, B. T. and Agoi, G.A. (2007). An Analysis of Food Security Situation Among Nigerian Urban Households: Evidence from Lagos State, Nigeria. Journal of Central European Agriculture Volume 8 No. 3 (397-406)

Ostry, J. D., Berg, A., & Tsangarides, C. G. (2014). Redistribution, Inequality, and Growth. Washington, DC: IMF.

Otekunrin, O. A., Ayinde, I. A., Sanusi, R. A., & Otekunrin, O. A. (2022). Assessing the determinants of agricultural commercialization and challenges confronting cassava farmers in Oyo State, Nigeria. Journal of Socioeconomics and Development, 5(1), 76-87.

Otekunrin, O. A., & Sawicka, B. (2019). Cassava, a 21st century crop: How can nigeria harness its enormous trade potential? Acta Scientific Agriculture 3(8), 194-202.

Quisumbing, A. Lawrence, H., and Christine P. (2001). "Are Women Overrepresented Among the Poor? An Analysis of Poverty in Ten Developing Countries." *Journal of Development Economics*, 66(1): 225-269.

SAHEL, (2016). Cassava: A staple Crop in Nigeria. SAHEL Newsletter, Volume 13(Issue 5), 1–5.

Sallawu H., Tanko L., Nmadu J.N., Ndanitsa A.M. (2016). Determinants of Income Diversification Among Farm Households In Niger State, Nigeria Russian Journal of Agriculture and Social Sciences, 2(50), 55-65

Stewart, F. (2010). Horizontal Inequalities as a Result of Conflict: A Review of CRISE Findings. Oxford: Centre for Research on Inequality, Human Security and Ethnicity.

Stewart, M. J., Makwarimba, E., Reutter, L. I., Veenstra, G., Raphael, D., & Love, R. (2009). Poverty, Sense of Belonging and Experiences of Social Isolation. *Journal of Poverty,* 13(2), 173-195. https://doi.org/10.1080/10875540902841762

Sumner, A. (2013). Who are the poor? New Regional Estimates of the Composition of Education and Health 'Poverty' by Spatial and Social Inequalities (Working Paper 378). London: ODI.

UNDP Human Development Report, (2005). International cooperation at Crossraods: Aid, Trade and Security in an Unequal World: United Nations Development Programme.

UNICEF, UN Women, UNDP, & OHCHR. (2014). TST Issues Brief: Promoting Equality, Including Social Equity Sustainable Development.

United Nation Population Funds Agency – UNFPA. (2002). State of World Population 2002. People Poverty and Possibilities: Making Development Work for the Poor

United Nations (UN), (2000).United Nations Millennium Declaration, UN General Assembly Distr.: General 18 September 2000.

World Bank-Nigeria, (2012). World Bank Country Briefing. Retrieved from https://www.worldbank.org/

Yamane, T. (1967). statistics. An *Introductory Analysis*, 2nd Ed., New York: Harper and Row.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 41-48
DOI: https://doi.org/10.31015/jaefs.2023.1.5

Effect of plant population and row orientation on crop yield under sorghum-cowpea intercropping systems in semi-arid Zimbabwe

Tafadzwa Talent Haripo[1,2] • **Veronica Makuvaro**[2] • **Tendai Madanzi**[2,3] • **Nyasha Sakadzo**[3]

[1] Solusi University (SU), Faculty of Applied Sciences, Department of Agribusiness, Bulawayo, Zimbabwe
[2] Midlands State University (MSU), Faculty of Agriculture and Natural Resources, Agronomy and Horticulture Fepartment, Gweru, Zimbabwe
[3] Department of Agricultural Economics and Development, Manicaland State University of Applied Sciences (MSUAS) Mutare, Zimbabwe

Citation: Haripo, T.T., Makuvaro, V., Madanzi, T., Sakadzo, N. (2023). Effect of plant population and row orientation on crop yield under sorghum-cowpea intercropping systems in semi-arid Zimbabwe. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 41-48

Received: 07 October 2021
Revised: 01 December 2022
Accepted: 04 December 2022
Published Online: 09 February 2023

Correspondence: Tafadzwa Talent Haripo
E-mail: tharipo52@gmail.com

Abstract

Smallholder farmers commonly practise intercropping to improve crop yield per unit land area. Proper combination of plant population and row orientation of the component crops needs to be established and this prompted this investigation. A 2x7 factorial experiment was laid in a RCBD with three replications, at Matopos Research Station in Natural Region IV of Zimbabwe. Treatments consisted of sorghum planted at a constant population of 55556plants/ha intercropped with cowpea (C) simultaneously planted at varying populations of 111111plants/ha (C1), 166667plants/ha (C3) and 222222plants/ha (C3) in East-West (EW) and North-South (NS) row orientation. Interaction of cowpea population density and row orientation significantly ($p<0.05$) influenced crop yield and its attributes. Treatment NS-SC3 produced lowest number of pods/plant and grains/pod of 2.6 and 6.1 respectively. Highest cowpea grain yield (637.2kg/ha) was obtained in EW-C3 and lowest (92.4kg/ha) in EW-SC3. Sorghum yield was highest in NS-S (1296.5kg/ha) and lowest in EW-SC3 (491.9kg/ha). LER showed that intercropping performed better than sole crops except for EW-SC3 and NS-SC3 which had LER of 0.800 and 0.905 respectively. Highest LER of 1.312 was obtained in EW-C2. Farmers should plant sorghum-cowpea intercrops in EW row orientation for increased cowpea grain yield but NS row orientation for sole sorghum.

Keywords: Intercropping, Intercrop population, Row orientation, LER

INTRODUCTION

Many rural households are currently food insecure and food demand is expected to increase in coming decades due to growing population and changing patterns of food consumption (Thornton *et al*., 2011). The food insecurity situation has been aggravated by numerous challenges faced since the late 1990s (USAID, 2020), alteration of temperature and rainfall pattern, drastic reduction in agricultural production following erratic rainfall and gross lack of key farming inputs (FAO/WFP, 2008). Furthermore, indigenous staple food crop production has declined in semi-arid Zimbabwe due to introduction of exotic cash crops during colonization (Muyambo and Shava, 2020). Production of main staple food crops is anticipated to grow less in coming decades if adaptation-based agriculture systems are not adopted (Lobell *et al*., 2008; Thornton *et al*., 2011).

Intercropping is one of these adaptation-based systems commonly practised by smallholder farmers in Zimbabwe with the potential contribution to weed management (Mandumbu and Karavira, 2012), improve crop yield and impac-

ting positively to future food problems for smallholder farmers in the semi-arid regions of developing countries (Egbe, 2005). Intercropping is the practice of growing two or more crops simultaneously in the same field for entire or part of their growing period (Khanal *et al*., 2021). Intercropping is widely practised to increase efficiency of resource utilisation, reduce negative externalities of monoculture, improve agricultural productivity and reduce business risk (Bernard and Lux, 2017).

Practising intercropping increases productivity and yield in dry areas by increasing plant densities thus optimizing land use. The component crops should be adequately spaced to maximize production and reduce competition which can be accomplished by plant density, spatial arrangement, plant architecture, maturity dates of the crops grown (Banik *et al*., 2006) and row orientation (Kanjara *et al*., 2014). These gaps in intercropping have prompted this investigation.

MATERIALS AND METHODS

Site description

A field experiment was conducted at Matopos Research Station. The station is located about 40 kilometres south of Bulawayo city in South-West Zimbabwe on latitude 17°42'01 64'' S and longitude 30°56'33 24'' E and at an altitude of 1 353 m. The site is in Natural Region IV which receives annual rainfall of 400 - 650 mm. The area experiences mean annual minimum temperature range of 11-20°C, mean maximum temperature range of 19-26°C and mean annual temperature range of 18-24°C (Mugandani *et al*., 2012). The site has red Fersiallitic loamy clay soils. Supplementary irrigation was provided when necessary.

Experimental design and treatments

The experiment was laid out in a 2 x 7 factorial arrangement of a Randomised Complete Block Design (RCBD) with three replications. The experiment consisted of 14 treatments (Table 1) namely four sole crops, sorghum (S) spaced at 90 x 20 cm (55 556 plants per hectare) and sole cowpea spaced at 45 x 20 cm (C1), 30 x 20 cm (C2) and 22.5 x 20 cm (C3) and three intercrop treatments of sorghum at 55 556 plants per hectare intercropped with cowpea at 111 111 (SC1), 166 667 (SC2) and 222 222 cowpea plants per hectare (SC3) in two row orientations; East-West (E-W) and North-South (N-S).

Table 1. Treatment structure

Row orientation	Intercrop population						
	S	**C1**	**C2**	**C3**	**SC1**	**SC2**	**SC3**
N-S	T1	T2	T3	T4	T5	T6	T7
E-W	T8	T9	T10	T11	T12	T13	T14

Gross plot dimensions were 71 m by 16 m (1 136 m^2) including 1 m boarders at all the edges of the plot and net plot was measuring 4 m x 4 m (16 m^2). At the edges of each plot, 2 boarder rows of sorghum were planted to avoid boarder effects. The field borders were cleared up to a width of 1 m and kept weed free to avoid the effect of the external environment.

Data collection

Cowpea yield and yield components

Number of pods per plant for five plants from each plot and number of grains per pod for five pods from each plot were determined at harvesting. Biomass and grain yield of cowpea were determined and recorded separately for each plot using an electronic scale.

Sorghum yield and yield components

Biomass and grain yield of sorghum were measured separately for each plot using an electronic scale.

Land Equivalent Ratio (LER)

LER was calculated to determine the intercrop advantage. It measures the effectiveness of intercropping in utilization of resources compared to sole cropping (Dhima *et al*., 2007; Takim, 2012). LER is the sum of fractions of intercrop yields divided by the sole crop yield and can be used as an agronomical index for assessing yield advantages derived from intercropping. The index is calculated as follows;

$$LER = \frac{I_a}{S_A} + \frac{I_b}{S_B}$$

Where

I_a = intercrop yield of crop A

I_b = intercrop yield of crop B

S_A = sole crop A yield

S_B = sole crop B yield

A LER greater than 1.0 shows that intercropping is more efficient than sole cropping and a LER less than 1.0 shows that intercropping is disadvantageous. Willey (1985) indicated that a LER of 1.25 can be interpreted as 25% greater yield for intercropping or as 25% greater area requirement for the monocrop system.

Data analysis

Analysis of variance (ANOVA) was done using Genstat version 14th Edition (2013). Separation of means at α = 5% was done using Fischer's Least Significant Difference (LSD) where significant differences were noted (p-value < 0.05). The Land Equivalent Ratio (LER) was used to determine the intercrop advantage.

Results

Effect of cowpea population density and row orientation on cowpea pods per plant

Interaction effects of cowpea population density and

row orientation significantly (P<0.001) influenced the number of pods per plant. The number of pods per plant ranged from 2.6 in the treatment sorghum intercropped with cowpea at 222 222 plants/ha in NS row orientation (NS-SC3) to 12.6 in treatment with sole cowpea at 111 111 plants/ha in EW row orientation (EW-C1). Generally, EW row orientation produced the highest number of pods per plant, ranging from 3.0 to 12.6 as compared to the NS row orientation which produced 2.6 to 5.7 pods per plant (Figure 1). Increasing the cowpea population density from 111 111 to 166 667 plants/ha resulted in 7.7 % and 25.0 % increase in the number of pods per plant for EW and NS row orientation, respectively. Further increase of the population of cowpea from 166 667 to 222 222 plants/ha in both sole and intercropped treatments in NS and EW row orientation, reduced the number of pods/plant. The number of pods per plant in intercropped treatments were reduced by 35.0% and 28.6% in EW and NS row orientation respectively compared to sole cropping.

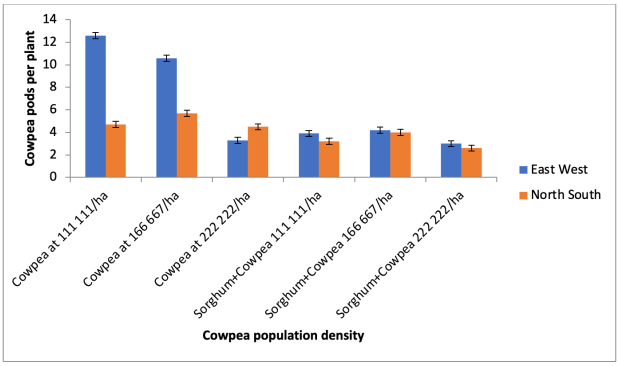


Figure 1. Effect of cowpea population density and row orientation on cowpea pods per plant in sorghum-cowpea intercropping systems

Effect of intercrop population and row orientation on number of cowpea grains per pod

Interactive effects of cowpea population density and row orientation significantly (P<0.001) influenced cowpea grains per pod (Figure 2). The treatment with sorghum intercropped with cowpea at 111 111 plants/ha and EW row orientation (EW-SC1), sole cowpea at 166 667 plants/ha and EW row orientation (EW-C2), sole cowpea at 111 111 plants/ha and NS row orientation (NS-C1) and sole cowpea at 166 667 and NS row orientation (NS-C2) produced the highest number of grains per pod ranging from 13.3 to 13.6 which were not significantly different from each other. The lowest cowpea grain number per pod of 6.1 was produced in treatment with the highest cowpea population density and NS row orientation (NS-SC3). The results also show that intercropping gave lower number of grains than sole cropping, with the NS row orientation giving lower yields than EW.

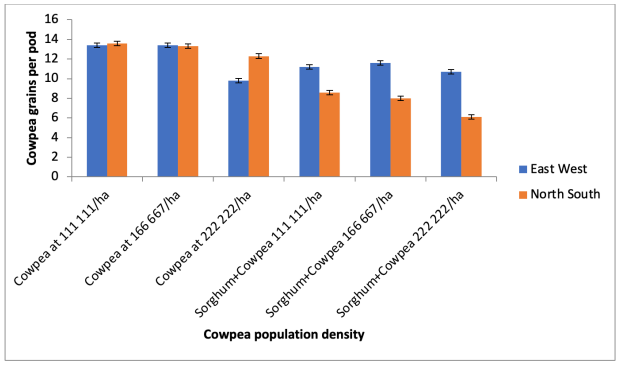


Figure 2. Effect of cowpea population density and row orientation on cowpea grains per pod in sorghum-cowpea intercropping systems

Effect of cowpea population density and row orientation on cowpea biomass

Cowpea biomass yield was significantly (P<0.001) influenced by the interaction effects of cowpea population density and row orientation (Figure 3). The biomass was generally higher, ranging from 368.5 to 578.5 kg/ha, under sole cowpea cropping in EW row orientation than under intercropping in both EW and NS row orientation which recorded low cowpea biomass ranging from 303.3 to 398.4 kg/ha.

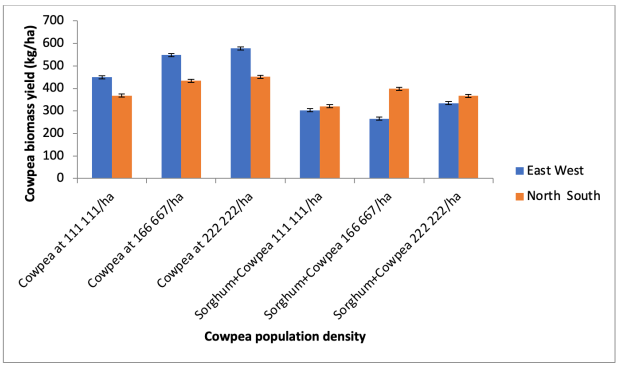


Figure 3. Effect of cowpea population density and row orientation on cowpea biomass yield in sorghum-cowpea intercropping systems

Effect of cowpea population density and row orientation on cowpea grain yield

The grain yield of cowpea was significantly (P<0.001) influenced by the interaction of cowpea population density and row orientation (Figure 4). The cowpea grain yield was higher in the treatments with sole cowpea in the EW row orientation ranging from 405.0 to 637.2 kg/ha and was lower in the treatments with intercropped cowpea in NS row orientation ranging from 92.4 to 206.3 kg/ha. The least grain yield of 92.4 kg/ha was produced in the treatment with highest cowpea population density in the NS row orientation (NS-SC3) and the highest cowpea grain yield of 637.2 kg/ha was produced in the treatment with sole cowpea in EW row orientation. The lowest cow-

pea grain yield was 88.5 % lower than the highest cowpea grain yield. The results also indicated that cowpea intercropping with highest population density produced significantly lower grain yield which was 70.9 % and 81.5 % lower in EW and NS row orientation respectively compared to their corresponding sole crops.

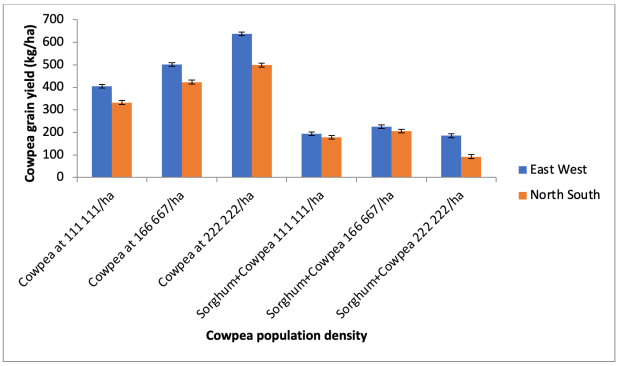


Figure 4. Effect of cowpea population density and row orientation on cowpea grain yield in sorghum-cowpea intercropping systems

Effect of cowpea population density and row orientation on sorghum biomass

Sorghum biomass was significantly ($p<0.05$) influenced by the interaction of row orientation and cowpea population density (Figure 5). Sole sorghum in both NS and EW row orientation (NS-S and EW-S) produced biomass which was significantly higher than that under intercropping, with sole sorghum in NS orientation producing significantly higher biomass than EW orientation. The lowest sorghum biomass of 1 366.4 kg/ha was produced in the treatment with cowpea intercrop at 111 111 plants/ha planted in EW row orientation, but was not significantly different from all the intercropped treatments. The highest sorghum biomass yield of 2487.4 kg/ha was produced in treatment with sole sorghum in NS row orientation.

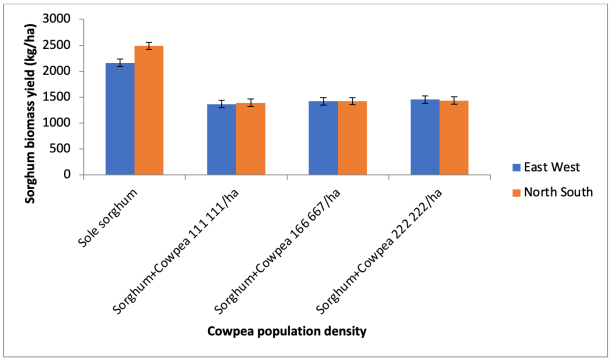


Figure 5. Effect of cowpea population density and row orientation on sorghum biomass in sorghum-cowpea intercropping systems

Effect of cowpea population density and row orientation on sorghum grain yield

Interaction between cowpea population density and row orientation significantly ($P<0.001$) influenced sorghum grain yield (Figure 6). Increasing the cowpea population density from 111 111 to 166 667 plants/ha produced significantly higher grain yields which were ranging from 906.4 to 988.5 kg/ha in NS row orientation compared to 491.9 to 831.9 in EW row orientation. The highest grain yield of 1 296 kg/ha was produced in sole sorghum planted in NS row orientation and the lowest sorghum grain yield of 491.9 kg/ha was produced in sorghum intercropped with cowpea at 222 222 plants/ha (highest cowpea population density) and planted in EW orientation. There was higher sorghum yield in sole sorghum in both row orientation which decreased by 29.2% and 30.1% with the introduction of the lowest cowpea population density of 111 111 plants/ha (SC1). As the cowpea population density was increased from 111 111 to 166 667 plants/ha, sorghum yield increased by 21.7% and 9.9% in EW and NS row orientation respectively. Sorghum yield decreased significantly by 40.9% and 5.6% in EW and NS row orientation respectively when cowpea population density was increased beyond critical of 166 667 (SC2) plants/ha (SC2).

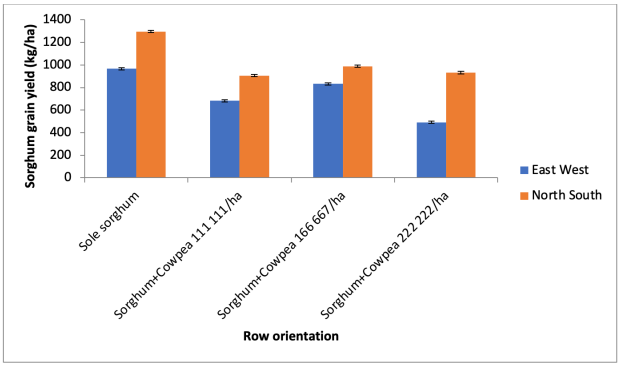


Figure 6. Effect of cowpea population density and row orientation on sorghum grain yield in sorghum-cowpea intercropping systems

Comparison of the productivity of sorghum-cowpea intercropping with that of sole crops using the Land Equivalent Ratio (LER)

The intercrop performance relative to the sole crop showed that sorghum-cowpea intercropping system performed better than sole crop except for those with highest cowpea population density planted in either EW or NS row orientation which had LERs of 0.800 and 0.905 respectively (Table 2). The intercrop system with the highest LER was the one with 166 667 cowpea plants/ha in EW row orientation. Sorghum with cowpea population density of 222 222 plants/ha in EW row orientation had the lowest LER. When the cowpea population density was increased from 111 111 to 166 667 cowpea plants/

ha, the LER increased by 10.4% from 1.188 to 1.312 in EW row orientation and by 1.2% from 1.233 to 1.248 in NS row orientation. The results also indicate that further increase in the cowpea population density from 166 667 to 222 222 plants/ha, reduced the LER by 31.4 and 27.6% in EW and NS row orientation respectively resulting in LERs which are less than a unit.

Table 2. LERs for sorghum intercropped with varying population density of cowpea

Treatment	Partial LER		LER
	Sorghum	Cowpea	
EW-Sorghum + cowpea at 111 111 plants/ha	0.480	0.708	1.188
EW- Sorghum + cowpea at 166 667 plants/ha	0.451	0.861	1.312
EW- Sorghum + cowpea at 222 222 plants/ha	0.291	0.509	0.800
NS- Sorghum + cowpea at 111 111 plants/ha	0.536	0.699	1.235
NS- Sorghum + cowpea at 166 667 plants/ha	0.487	0.762	1.249
NS- Sorghum + cowpea at 222 222 plants/ha	0.185	0.720	0.905

DISCUSSION

Effect of cowpea population density and row orientation on cowpea yield and yield attributes

The number of pods per plant was higher in the sole cowpea as compared to the intercropped cowpea. This can be attributed to the absence or reduced interspecific competition which led to the production of more branches and probably taller plants with more pod/plant and higher number of grains per pod as compared to the intercropped plants. The reduction in number of pods per plant in intercropped cowpea plants could also, presumably, be attributed to better growth of the more aggressive sorghum plants during the dry spells which might have outcompeted the cowpea plants for radiation. More and well-distributed rainfall could have produced taller cowpea plants which would access more solar radiation allowing the crops to produce more pods per plant, number of grains per pod and yield more biomass and grain yield.

Cowpea biomass and grain yield reduction in intercropping might be due to the aggressive effects of sorghum plant on cowpea, similar to the case of reduced number of pods per plant under intercropping. Sorghum which is a C_4 plant probably had the ability to out compete cowpea which is a C_3 plant, for resources during the long dry spell experienced during the growing season resulting in lower biomass and grain yield for the cowpea crop. Crops with C_4 photosynthetic pathways have been known to be dominant when intercropped with C_3 crop species like cowpea (Hiebsch *et al*., 1995). The yield reduction of intercropped cowpea can also be attributed to the shading effect of taller sorghum plants as reported by Egbe (2010) who alluded that the photosynthetic rate of the lower growing plants can be reduced by the shading of the taller growing plants in a mixture thereby reducing the final grain yield. Interaction between plant population and row orientation influences solar radiation interception by the plant canopy and soil moisture and nutrient uptake by the crops (Tsubo & Walker, 2003).

This reduction in biomass and grain yield as the cowpea intercrop population is increased can be attributed to severe intra-specific and interspecific competition for growth resources such as soil moisture, solar radiation, nutrients and air between the intercrop components. In addition to these factors, depressive effects like shading from sorghum plants and high population density have also contributed to the decrease in the cowpea grain yield as reported by Egbe (2010). Pal *et al*. (1992) and Muoneke *et al*. (2007) reported similar yield reductions in Benue State, Nigeria in soybeans intercropped with maize and sorghum and associated the yield depression to interspecific competition and the depressive effect of cereals. These results were further explained by Ghosh (2004) in a report where the differences in yield were reported to be due to the differences in canopy height of soyabean and sorghum and added that the two species did not only compete for nutrients and water but also for sunlight.

Row orientation also influences the interception of solar radiation by the plant canopy. Borger *et al*. (2010) found that light influences flowering and fruit set thereby significantly determining number of pods per plant, number of grains per plant and crop productivity. This implies that light is a determinant of both biomass and grain yield. Reducing the crop row spacing or changing the crop row orientation at near right angle to the sunlight direction (NS) increases shading of the intercrop (cowpea) by the main crop (sorghum). Cowpea yields achieved in this research were far less than the varietal yield potential of 4 000 kg/ha reported by DR&SS (2015). The differences in yield can be due to differences in soil fertility and the poor rainfall season.

Effect of cowpea population density and row orientation on sorghum yield

There was higher biomass and grain yield in sole sorghum than in sorghum-cowpea intercrops probably due to absence of or reduced competition under the former system. This is in contrast to Lemlem (2013) and Mashingaidze (2004) who independently observed the attainment of higher yield under intercropping systems due to

more efficient utilisation of resources available. Pathak *et al*. (2013) reported higher total green fodder yield in sorghum intercropped with cowpea in 2:1 row ratio. Competition for resources such as nutrients, soil moisture, air, solar radiation and space is reduced under sole cropping than under intercropping if same plant population for the main crop is maintained. When cowpea intercrop population was increased from 166 667 to 222 222 plants/ha there was a reduction in both biomass and grain yield and this could be due to the plant density of cowpea which had exceeded the optimum for intercropping. In intercropping, the plant density should be optimised to reduce competition from overcrowding by adjusting the seeding rate of each crop on the mixture below the full rate to allow the crops to yield well in the mixture as reported by Hiesbick, (1980) and Prabhakar, *et al*. (1983). These results are similar to those found by Kanjara *et al.* (2014). The results are also similar to those found by Tsubo *et al.* (2003) who reported that maize crops oriented in NS row orientation intercepted more Photosynthetically Active Radiation (PAR), increasing the rate of photosynthesis and thereby increasing the ear length, ear weight and grain yield in maize-beans intercrop experiments in semi-arid conditions of South Africa.

Generally, row orientation produced contrasting results for sorghum and cowpea biomass and grain yields. NS row orientation produced significantly higher sorghum biomass and grain yield than the EW oriented intercrop crops. On the contrary, EW row-oriented crops produced higher cowpea biomass and grain yield than NS oriented crops. This can be attributed to more solar radiation interception by the taller sorghum plants resulting in increased photosynthesis and consequently more dry matter and grain yields production. Cowpea plants in NS row orientation received less solar radiation due to more shading effects of the taller sorghum plants.

Comparison of the productivity of sorghum-cowpea intercropping with that of sole crops using the Land Equivalent Ratio (LER)

Land Equivalent Ratio (LER) was used to determine yield advantage of intercropping. The results indicated that intercropping had advantages up to a cowpea population density of 166 667 plants per hectare as indicated by the LERs which are greater than 1. The LERs which are less than a unity in the treatments with higher cowpea population mean that there was more competitive interference than complementary facilitation. This indicates that the performance of the intercrop was affected by competition from the cowpea component. The treatments which resulted in a LER above 1 had yield advantage as compared to sole cropping and the results could stem from low interspecific competition or strong facilitation (Kipkemoi *et al*., 1997). Intercropping was found to be more beneficial (indicated by LER greater than a unit) in less fertile fields and more marginal environments compared to fertile fields (Kermah *et al*. (2017). Choudhary *et al*. (2016) observed that intercropping increased land-use efficiency by 17-53 % and produced a LER of 1.21-1.56.

According to Van der Meer (1989) it is possible to obtain the net result of Land Equivalent Ratio (LER) where the complimentary facilitation is contributing more to the interaction of the crop species intercropped than the competitive interaction since both competition and facilitation take place in many intercropping systems. Thus, a LER < 1 could result from high interspecific competition or weak to no facilitation while a LER > 1 could result from low interspecific competition and strong facilitation among the intercropped crop species.

CONCLUSION

The yield and yield components of both the main crop (sorghum) and the intercrop (cowpea) were significantly influenced by the interaction of cowpea population density and row orientation. The treatment which had sole cowpea at 166 667 plants/ha in EW row orientation produced the highest number of pods per plant and the treatment which had sorghum intercropped with cowpea at 222 222 plants/ha in NS row orientation produced the least number of pods per plant. The least cowpea biomass and grain yields were produced in the treatment which had sorghum intercropped with cowpea at 166 667 plant /ha while sole cowpea at 222 222 plants/ha produced the highest cowpea biomass. The highest sorghum biomass and grain yield was produced in the treatment which had sole sorghum in NS row orientation (N-S) and the least sorghum biomass and grain yield was produced in the treatment which had sorghum with cowpea at 222 222 plants/ha in EW and NS row orientation, respectively. The LER results indicated that sorghum-cowpea intercrop systems performed better than their corresponding sole crops except for the treatments which had the highest cowpea population density of 222 222 plants/ha in both EW and NS row orientation which had LERs of 0.800 and 0.905 respectively.

Recommendations

We recommend farmers in Matobo District and other semi-arid areas to plant cowpea intercrops in sorghum under the ES row orientation at populations ranging from 111 111 to 166 667 plants/ha to produce relatively high yields from cowpea plants as it allows more light penetration and interception by the cowpea canopy. This row orientation would enhance higher light interception hence higher photosynthesis by cowpea plants and ultimately produce better yields that would vary depending on amount of rainfall received. LER which is above unit for the same treatment combinations further supports this recommendation. Sorghum-cowpea intercrops should be planted in EW row orientation to enhance sorghum yield and NS row orientation for sole sorghum. More studies are recommended across rainfall season, soil types, agroecological regions and varietal or crop diversity to fully appreciate the effects of cowpea population density

and row orientation on crop yield.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

Authors do not declare any conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

We would like to express our sincere gratitude to Matopos Research Centre for providing the experimental site and support for this study.

REFERENCES

Banik, P., Midya, A., Sarkar, B.K. & Ghose, S.S. (2006). Wheat and chickpea intercropping systems in an additive series experiment: Advantages and weed smothering. *European J. Agronomy*, 25, 202-207.

Bernard, B. and Lux, A. (2017). How to feed the world sustainably: An overview of the discourse on agroecology and sustainable intensification. *Regional Environmental Change*, 17, 1279-1290. https://doi.org/10.1007/s101113-016-1027-y

Borger, C.P.D., Hashem, A. & Pathan, S. (2010). Manupulating Crop Row Orientation to Suppress Weeds and Increase Crop Yield. *Weed Science Society of America*, 58, 174-178.

Buchanan, C. *et al*. (2005). Sorghum bicolor's transcriptome response to dehydration, high salinity. *Plant Mol. Biol.*, 58, 699-720.

Choudhary, V.K., Dixit, A. and Chauhan, B.S. (2016). Resource-use maximization through legume intercropping with maize in the eastern Himalayan region of India. *Crop and Pasture Science*, 67 (5), 508-519. https://doi.org/10.1071/CP15233

Dhima, K.V., Lithourgidis, A.A., Vasilakoglou, I.B. and Dordas, C.A. (2007). Competition indices of common vetch and cereal intercrops in two seeding ratios, *Field Crop Research*, 100, 249-256.

DR&SS (2015). Department of Research and Specialist Services (DR&SS), Harare, Zimbabwe. Available at: http://www.drss.gov.zw/index.php?option=com_content&view=article&id=111&Itemid=129

Egbe, O.M. (2005). Evaluation of some agronomic potentials of pigeon pea genotypes for intercropping with maize and sorghum in Southern Guinea Savanna. PhD Thesis, University of Agriculture, Makurdi, Nigeria.

Egbe, O.M. (2010). Effects of plant density of intercropped soybeans with tall sorghum on competitive ability of soybeans and economic yield at Otobi, Benue State, Nigeria. *Journal of Cereal and Oilseeds*, 1 (1), 1-10.

FAO/WFP (2008). Special Report: FAO/WFP Crop and Food Supply Assessment Mission to Bangladesh, 28 August 2008. Food and Agriculture Organisation of the United Nations and World Food Programme, Rome. Retrieved from https://allafrica.com/view/resource/main/main/id/00011566.html

Ghosh, P.K. (2004). Growth, yield, competition and economics of groundnut/cereal fodder intercropping in the semi-arid tropics of India. *Field Crops Res.* , 88, 227-237.

Hiebsch, C., Tetio-kegho, F. & Chirembo, F.P. (1995). Plant density and soybean maturity in maize soybean intercrop. *Agronomy Journal*, 87, 965-970.

Hiesbick, C.K. (1980). Principles of intercropping: Effects of N. Fertilisation, Plant population and crop duration on equivalent ratios in intercrop versus Monoculture. PhD dissert. North Carolina State University. Raleigh Abstr.

Kanjara, S.M.S.M., Kibe, A., Karogo, P. & Mwangi, M. (2014). Effects of intercropping population density and row orientation on growth and yields of sorghum-cowpea systems in semi arid Rongai, Kenya. *Journal of Agricultural Sciences*, 6 (5), 34-43.

Kazi, B.R., Buriro, A.H., Kubar, R.A. & Jagirani, A.W. (2007). Weed spectrum frequency and density in wheat (*Triticum aestivum*) under Tandojam conditions. *Pak J. Weed Sci. Res.*, 13 (3-4), 241-246.

Kermah, M., Franke, A.C., Adjei-Nsiah, S., Ahiabor, B.D.K., Abaidoo, R.C. and Giller, K.E. (2017). Maize-grain legume intercropping for enhanced resource use efficiency and crop productivity in the Guinea savanna of northern Ghana. *Field Crop Research*, 213, 30-50.

Khanal, U., Stott, K.J., Armstrong, R., Nuttall, J.G., Henry, F., Christy, B.P., Mitchell, M., Riffkin, P.A., Wallace, A.J., McCaskill, M., *et al*. (2021). Intercropping-Evaluating the advantages to broadacre systems. *Agriculture*, 11, 453. https://doi.org/10.3390/agriculture11050453

Kipkemoi, P.L. *et al*. (1997). Effects of intercropping pattern on soyabean and maize yield in Central Rift Valley of Kenya.

Lemlem, A. (2013). The effects of intercropping maize with cowpea and lablab on crop yield, *Journal of Agriculture and Food Science Research*, 2 (5), 156-170.

Liebman, C. L. Mohler and C. P. (2003). Staver, eds Ecological Management of Agricultural Weeds. Cambridge, UK: Cambridge University Press.

Lima-Fihlo, J. (2000). Physiological Response of Maize and Cowpea to intercropping. *Agropec. Brasillia*, 35, 915-921.

Lobell, D.B., Burke, M.B., Tebaldi, C., Mastrandrea, M.D., Falcon, W.P. and Naylor, R.L. (2008). Priotising climate change adaptation needs for food security in 2030, *Science*, 319 (5863), 607-610.

Mandumbu R. and Karavira C. (2012). Weed suppression and component crops response in maize/pumpkin intercropping systems in Zimbabwe, *Journal of Agricultural Sciences*, 4 (7).

Mashingaidze, A.B. (2004). Improving weed management and crop productivity in maize systems in Zimbabwe. PhD thesis, Wageningen University, Wageningen.

Mohler, C.C. (2001). Enhancing the competitive ability of crops pgs 269-321. In M. Liebman, C.L. Mohler and C.P. Staver, eds Ecological management of weeds. Cambridge, UK: Cambridge University Press.

Morgan, J.M. (1984). Osmoregulation and drought stress in higher plants. *Annu. Rev. Plant Physiol.*, 35, 299-319.

Mugandani, R., Wuta, M., Makarau, A. and Chipindu, B. (2012).

Re-classification of agro-ecological regions of Zimbabwe in conformity with climate variability and change, *African Crop Science Journal*, 20 (s2), 361-369.

Muoneke, C.O., Ogwuche, M.A.O.and Kalu, B.A. (2007). Effect of maize planting density on the perfomance of maize/soybean intercropping system in a Guinea savanna agro-ecosystem. *Afr. J. Agric. Res.*, 2, 667-77.

Mupeperi S. (2016). Effects of intercropping maize (*Zea mays*) with butternut (*Curcubita moschata*) at varying butternut population levels on maize growth, yield and weed suppression, BSc thesis, Midlands State University, Gweru.

Muyambo, T.M. and Shava, S. (2020). Indigenous crop production for sustainable livelihoods: A case of *uninga* in the rural areas of South-Eastern Zimbabwe, *International Journal of Community Well-Being*. https://doi.org/10.1007/s42413-020-00102-6

Pal, U.R., Oseni, T.O. and Norman, J.C. (1992). Effect of component densities on productivityof soybean/maize soybean/sorghum intercrop. *J. Agronomy Crop Sci.*, 170, 66-70.

Prabhakar, B.S., Shulka, V. and Srinwa, K. (1983). Nutritional potential of vegetable intercropping system. *Indian J. Hort.* 2 (3), 258-68.

Reddy, K.C., Vissel, P. and Buckner, (1992). Pearl/millet and cowpea yields in sole and intercrop systems and their after effects on soil and crop productivity. *Field Crop Res.*, 4, 13-124.

Reddy, M.S. & Willey, R.W. (1981). Growth and resource use studies in an intercrop of pearl millet/groundnut. *Field Crop Res.*, 4, 13-24.

Takim, F.O. (2012). Advantages of maize-cowpea intercropping through competition indices, *Journal of Agriculture and Biodeversity Research*, 1 (4), 53-59.

Tharp, B.E. & Kells, J.J. (2001). Effect of glufosinate resistant corn (*Zea mays*) population and row spacing on light interception, corn yield and common lambsquarters (*Chenopodium album*) growth. *Weed technol.*, 15, 413-418.

Thornton, P.K., Jones, P.G., Ericksen, P.J. and Challinor, A.J. (2011). Agriculture and food systems in sub-Saharan Africa in a 4 + world, *Philosophical Transactions of the Royal Society*, 369, 117-136. https://doi.org/10.1098/rsta.2010.0246

Tsubo, M. & Walker, S. (2003). Shade effect on *Phaseolus vulgaris* L. intercropped with *Zea mays* L. under well-watered conditions. *J. Agronomy and Crop Science*, 190, 168-176.

Tsubo, M., Mukhala, E., Ogindo, H.O. & Walker, S. (2003). Productivity of maize-beans intercropping in a semi-arid region of South Africa. *Department of Soil, Crop and Climate Science, University of Free State, P.O. Box 339, Bloemfontein 9300, South Africa*, 381-87.

Vandermeer, J. (1989). *"The Ecology of Intercropping"*. Cambridge: University of Cambridge.

Willey, R.W. (1985). Evaluation and presentation of intercropping advantages. *Experimental Agriculture*, 21 119-133.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 49-61
DOI: https://doi.org/10.31015/jaefs.2023.1.6

The agricultural mechanization situation of agricultural enterprises involved in organic and conventional hazelnut cultivation in Türkiye

Onur Kahraman[1] • **Ebubekir Altuntaş**[2] • **Osman Gökdoğan**[3]

[1] Tokat Gaziosmanpaşa University, Institute of Science, Biosystem Engineering Department, Tokat, Türkiye
[2] Tokat Gaziosmanpaşa University, Agriculture Faculty, Biosystem Engineering Department, Tokat, Türkiye
[3] Isparta University of Applied Sciences, Agriculture Faculty, Agricultural Machinery and Technologies Engineering Department, Isparta, Türkiye

Citation: Kahraman, O., Altuntas, E., Gokdogan, O. (2023). The agricultural mechanization situation of agricultural enterprises involved in organic and conventional hazelnut cultivation in Turkiye. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 49-61

Received: 28 October 2022
Revised: 01 December 2022
Accepted: 06 December 2022
Published Online: 12 February 2023

Correspondence: Ebubekir Altuntaş
E-mail: ebubekir.altuntas@gop.edu.tr

Abstract

The aim of the research is to examine and evaluate the agricultural mechanization status of organic and traditional hazelnut farming enterprises in Alaplı and Karadeniz Ereğli districts of Zonguldak province in Turkey. A survey was conducted with a total of 209 enterprises from 34 villages in 2 districts where hazelnut production is intense in the research. The survey study includes information about the land assets of the enterprises, tractor, agricultural-tool and machinery assets, and hazelnut production stages. In addition, mechanization applications in all agricultural processes from tillage to harvest in hazelnut production were examined and evaluated. It has been determined that 24 of the organic hazelnut production enterprises and 30 of the traditional hazelnut production enterprises have tractors and it has been determined that the tractor brand preferences of the enterprises vary independently from each other. Massey Ferguson and New Holland brands are mostly preferred. It has been determined that the vast majority of businesses prefer to use motorized hoeing machines. As a result, when organic hazelnut enterprises used traditional hazelnut production in previous years and gave up this production system, they reduced the use of tools and machinery. For this reason, it has been determined that there are fewer tools and machines in organic hazelnut producer enterprises compared to traditional hazelnut producer enterprises. While the tractor power (kW/ha) value per unit area processed in organic hazelnut producer enterprises was 1.344, this value was found as 6.320 kW/ha in traditional hazelnut producer enterprises. It is seen that traditional hazelnut producer enterprises are in a better situation in terms of mechanization level than organic hazelnut producer enterprises

Keywords: Hazelnut, Production, Organic hazelnut, Tractor, Mechanization Level

INTRODUCTION

Hazelnut (*Corylus avellana*) is a hard-shelled fruit belonging to the Betulaceae (Birch) family of the Fagales (Beechaceae) family and has over 25 species (Polat, 2014). Being the most cultivated product in the world following almond, the cultural varieties of hazelnut are being widely produced in Turkey, Italy, Georgia, Azerbaijan, USA and Spain (TMO, 2021). Hazelnut is a long-lived culture plant that grows in 36°41' northern latitudes around the world, has a unique climate need, in the form of a bush and can be grown up to 6-7 m (Sobutay, 2006; Doğanay, 2012).). In order to obtain efficient results in hazelnut production, the annual

average temperature should be between 13-16°C, and the temperature should not be less than -8°C or -10°C in winter months and should not exceed 36°C or 37°C in summer months (Ünsal, 2020).

A total of 18 hazelnut varieties are grown in Turkey. Among these, Tombul, Çakıldak, Foşa, Kara Hazelnut, Mincane, Palaz and Sivri are commercially important hazelnut varieties (Pelvan et al., 2012). The regions where hazelnut cultivation is carried out in Turkey are examined in two groups as old and new regions. The Eastern Black Sea Region, which is considered to be the more important region and includes the provinces of Artvin, Rize, Giresun, Ordu and Trabzon, is the first region. The hazelnut production area of this region was 7.3 million hectares in 2020. The second region is the provinces of Düzce, Sakarya, Zonguldak, Bartın, Kocaeli, Kastamonu and Sinop, known as the Central and Western Black Sea region. According to TUIK 2021 data, Ordu, Samsun, Düzce, Trabzon, Zonguldak, Kocaeli, Artvin, Giresun, Bartın, Rize provinces meet 82% of Turkey's hazelnut production (Öztürk and İslam, 2019; Anonymous, 2021a).

Hazelnut consumption has reached significant levels in the world and in Turkey, and every year people prefer cleaner, chemical-free, and pesticide-free products. For this reason, organic production has gained importance in hazelnut production and this type of cultivation is becoming increasingly common. There are important differences between organic and traditional (conventional) hazelnut cultivation. In the traditional (conventional) production method, enterprises can apply their preferred chemical fertilizers or pesticides to the soil and the plant. In organic hazelnut cultivation, animal manures, compost, and green manures are applied. In organic hazelnut cultivation, labour costs are less when compared to traditional hazelnut cultivation. The main purpose of organic hazelnut cultivation is to provide quality, low-cost and healthy production without harming nature and preserving the ecological balance.

Hazelnut cultivation includes agricultural practices such as soil preparation, planting, fertilization, spraying, harvesting and threshing. In hazelnut cultivation, about 50% of total production cost consists of collection costs of the harvested product. Hazelnut cultivation is generally carried out in very inclined areas in the Black Sea Region and mechanic harvesting is very difficult in the region. Despite ranking first in the world hazelnut production, pre-harvest and harvest mechanization applications are almost non-existent in Turkey. Such applications require labour-intensive human labour and this significantly increases production costs in Turkey. However, post-harvest mechanization applications are continuously improving (Yıldız, 2020).

In the literature, there are some studies on mechanization in hazelnut cultivation on a regional basis in Turkey. Alkan and Kılıç (2007) interviewed 151 enterprises in Samsun province and concluded that hazelnut cultivation is mostly carried out in the high-altitude regions of the province, but the desired yield cannot be obtained due to the training status of the enterprises and farmers and the structure of the land. They have further reported that organic hazelnut cultivation is the popular choice in the area. According to Beyhan and Sauk (2018), Turkey is at the forefront of hazelnut production in the world, but due to the slope of the hazelnut growing areas, Turkey is not suitable for machine harvesting. In the study conducted by Güney and Güner (2018), especially based on Ordu, Trabzon and Giresun provinces, it was stated that hand harvesting is common due to the sloping land structure of these provinces. Öztürk and İslam (2019) conducted a survey with hazelnut farms in 183 old (Eastern Black Sea) production areas and 93 new (Central and Western Black Sea) production areas in hazelnut agricultural lands in Turkey. In this study, they also explained that the mechanization situation in the old production areas was lower than the new production areas in terms of differences in agricultural mechanization, irrigation and similar issues between these two regions. Taylan and Durul (2019) concluded that Kocaeli province is unsuitable for mechanization in hazelnut farming. Yıldız (2020) drew attention to the difficulty of mechanization in hazelnut farming in Turkey. In addition, he concluded that the mechanization status of hazelnut in Turkey is lower than in other developed countries, especially in the Black Sea Region, due to the high slope of the soil and the scattered planting areas.

There is no comparative study in terms of mechanization between organic system and traditional cultivation in hazelnut cultivation in Turkey. For this purpose, considering its contribution to the literature, it is planned to examine the mechanization status in organic and traditional hazelnut cultivation. For this purpose, Zonguldak province, which is one of the provinces where organic hazelnut cultivation is important, has been considered as a research subject in this study. In the study, the mechanization levels of producers in traditional and organic hazelnut cultivation were compared.

MATERIALS AND METHODS

The data obtained through face-to-face interviews with the farmers formed the main material of the study. Black Sea Ereğli and Alaplı districts, where hazelnut production is intense in Zonguldak province, were determined as research areas. The sampling study of the research was carried out in two stages. The Black Sea Ereğli and Alaplı districts of Zonguldak province were determined as the districts with the highest hazelnut production and the mostly sloping rural settlements in these districts were taken into account in the study. Afterwards, the villages where the survey will be conducted were determined among these villages by *Purposeful Sampling Method*. The information obtained from the Provincial Directorate of Agriculture and Forestry, TUIK data, Chambers of Agriculture and Headmans were used to determine the

enterprises in the villages included in the sampling area.

Karadeniz Ereğli and Alaplı districts of Zonguldak province have been determined as the most intense hazelnut producers, and then the rural settlement areas of these districts have been defined. Organic hazelnut is being produced in 59 villages of Karadeniz Ereğli district and 47 villages of Alaplı district. Traditional hazelnut, on the other hand, is produced in 126 villages of Karadeniz Ereğli district and in 55 villages of Alaplı district. Villages with 4 or less producers have been removed from the long list leaving behind 22 villages producing organic hazelnut in Karadeniz Ereğli and 20 in Alaplı, and 91 villages producing traditional hazelnut in Karadeniz Ereğli district and 42 villages in Alaplı district. These villages have then been ranked with regards to number of producers and land size. Based on *Purposeful Sampling Method*, it has been decided to study 20% of these villages and the villages with the highest number of producers and area size have been determined. Hazelnut producers from 34 villages comprised the main mass of the study. 8 of these villages (Karadeniz Ereğli 4, Alaplı 4) did organic production and 26 villages (Karadeniz Ereğli 18, Alaplı 8) did traditional production. Based on hazelnut production area and using *Simple Random Sampling Method* (Altunışık et al., 2012), the sample volume has been determined as 209 with 95% confidence interval and 10% deviation from average. Based on districts and product method (organic-traditional), sample volume has been shared by Karadeniz Ereğli organic hazelnut cultivation (43), Karadeniz Ereğli traditional hazelnut cultivation (53), Alaplı organic hazelnut cultivation (63) and Alaplı traditional hazelnut cultivation (50).

The sample numbers to be studied were distributed proportionally to the villages and interviews were made with the enterprises determined through the random numbers table. Questionnaire forms were prepared for the purpose of the research and for businesses producing organic and traditional hazelnuts, and a survey was conducted considering the sample volume determined. For the study, an ethical committee report was received from Tokat Gaziosmanpasa University University Social and Human Sciences Ethics Committee. The data compiled by the survey study were transferred to digital media, evaluated and interpreted in Microsoft Excel program through frequency tables, average, minimum-maximum values and percentage distributions. In the study, the agricultural mechanization level was determined by taking into account the existing agricultural land and tractor numbers in Zonguldak province, Karadeniz Ereğli, Alaplı and other districts throughout Turkey. In addition, the mechanization levels of organic and traditional hazelnut producer enterprises were also tried to be determined.

The condition of the tractor park of a commercial enterprise by years and its relationship with agricultural machinery is the most important indicator to reveal the agricultural mechanization level of the region. Tractors, which have an important place in agricultural mechanization, constitute an important part of mechanization investments. In order to have a good idea about the agricultural mechanization level, the tractor power per unit area (kW/ha), the number of tractors per hectare (tractor /1000 ha), the worked area per one tractor (ha/tractor) values should be determined as the fundamental criteria. Tractor power per hectare (kW/ha) is one of the tractor-related criteria and is the most widely used indicator in the world to explain the current situation and to obtain comparative results (Sümer et al. 2003; Özgüven et al. 2010). As the tractor power grouping, generally 1-5 HP, >5 HP power ranges for single axle tractors, tractors with 1-10 HP, 11-24 HP, 25-34 HP, 35-50 HP, 51-70 HP and >70 HP are taken into account for double-axle tractors, respectively. In determining the tractor power per unit area on the basis of districts for both Zonguldak province and traditional and organic hazelnut producer businesses, the average power value of each tractor power group is multiplied by the number of tractors for the desired year and the unit is proportioned to the processed area (Aybek et al., 2021).

RESULTS AND DISCUSSION

Socio-economic characteristics of the enterprises

Table 1 lists the age distributions and education levels of traditional and organic hazelnut cultivation enterprise owners. The gender distribution of the 209 operators included in the survey was examined and it was determined that 88.68% of the organic production enterpris-

Table 1. Age distributions and education levels of traditional and organic hazelnut cultivation enterprise owners

	Traditional enterprises		Organic enterprises		Traditional enterprises			Organic enterprises	
Age distribution	**Freq.**	**Rate %)**	**Freq.**	**Rate (%)**	**Education level**	**Freq.**	**Rate (%)**	**Freq.**	**Rate (%)**
20-30	1	0.97	-	0.00	Literate	11	10.68	17	16.04
31-40	5	4.85	2	1.89	Primary	34	33.01	59	55.66
41-50	20	19.42	9	8.49	Secondary	36	34.95	23	21.70
51-60	36	34.95	35	33.02	High School	22	21.36	7	6.60
>61	41	39.81	60	56.60	University-Postgrad.	-	0.00	-	0.00
Total	103	100	106	100	Total	103	100	106	100

es were male and 11.32% were female. In the survey of traditional businesses, it was determined that 87.38% of them were male and 12.62% were female.

As Table 1 indicates, average age of traditional-production enterprises is 56 and average age of organic-production enterprises is 58. It was noted that the youngest operator among the traditional production enterprises participating in the survey was 33 years old and the oldest operator was 81 years old. The rate of literate enterprises in organic production enterprises is 16.04%. This rate was recorded as 10.68% in traditional production operators in terms of educational status. The educational status of the operators was examined. According to this analysis, while the rate of operators who state that they are primary school graduates and engage in organic production is 55.66%, this rate is 33.01% for those who make traditional production. The rate of those who state that they are secondary school graduates in traditional production enterprises is 34.95%. The distribution of secondary school graduates in organic production enterprises is 21.70%.

and are engaged in farming. It is seen from the survey results that 16.07% of the operators work in various factories. Most of the surveyed enterprises are retired and have been producing hazelnut for many years.

Land assets of enterprises

The area where hazelnut is grown belonging to the enterprises producing traditional and organic hazelnut is given in Table 2 in terms of 'da'. Accordingly, most of the enterprises engaged in traditional and organic production stated that the lands belonged to them (90.56% and 97.22%, respectively). It was determined that the remaining 2.56% of the land in 6 731 da area was rented and 0.22% of the land was common use. It has been determined that some of the enterprises, where the cultivated lands are rented and shared, produce hazelnuts in rental lands in addition to their own lands.

In Table 3, the sizes of the hazelnut production lands included in the survey are given. In the survey analysis conducted with 209 enterprises in the Black Sea Ereğli and Alaplı districts, a survey was conducted with the enterprises with a land of 15 da or more, according to the total

Table 2. Hazelnut cultivation areas (da) of enterprises engaged in traditional and organic hazelnut production

	Traditional enterprises		Organic enterprises	
Enterprise area	**Processed area (da)**	**Rate (%)**	**Processed area (da)**	**Rate (%)**
Self-owned	1889.771	90.56	6544	97.22
Rented	174.875	8.38	172	2.56
Co-owned	15	0.72	15	0.22
Other	7.21	0.34	-	-
Total	2086.856	100	6731	100

Table 3. Land sizes of hazelnut producers that participated in the survey

Enterprise size (da)	**Frequency**	**Rate (%)**
15-20 da	132	63.16
21-30 da	49	23.44
31-40 da	16	7.66
41-50 da	4	1.91
[3] 51 da	8	3.83
Total	209	100

Each one of the 209 business owners participating in the survey was asked "*Do you always reside in the village*?". According to the answers received from the enterprises, 196 (93.78%) of the 209 enterprises were permanently residing in the village, and 13 (6.22%) were not residing in the village. 37.73% of the businesses producing organic and traditional hazelnuts are operated by people retired from different fields of business. Although there is no various job distributions in the enterprises, it has been observed that 16% of the enterprises work in the private sector. 19% of the enterprises surveyed stated that they do not work in any job, 11.20% of the enterprises stated that they are coffee shop operators, bakery operators

land assets of the enterprises. There are 132 enterprises with land in the range of 15-20 da. The size of the land of the enterprises varies. Among the enterprises participating in the survey, the land size of the enterprise with the most land is 70 da. Looking at the other product patterns of organic hazelnut producers, in the Black Sea Ereğli and Alaplı districts, the walnut production as a by-product of the enterprises engaged in organic farming is 85.30%, and 56.50% in traditional producers.

Hazelnut production experiences of enterprises

Enterprises producing organic hazelnuts were asked how many years they had been producing this type of

production. The results are given in Table 4. Accordingly, it has been determined that 42% of the organic producer enterprises have between 16 and 20 years of experience. Again, enterprises producing organic hazelnuts were asked the question "*For how many years did you produce traditional hazelnut*?" According to the answers received, 41% of the enterprises answered that they produce traditional hazelnut in the range of 21-30 years.

Table 5 provides the experience durations of traditional hazelnut production enterprises. As the Table 5 indicates, 33.98% of the enterprises have been engaged in traditional production for 21 to 30 years.

The enterprises engaged in traditional production have been asked about the reasons for preferring traditional production. Enterprise owners stated that traditional production is more effective in combating diseases and pests and they prefer traditional production because it is a method they know better. The same enterprises were asked, "*Do you think there is a difference between organic and traditional production systems in terms of tool-machine use?*". Traditional production enterprises stated that the labour costs increased due to the limitation of the use of agricultural tools and machinery in organic production, and therefore they did not switch to organic production. In addition, the fact that organic production requires less labour and the tool-machine costs of traditional production are increasing is an important difference between the organic and traditional production systems. Although some of the traditional production enterprises are aware that chemicals can harm the soil and beings, they do not prefer organic production because there is no limitation in the use of tools and machinery. At the same time, they do not give up on traditional production because they have adopted an understanding of agriculture that has been going on for years.

Pesticides and fertilizers used by enterprises

In order to determine whether the fertilizers and pesticides they use are used consciously, the question "*how often do they have an analysis done on the soil where they grow hazelnut*" was posed to the enterprises. It has been observed that 67.92% of the enterprises producing organic hazelnuts have their soils analysed, although not at regular intervals, and 26.42% of them have their soil analysed at regular intervals. However, a significant proportion of the enterprises producing traditional hazelnut have never had a soil analysis and this rate corresponds to 51 enterprises out of 103 enterprises, that is, almost half of the enterprises. Table 6 shows the fertilizers and pesticides are used by organic and traditional hazelnut cultivation enterprises.

The pesticides and fertilizers used by traditional hazelnut producers were examined. It was taken into account that more than one answer was given to the questions asked.

Table 4. Hazelnut production experiences of organic production enterprises

Traditional production experience	Frequency	Rate (%)	Organic hazelnut production experience	Frequency	Rate (%)
1-10 years	3	2.83	1-5 years	2	1.89
11-20 years	31	29.25	6-10 years	41	38.68
21-30 years	43	40.57	11-15 years	19	17.92
31-40 years	22	20.75	16-20 years	44	41.51
41-50 years	5	4.72	-	-	-
51- + years	2	1.89	-	-	-
Total	106	100	Total	106	100

Table 5. Hazelnut production experiences of traditional production enterprises

Traditional production length	Frequency	Rate (%)
1-10 years	2	1.94
11-20 years	10	9.71
21-30 years	35	33.98
31-40 years	26	25.24
41-50 years	21	20.39
51-60 years	8	7.77
61- > years	1	0.97
Total	103	100

Accordingly, 58 enterprises among 103 traditional production enterprises used insecticides to combat pests in hazelnut production. Enterprises were asked how often they use insecticide. According to the answers received, it was determined that the ratio of the insecticide used varied between 50 cc/100 litres of water and 100 cc/100 litres of water. The enterprises also stated that they increased the amount of pesticides according to the size of the land. Apart from this, it has been determined that the rate of fungicide used in pest control varies between 250 cc/100 litres of water and 600 cc/100 litres of water.

Soils in the Black Sea region are generally poor in terms of nitrogen and phosphorus. Therefore, enterprises engaged in organic production frequently use farmyard manure, which is one of the organic fertilizers, in order to meet this need of soils (Doğanay, 2012). The enterprises participating in the survey gave more than one answer to the questions asked about the fertilizer and pesticide types. According to the information obtained from the survey data, all of the enterprises use organic solid fertilizer and organic foliar fertilizer. The amount of fertilizer used by enterprises using organic solid fertilizers varies between 3 kg/pit and 5 kg/pit. Enterprises using organic foliar fertilizer are using it in a ratio of 200 cc/100 litres water and 250 cc/100 litres water. 7 out of

Table 6. Distribution of the fertilizers and pesticides are used by organic and traditional hazelnut cultivation enterprises

	Traditional enterprises			Organic enterprises	
Fertilizer	**Frequency**	**Rate (%)**	**Fertilizer**	**Frequency**	**Rate (%)**
Farm manure	32	17.68	Organic solid fertilizer	98	46.23
Base fertilizer (artificial)	67	37.02	Organic foliar fertilizer	90	42.45
Top fertilizer (artificial)	63	34.81	Farm manure (cattle manure)	24	11.32
Lime	19	10.50	-	-	-
Total	181	100	Total	212	100
Pesticide	Frequency	Rate (%)	Pesticide	Frequency	Rate (%)
Insecticide	58	31.02	Burgundy slurry	27	60
Fungicide	22	11.76	Sulphur	11	24.44
Herbicide	79	42.25	Lime	7	15.56
Burgundy slurry	18	9.63	-	-	-
Sulphur	10	5.35	-	-	-
Total	187	100	Total	45	100

Table 7. Reasons for enterprises to prefer either organic or traditional production

What are the reasons for your organic production preference?	**Frequency**	**Rate**	**What are the reasons for your traditional production preference?**	**Frequency**	**Rate**
Marketed at a higher price	40	22.10	Marketed at a higher price	18	8.91
Easier to find buyers	25	13.81	Easier to find buyers	13	6.44
Not harmful to the environment	33	18.23	Not harmful to the environment	9	4.46
Input costs are lower	11	6.08	Input costs are lower	20	9.90
Requires less labour	5	2.76	Requires less labour	43	21.29
More effective control of diseases and pests	21	11.60	More effective control of diseases and pests	29	14.63
Higher yield	27	14.92	Higher yield	17	8.42
A method I know better	13	7.18	A method I know better	49	24.26
Other	6	3.31	Other	4	1.98
Total	181	100	Total	202	100

Table 8. Differences between organic and traditional hazelnut production

What are the differences between organic and traditional production?	Frequency	Rate (%)
Organic production is a more labour-intensive (labour-based) system	45	13.76
In organic production, I produce with fewer tools and machines	30	9.17
Since I produce organically, my tool-machine cost decreases	23	7.03
Since there is a limitation on the use of tools and machinery in organic production, my labour costs increase	36	11.01
Traditional production is a more labour-intensive (labour-based) system	20	6.12
The use of tools and machines in traditional production can harm the soil and beings	41	12.54
The fact that there is no limitation in the use of tools and machines in traditional production makes my production easier	63	19.27
Being able to use tools and machines as I want in traditional production is one of the reasons why I did not switch to organic agriculture	56	17.13
Other	13	3.98
Total	327	100

Table 9. The number and brands of tractors belonging to enterprises engaged in traditional and organic production

Tractor brand	Traditional enterprises		Organic enterprises		
	Number (pcs)	Rate (%)	Tractor brand	Number (pcs)	Rate (%)
Massey Ferguson	8	26.67	New Holland	8	33.33
New Holland	6	20.00	Erkunt	2	8.33
Tümosan	1	3.33	Massey Ferguson	5	20.83
John Deere	4	13.33	Fiat	3	12.50
Başak	2	6.67	Ford	2	8.33
Erkunt	3	10.00	International	2	8.33
Deutz	2	6.67	Tümosan	1	4.17
Case IH	3	10.00	John Deere	1	4.17
Fiat	1	3.33	-	-	-
Total	30	100	Total	24	100

Table 10. Tractor powers of enterprises engaged in traditional and organic production

Power groups of tractors	Traditional enterprises		Organic enterprises	
	Number of tractors	Rate (%)	Number of tractors	Rate (%)
1-10 HP	-	-	-	-
11-24 HP	-	-	-	-
25-34 HP	2	6.67	2	8.33
35-50 HP	6	20.00	13	54.17
51-70 HP	16	53.33	6	25.00
71-100 HP	6	20.00	3	12.50
Total	30	100	24	100

106 organic hazelnut production enterprises stated that they use lime for pest control in hazelnut production, 27 enterprises use burgundy slurry and 11 enterprises use sulphur. It has been determined that 24 enterprises engaged in organic production use farm manure and this is generally cattle manure.

The enterprises that participated in the survey and preferred organic (106) and traditional (103) production methods were asked the question *'which method of control they use against weeds in hazelnut orchards'*. It was noted that 62.20% of the enterprises scythe weeds and 37.80% of them use weed control in the fight against weeds instead of scythes. The results obtained show that all of the organic production enterprises apply scythe. It was determined that 27 of 130 enterprises preferred the traditional production method, but they used scythe in-

Table 11. Tractor age distributions of enterprises engaged in traditional and organic production

Age distribution of tractors	Traditional enterprises		Organic enterprises	
	Number of tractors (pcs)	Ratio among all tractors (%)	Number of tractors (pcs)	Ratio among all tractors (%)
5-10 years	3	10	8	33.33
11-15 years	8	26.67	2	8.33
16-20 years	2	6.67	5	20.83
21-25 years	4	13.33	1	4.17
> 25 years	13	43.33	8	33.33
Total	30	100	24	100

Table 12. Agricultural tools and machines used in enterprises engaged in traditional and organic production

	Traditional enterprises		Organic enterprises	
Tools and machines	Number (pcs)	Rate (%)	Number (pcs)	Rate (%)
Motorized hoeing machine	61	19.00	82	32.03
Handsaw	109	33.96	112	43.75
Motorized garden sprayer	96	29.91	36	14.06
Motorized back sprayer	27	8.41	10	3.91
Trailer	14	4.36	9	3.52
Portable chain-saw	10	3.12	7	2.73
Pull-type hazelnut harvester	2	0.62	-	-
Disc plough	1	0.31	-	-
Harrow	1	0.31	-	-
Total	321	100	256	100

stead of herbicide in weed control. The majority of the enterprises stated that they use motor scythes in the fight against weeds. On the other hand, 79 enterprises use herbicides to dry and destroy weeds. Although the use of this pesticide varies according to the size of the land, it has been determined that they are used in the range of 200 cc/100 litres of water and 500 cc/100 litres of water. Enterprises also use burgundy slurry. They stated that they preferred 1% and 2% levels in the selection of burgundy slurry. As another pesticide, sulphur is also used in hazelnut to prevent disease. The enterprises stated that the sulphur usage rate varies between 150 cc and 200 cc/100 litres of water.

According to the results obtained from the surveys, some enterprises use only chemical fertilizers and some only apply chemical pesticides. According to the survey data, 17.68% of the enterprises use farm manure. The usage rates of fertilizers vary between 1 kg/pit and 8 kg/pit. In enterprises using base fertilizer, this rate varies between 2 kg/pit – 6 kg/pit. Since the soil of the Black Sea Region is not very sufficient in terms of nitrogen, enterprises use nitrogen fertilizers a lot. 63 of the enterprises participating in the survey stated that they use top fertilizer. It was determined that the fertilizer was applied in varying amounts between 2 kg/pit - 5 kg/pit. According to the answers given by the enterprises to the questionnaires, it has been revealed that they add lime to the hazelnuts in certain years, if not every year, for fertilization purposes. The amount of lime used in hazelnut production varies between 1.5 kg/pit - 7 kg/pit.

In the survey study, 8 enterprises out of 106 organic production enterprises stated that they do not use organic fertilizers and 36 enterprises stated that they do not apply pesticides. Likewise, it was determined that 11 enterprises among the enterprises producing traditional hazelnut do not fertilize and 6 enterprises do not apply pesticides. According to the answers received from the enterprises, it is also known that the reasons why the enterprises do not spray and fertilize are due to economic reasons.

It is known that there are certain differences between the two production patterns known as organic and traditional. In order to identify these differences, businesses were asked "*why they prefer organic or traditional production*" and "*what are the differences between the two production patterns*". The answers obtained from the enterprises are given in Table 7 and Table 8. According to Table 7, the first reason why 22.10% of organic production enterprises prefer this type of production is that the product is marketed at a higher price. Another reason that is effective in the preference of businesses is that it is not harmful to the environment (18.23%). On the other hand, 24.26% of the enterprises engaged in traditional production, that is, the majority, argued that their preference for traditional production is due to the fact that it is a method they know better. 14.63% of businesses think that traditional production is more effective in combating diseases and pests. At the same time, according to the survey results, considering the use of machinery and tools, enterprises stated that less labour is required (21.29%) due to the

lack of limitations in the use of tools and machinery and the use of pesticides and fertilizers in the fight against pests.

Enterprises engaged in organic and traditional production were asked "What are the differences between organic production and traditional production?" In the answers given, the idea that there is more intensive labour in production in organic production enterprises is noteworthy, while the other important difference is the thought that traditional production harms the soil and soil creatures with a ratio of 12.54% compared to organic enterprises. Traditional production enterprises, on the other hand, think that the most important one among the two production methods is the lack of tool-machine limitation (19.27%). At the same time, enterprises stated that they did not switch to organic agriculture because they used tools and machines as they wished (17.13%).

Mechanization status of hazelnut-producing enterprises

In order to determine the mechanization status of all 209 enterprises included in the study, the number of tractors belonging to the enterprises and the tools-machines they use were determined (Table 9). There are a total of 30 enterprises that have tractors in traditional production enterprises. It is striking that the tractor preferences of the enterprises are very different from each other. Majority of the enterprises prefer Massey Ferguson and New Holland brands. In the brand analysis, it was determined that the enterprises producing organic hazelnuts preferred the New Holland brand at a significant rate (33.33%), the tractors used by the enterprises were garden type tractors, and the tractor preferences of the enterprises were very different from each other. It has been determined that a total of 24 enterprises have tractors. On the basis of enterprises, the rate of ownership of tractors in enterprises engaged in traditional production was 29.13%, while the rate of ownership of tractors in enterprises engaged in organic production was determined as 22.64%.

Horsepower of tractors belonging to enterprises engaged in traditional and organic production is given in Table 10. From the information given, it is seen that tractors with power distribution in the range of 51-70 HP are mostly preferred among the tractor preferences of traditional production companies (53.33%). It is known that enterprises engaged in traditional production prefer high-powered tractors for spraying, fertilizing and transporting. These tractors are generally garden tractors. Enterprises participating in the research stated that they prefer vehicles that can manoeuvre easily in the garden due to the terrain structure. It has been noted that 54.17% of the tractors used by organic production enterprises are in the 35-50 HP power range. 25% of the enterprises have tractors with 51-70 HP, 12.50% with 71-100 HP and 8.33% with 25-34 HP power ranges. Garden type tractors are mostly used in Alaplı and Karadeniz Ereğli districts due to the slope of the hazelnut fields and the frequent planting of the quarries. There are a total of 6 tractors in the range of 71-100 HP in traditional production enterprises

It has been observed that the enterprises make their tractor selections according to the size of the land. It has been determined that enterprises with 31 da of land or more prefer higher powered tractors. Considering the land assets of the enterprises, it is known that the number of enterprises with a production area of 31 da or more is 28. Among these enterprises, 6 of them prefer high power tractors. At the same time, it is known that the enterprises use tractors according to the size of the land, and the tools and machines also vary. Data relating to age distributions of tractors used in traditional and organic production facilities are provided in Table 11. According to this, when the age distribution of tractors of the enterprises engaged in traditional production is reviewed, it is observed that 13 of the 30 enterprises have tractors older than 25 years (43.33%). 3 of the enterprises have tractors between 5-10 years of age. The average tractor age of the enterprises is 25. The average age of the tractors used by the organic production enterprises is 22 years. There are 8 tractors older than 25 years, and 8 tractors between 5 to 10 years.

Tractor assets and tractor power data are not sufficient on their own to determine the mechanization level of the enterprises, therefore the existence of agricultural tools and machines belonging to the enterprises have also been examined (Table 12).

According to the data given in Table 12, 19% of traditional production enterprises have motorized hoeing machines. Majority of these enterprises use these machines for transportation purposes. Due to the land structures of the enterprises and the spraying machines used, many enterprises prefer the use of tractors instead of motorized hoeing machines. 29.91% of the enterprises prefer motorized garden sprayers with 200 litres and 400 litres tank capacity when spraying. In traditional production, the proportion of motorized garden sprayers is higher than those that make organic production, due to the greater number and amount of spraying and fertilization. Among 103 traditional production enterprises, 2 enterprises have hazelnut harvesting machines. These enterprises own large plots and therefore they prefer hazelnut harvester to reduce labour costs. However, due to the slope of the lands, most of the enterprises producing traditional and organic hazelnuts prefer to collect manually.

32.03% of organic production enterprises use motorized hoeing machines. Significant portions of these enterprises use hoeing machines for transportation and some enterprises have two motorized hoeing machines for both hoeing and transportation. 14.06% of organic production enterprises use garden sprayers. These sprayers have two different tank capacities, 200 litres and 400 litres. It was determined that motorized garden sprayers

Table 13. To what extent do the existing tools-machines and tractors meet the needs of enterprises engaged in traditional and organic production enterprises

Do your existing tractors and tools-machines meet your needs?	Traditional enterprises		Organic enterprises	
	Frequency	Rate (%)	Frequency	Rate (%)
Not at all	13	12.62	8	7.55
To some extent	48	46.60	39	36.79
They meet all my needs	45	43.69	59	55.66
Total	103	100	106	100

Table 14. Number of tractors and power values in Zonguldak province, its districts and Turkey in general (Anonymous, 2021b)

Tracto Dist. (BG) Axle	Power		Alaplı	Karadeniz Ereğli	Other Districts	Zonguldak	Turkey
Single-axle	1-5		75	-	102	177	19 416
	>5		215	3400	586	4201	73 782
Tractors with single axle			290	3400	688	4378	93 198
Two-axle		1-10	105	-	3	108	6969
		11-24	102	-	75	177	20 944
		25-34	4	-	105	109	68 157
		35-50	42	360	665	1067	517 899
		51-70	150	-	1319	1469	544 909
		>70	83	-	657	740	190 677
Total number of two-axle tractors			486	360	2824	3670	1 349 555
Total number of tractors			776	3760	3512	8048	1 442 753

(14.06%) were used more than back sprayers (3.91%) in spraying and fertilization. Due to the large number of hazelnut orchards, enterprises tend to prefer garden-type sprayers. It is also noted from the study data that most of the garden sprayers are common property. It is determined that the enterprises use hand saw and tree motor for cleaning and pruning hazelnut trees. It is determined that there are 112 hand saws in total in the 106 enterprises included in the survey that make organic production. It is also noted that some businesses have more than one hand saw. In the same way, it is seen that tree engine is used in 7 enterprises. 9 enterprises stated that they have agricultural carts.

Enterprises engaged in organic and traditional (209) production were asked, "*What do you use to harvest hazelnuts*?" It was concluded that almost all of the enterprises (99.04%) harvested hazelnut by hand, and only 2 enterprises harvested hazelnut with machinery (0.96%). Another question asked to enterprises was "*Do you dry after harvesting*". According to the answers obtained from this question, all of the enterprises dry after the hazelnut harvest.

In addition to the characteristics of the mechanization status of the hazelnut producer enterprises such as tractors, tools-machines, tractor powers and ages, it is also important to know how much mechanization tools benefit the enterprises. For this reason, businesses were asked to what extent their existing tools-machines and tractors can meet their needs (Table 13).

It has been observed that the existing tools and machines do not meet the needs of 12.62% of the traditional production enterprises, they moderately meet the needs of 46.60% of them, and they completely meet the needs of 43.69% of them. It has been determined that the existing tractors and tools-machines fully meet the needs of 55.66% of the organic production enterprises, do not meet any of the needs of 7.55%, and moderately meet the needs of 36.79%. Since organic production enterprises do not need too many tools-machines, enterprises think that the existing tools-machines are sufficient for them.

Comparing the mechanization levels of Zonguldak province, its districts and Turkey in general

Zonguldak province has an important position in hazelnut production. In terms of measuring the level of mechanization in Zonguldak, the number of tractors and their power were evaluated by taking into account the TUIK

Table 15. Mechanization level comparison between Zonguldak province, and Turkey in general

	Agricultural land (ha)*	Number of tractors (pcs)*	Tractor power per unit area (kW/ha)	Number of tractors per 1000 ha (tractor/1000 ha)	Processed area per tractor (ha/tractor)
Alaplı	10 716.1	776	1.527	72.414	13.809
Karadeniz Ereğli	16 621.3	3760	1.807	226.216	4.421
Other districts	21 306.8	3512	5.977	164.830	6.067
Zonguldak	48 644.2	8048	3.572	165.446	6.044
Turkey	23 145 133.7	1 442 753	2.360	62.323	16.045

*: Anonymous, 2021c.

2020 data (Table 14). Accordingly, there are a total of 1 349 555 two-axle tractors used for agricultural mechanization in Turkey. 3670 of those tractors are found in Zonguldak province. Considering the ratio of single-axle tractors, which are generally used in small areas, there are 4378 tractors in total in Zonguldak. 290 of those tractors are found in Alaplı and 3400 of them are found in Karadeniz Ereğli district. About 5% of all single-axle tractors in Turkey are found in Zonguldak. Due to the sloping lands of Zonguldak province, 77.66% of the use of these vehicles belongs to the Black Sea Ereğli district. It is seen that the use of tractors is higher in the Black Sea Ereğli district, where hazelnut production is higher than other districts.

The average power values used to calculate the tractor powers were determined according to the average power values of each tractor power group determined by Aybek et al (2021). While determining the mechanization level, single and double axle tractors were evaluated together as tractor power groups and accordingly, the average tractor power value was determined as 31.70 kW for organic enterprises and 43.96 kW for conventional enterprises according to tractor power groups. In this study, for the fundamental criteria agricultural mechanization level, the tractor power per unit area (kW/ha), the number of tractors per hectare (tractor /1000 ha), and the worked area per tractor (ha/tractor) values were determined. A comparison of the agricultural mechanization level of Zonguldak province, its districts and Turkey is given in Table 15.

According to the 2020 data given in Table 15, the tractor power (kW/ha) per unit area worked in Zonguldak province and Turkey is 3.572 kW/ha for Zonguldak province and 2.360 kW/ha for Turkey. It has been revealed that the value of Zonguldak province is lower than that of Turkey. On the basis of districts, the sum of 1.527 kW/ha in Alaplı and 1.807 kW/ha in Karadeniz Ereğli is close to the value of Zonguldak province. While the number of tractors per 1000 ha in the province of Zonguldak is 165.446, approximately 3.63 times the average of Turkey, the district of Alaplı is above the average of Turkey. On a district basis, the number of tractors per 1000 ha in Karadeniz Ereğli is 226.216, which is nearly 4 times the average of Turkey. The processed land value of 16.045 per tractor in Turkey is 2.65 times higher than the value of 6.044 ha/tractor in Zonguldak.

Table 16. Hazelnut production areas and number of tractors per enterprise on the basis of traditional and organic producers

	Hazelnut production area (ha)	Number of tractors (pcs)	kW/ha	tractor/1000 ha	ha/tractor
Traditional enterprises	208.686	30	6.320	143.757	6.956
Organic enterprises	673.1	24	1.344	35.656	28.046

Mechanization levels of enterprises engaged in traditional and organic production

Calculations were made to determine the mechanization levels of traditional and organic production enterprises and the values obtained are given in Table 16.

Enterprises engaged in organic production have a production area of 6731 da, while enterprises engaged in traditional production have a production area of 2 086.856 da. The difference is 3.23 times. However, in terms of the number of tractors, traditional manufacturing enterprises have 1.25 times less tractors. According to 2020 data, the tractor power (kW/ha) per unit area processed in organic hazelnut producing enterprises is 1.344, while the tractor power per unit area in traditional hazelnut

producing enterprises is 6.320 kW/ha. This criterion was found to be 4.70 times higher in traditional enterprises compared to organic enterprises. This is because the hazelnut production areas of traditional enterprises are less, but the number of tractors is higher.

While the number of tractors per 1000 ha in traditional hazelnut producer enterprises was 143.757 in total, this value was found to be 35.656 in organic producer enterprises, which is 4.03% times higher. On the other hand, while the processed area value of traditional hazelnut producer enterprises was 6.956 per tractor, this value was 4.03 times higher with 28.046 in organic enterprises. Accordingly, traditional hazelnut producer enterprises are seen to be in a better position in terms of mechanization level compared to organic hazelnut producer enterprises, this situation is related to the current situation of agricultural land and tractor numbers.

CONCLUSION

In the present study, a survey was conducted with enterprises producing organic and traditional hazelnuts in Karadeniz Ereğli and Alaplı districts of Zonguldak province, and the number of agricultural tools and machinery and tractor numbers were evaluated within the scope of the enterprises' socioeconomic structures, land assets, agricultural structures, mechanization status of the producers and a comparison of the agricultural mechanization level of producer enterprises was made.

The age distribution of the owners of enterprises producing hazelnuts was analysed and it was determined that the average age of enterprises producing with organic and traditional methods was over fifty years old. Educational status of enterprise owners was also examined and it was determined that many enterprises owners were secondary and primary school graduates. In the study, the total processed area is 8817.856 da in two production patterns separated as organic and traditional, and 76.33% (6731 da) of this is organic production area. Enterprises argued that traditional production is harmful to human health and that organic production is a more profitable method. Enterprises that perform traditional production use too many chemical inputs and they continue traditional production because they know this method better. It has been observed that enterprises engaged in organic farming have 24 tractors and traditional producers have 30 tractors, and there are differences in terms of tractor brand and power preferences. It has been determined that both manufacturers prefer Massey Ferguson and New Holland brands the most. As a result of the study, it has been observed that 14.06% of the enterprises engaged in organic production have garden sprayers and the sprayers are mostly 200 litres car/roller type garden sprayers. It has been determined that the tractor power group preferences of traditional production enterprises are mostly medium power tractors (35-70 HP). The age of the tractors used by the enterprises was determined as 25 in traditional producers and 22 in organic enterprises.

Within the scope of the study, the mechanization levels of the enterprises in Zonguldak and its districts and Turkey in general were examined and according to the results obtained, it was determined that the tractor power per unit area in Zonguldak was above the Turkey average of 2.360 kW/ha. When a comparison is made with the whole of Turkey on the basis of districts, it has been determined that the tractor power per unit area of Alaplı and Karadeniz Ereğli districts is below the Turkey average. It has been observed that the tractor power (kW/ha) value per unit area processed in traditional hazelnut producer enterprises is 4.70 times higher than the organic production enterprises, and the number of tractors per 1000 ha is also 4.03% higher.

The production amount of organic hazelnut producer enterprises is increasing, but the use of tractors, tools and machinery remains limited. Therefore, in order to improve the current level of agricultural mechanization and to increase the number of tractors and tools and machinery they use in agricultural production, providing incentives and supports for the purchase of tractors and tools-machines to the enterprises that will abandon the traditional production approach and adopt the organic production approach will contribute to the more profitable production of organic enterprises.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

Authors do not declare any conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are
original and that they have not been published before.

Ethical approval

Ethics Committee Report was received from Tokat Gaziosmanpasa University Social and Human Sciences Ethics Committee (30.06.2021, session 14, 1-16 issues).

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

This study was summarised from Onur Kahraman's master's thesis. This study was submitted as poster abstract in 34th National Agricultural Mechanization and Energy Congress 7-9 Sep 2022, Bilecik, Turkey.

REFERENCES

Altunışık, R., Coşkun, R., Bayraktaroğlu, S., Yıldırım, E. (2012). Sosyal Bilimlerde Araştırma Yöntemleri SPSS Uygulamalı, Sakarya, Sakarya Yayıncılık (in Turkish).

Alkan, I., Kılıç, O. (2007). Samsun ili Terme ilçesinin ova ve yüksek kesimindeki fındık işletmelerinin karşılaştırmalı ekonomik analizi, OMÜ Ziraat Fakültesi Dergisi, 22(2): 171-178 (in Turkish).

Anonymous. (2021a). Fındık, Tarım Ürünleri Piyasaları, Strateji Geliştirme Başkanlığı, 1-4 (in Turkish).

Anonymous. (2021b). Türkiye İstatistik Kurumu, https://biruni.tuik.gov.tr/medas/ (Access date: 24 July 2021).

Anonymous, (2021c). Türkiye İstatistik Kurumu, https://biruni.tuik.gov.tr/medas/?locale=tr, (Access date: 10.12.2021).

Aybek, A., Kuzu, H., Karadöl, H. (2021). Türkiye'nin ve tarım bölgelerinin tarımsal mekanizasyon düzeyindeki değişimlerin son on yıl (2010-2019) ve gelecek yıllar (2020-2030) için değerlendirilmesi, KSÜ Tarım ve Doğa Dergisi, 24(2): 319-336 (in Turkish). https://doi.org/10.18016/ksutarimdoga.vi.747163

Beyhan, M., Sauk, H. (2018). Türkiye'de fındık tarımında mekanizasyon durumu, Türktob Dergisi, 27: 22-27 (in Turkish).

Doğanay, H. (2012). Türkiye fındık meyvacılığındaki yeni gelişmeler, Doğu Coğrafya Dergisi, 17(27), 1-22 (in Turkish).

Güney, M., Güner, F., (2018). Fındık tarımında durum analizi, makineli hasat gerekliliği ve hasat makinelerinin sınıflandırılması, Türk Tarım-Gıda Bilim ve Teknoloji Dergisi, 6(9): 1141-1147 (in Turkish).

Özgüven, M.M., Türker, U., Beyaz, A. (2010). Agricultural Structure and Mechanization Level of Turkey, Journal of Agricultural Faculty of Gaziosmanpaşa University, 27(2): 89-100 (in Turkish).

Öztürk, D., İslam, A. (2019). Türkiye'de eski ve yeni üretim bölgelerinde fındık yetiştiriciliği yapan işletmelerin tarımsal üretim açısından karşılaştırmalı analizi, Akademik Ziraat Dergisi, 8 (özel sayı): 99-106 (in Turkish).

Pelvan, E., Alasalvar, C., Uzman, S. (2012). Effects of roasting on the antioxidant status and phenolic profiles of commercial Turkish hazelnut varieties (*Corylus avellana* L.), Journal of Agricultural and Food Chemistry, 60: 1218-1223. https://doi.org/10.1021/jf204893x

Polat, S. (2014). Türk fındığının Türkiye'deki yeni yayılış alanı, Marmara Coğrafya Dergisi, 29 (Ocak): 136-149 (in Turkish).

Sobutay, T. (2006). Fındık Sektör Araştırması, İstanbul Ticaret Odası Dış Ticaret Şubesi Uygulama Servisi, 26 (in Turkish).

Sümer, S.K., Say, S.M., Has, M., Sabancı, A. (2003). Türkiye'de ekonomik traktör parkı ve gelişimi, Ç.Ü. Ziraat Fakültesi Dergisi, 18(4): 45-52 (in Turkish).

Taylan, E., Durul, M. (2019). Kocaeli'nde fındık tarımı ve Kandıra'da fındık, Uluslararası Marmara Fen ve Sosyal Bilimler Kongresi, (Bahar) 2019, Bildiriler Kitabı (2): 2294-2301 (in Turkish).

TUIK. (2021). Türkiye İstatistik Kurumu, https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1 (Access date: 9 Dec 2021).

TMO. (2021). 2020 Yılı Fındık Sektör Raporu, Toprak Mahsulleri Ofisi Genel Müdürlüğü, Ankara (in Turkish).

Ünsal, S. (2020). 1970'ten itibaren Türkiye'nin fındık üretimi ve ihracatı, (Proje Finali) Bilkent Üniversitesi, İstanbul (in Turkish).

Yıldız, T. (2020). Türkiye'de fındık tarımında hasat-harman mekanizasyonu, Tarım Makinaları Bilimi Dergisi, 16(1): 12-22 (in Turkish).

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 62-69
DOI: https://doi.org/10.31015/jaefs.2023.1.7

Evaluation of maize farmers' attitude towards risk management and preference for crop insurance in Nigeria

Olugbenga Omotayo Alabi[1] • **Shehu Bako Makarau**[2] • **Samuel Jeremiah Aluwong**[3]
Joseph Dauda Bayei[2] • **Jummai Sunday Lyocks**[2] • **Man Abdullahi**[2] • **Tijani Drisu**[1]
Chinwe Edith Anekwe[1] • **Godbless Friday Safugha**[1] • **Funso Omolayo Alabuja**[1]
Cynthia Onyinyechi Obinna-Nwandikom[1]

[1] Department of Agricultural-Economics, University of Abuja, PMB 117 Gwagwalada-Abuja, Federal Capital Territory, Nigeria
[2] Department of Agricultural-Economics and Extension, Kaduna State University (KASU), Kaduna State, Nigeria
[3] Department of Agricultural-Extension and Management, School of Agricultural Technology, Nuhu Bamali Polytechnic, Zaria, Samaru Kataf Campus, Kaduna State, Nigeria

Citation: Alabi, O.O., Makarau, S.B., Aluwong, S.J., Bayei, J.D., Lyocks, J.S., Abdullahi, M., Drisu, T., Anekwe, C.E., Safugha, G.F., Alabuja, F.O., Obinna-Nwandikom, C.O. (2023). Evaluation of maize farmers' attitude towards risk management and preference for crop insurance in Nigeria. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 62-69

Received: 04 December 2022
Revised: 22 December 2022
Accepted: 24 December 2022
Published Online: 12 February 2023

Correspondence: Olugbenga Omotayo Alabi
E-mail: omotayoalabi@yahoo.com

Abstract

This study focused on evaluation of maize farmers' attitude towards risk management and preference for crop insurance in Nigeria. Multi-stage method of sampling was used. One hundred (100) maize producers were sampled and selected. Primary sources of data were used for this study and the data were collected through the use of well-structured and well-designed questionnaire. Econometrics and statistical tools employed were used for data analysis. The results obtained show that 51% of maize farmers were risk averse, 21% were risk preferring, and 28% were risk neutral. Age, gender, and education level were statistically and significant predictors influencing risk averse attitudes of maize farmers. Age, farm size, household size, gender, risk aversion, education level, and access to agricultural extension services were the statistically and significant predictors influencing preference of maize farmers for crop insurance policy. Garrett index ranking technique employed for risk management strategies and crop insurance policy adopted by maize farmers show that crop diversification was ranked 1st, weather information was ranked 2nd, crop insurance was ranked 3rd, and off-farm activities was ranked 4th respectively. The results of constraints faced by maize producers revealed that lack of extension services was ranked 1st, lack of credit facilities was ranked 2nd, inadequate knowledge of agricultural insurance was ranked 3rd, high premium of agricultural insurance was ranked 4th, while lack of fertilizer was ranked 5th respectively. The constraints retained explained 74.85% of all constraints in the analysis. The study recommends that extension officers should be employed to disseminate research results, innovations and information on risk management strategies and crop insurance to maize farmers. Weather information should be made available to maize farmers, and credit facilities at low interest rate should be provided to maize farmers. Bureaucratic process and cumbersome administrative procedures in accessing credit facilities should be removed.

Keywords: Risk Management, Crop Insurance, Maize Farmers, Kaduna State, Nigeria

INTRODUCTION

Agricultural production is a risky business, and maize farming is faced or characterized with risk and uncertainties such as unforeseen weather conditions, drought, fire outbreak, flood, pests, disease infestations, theft, injury, changes in government policies and market conditions which cause variations in commodity output prices and yields (Yanuarti *et al*., 2019). Smallholder farmers

faces many risks in their maize farming business which makes their income unpredictable and unstable from year to year. The major risks in agricultural production include: marketing risk, production risk, financial risk, human risk, and institutional risk (Aminu *et al*., 2019). Risk in agriculture has negative effects on market stability, farmers' income, food security, and can lead to long term poverty (Akinola, 2014). Smallholder or resource poor or peasant farmers are risk averse, risk averse maize farmers are less willing to take investment and activities that have higher expected outcomes with associated risk of failure. Risk averse maize farmers were those trying to avoid taking risk, risk preferring maize farmers were those open to risky options, while risk neutral maize farmers were indifferent to risky options. Resource poor farmers naturally avoid taking risk that might threaten their livelihood, they avoid investment that involve risks which are capable of increasing output (Oparinde *et al*., 2018). In developing or sub-Saharan countries, smallholder or resource poor or peasant farmers are not willing to adopt new innovations or technologies even when these new modern technologies have higher returns to labour and land than traditional technologies. Smallholder maize farmers make difficult investment decisions on fertilizers, labour, repairs and equipment's during the production cycle, even when he does not know whether he will be able to pay back the loan obtained. Maize farmers differ in their various ways they make investment decisions under risk and uncertainties and this differences defined the differentials in their risk attitudes. Risk management strategies involves choosing among available alternative strategies with the aim of decreasing the impact of risks. Risk management strategies vary from farm to farm and it involves the use of risk assessment technique to evaluate the degree of risk, and to develop strategies to ameliorate the risk and minimize the extent of risk to acceptable level (Obike *et al*., 2017). The farmers' behaviors are critical for proper risk management in agricultural production. Appropriate risk perception is necessary for choosing an effective risk coping strategies, maize producers that is not aware of the risk faced cannot manage the risk effectively. It is important to understand risk information at the local farm level before developing an effective policy to assist farmers. Crop insurance is a risk management tool for maize farmers to mitigate against climate and adverse natural hazards (Ngango *et al.,* 2022; Ellis, 2017). Maize farmers lack basic knowledge about crop insurance and have difficulties in obtaining information on weather, which bring about low outreach and uptake of crop insurance in sub-Saharan Africa. Crop insurance help to insure maize farmers against any losses due to drought, theft, fire outbreak, outbreak of pest, disease infestations, and natural catastrophe. It is designed to provide cover for financial loss incurred by farmers due to reduction in expected maize output. When loss occur in agricultural production, the insurer pays the policy holder a certain amount of money known as premium to secure his life and property (Gbigbi and Ndubuokwu, 2022). The high premium payments created barrier for the maize farmers not to register for insurance, in developed countries, government subsidize premium and makes insurance coverage more attractive (Nwosu *et al*., 2012). Effective and sustainable risk management strategies for maize producers requires coordination at three different levels which involves the state, farm and markets (Sulewski and Kloczko-Gajewska, 2014). The unsatisfactory image of the insurance industry regarding low compensations, low income of maize farmers, poverty, small farm holdings, and burden of payments of premium were factors impeding willingness of maize farmers to register for insurance coverage.

Maize (*Zea mays*) ranks third after sorghum and millet as the most significant and important cereal in Nigeria. Maize can be used for food for man, for livestock feed and as resource input or raw materials for industries (Alabi and Abdulazeez, 2018). Maize is an essential material for the industrial production of fuel, starch, medicine, and food sweeteners (Egwuma *et al*, 2019; Amanza *et al.*, 2021). Nigeria produces 10 million metric tonnes of maize in 2020 and 11.6 million metric tonnes of maize in 2021, this is about 16% increase over the previous year 2020 (USDA, 2021). Maize is a source of income for smallholder farmers and also source of foreign exchange earnings for sub-Saharan or developing and developed countries. In Nigeria, maize is used by brewing industries for producing various types of beer, production of maize flour by milling industries, corn flakes and confectionary for human consumption. Maize is a good source of minerals, protein, carbohydrates, iron, and Vitamin B.

Objectives of the Study

This study focused on evaluation of maize farmers' attitude towards risk management and preference for crop insurance in Nigeria. Specifically, the objectives were:

- determine the attitudes of maize farmers towards risk and uncertainty,
- evaluate factors influencing risk attitudes of maize farmers,
- evaluate factors influencing preference for crop insurance policy by maize farmers,
- determine the risk management strategies, and crop insurance policy adopted by maize farmers, and
- examine the constraints facing maize farmers in the area of study.

METHODOLOGY

This study was carried out in Kaduna State, Nigeria. Kaduna State occupies between Longitudes 06^0 15| and 08^0 50| East and Latitudes 09^0 02| and 09^0 02|North of the equator. The State has land area totaling 4.5 million hectares. The state vegetation is divided into two (2), the

Southern guinea savanna and Northern guinea savanna. There are 2 seasons in Kaduna State. The seasons are: dry and wet seasons, the dry season is between October to March, and the wet season happens to starts from April to October, in between the dry and wet seasons is the brief harmattan period which span from November to February. The mean or average rainfall is about 1,482mm, the temperature of Kaduna State ranges from 35^{0}C to 36^{0}C, which can be as low as 10^{0}C to 23^{0}C during the harmattan period. The population of Kaduna as at 2021 was 8.9 million people. They are involved in agricultural activities. Crops grown include: tomatoes, okra, pepper, maize, ginger, sorghum, rice, yam, cassava, and millet. Animal reared include: cattle, goats, sheep, rabbit, and poultry. Multi-stage method of sampling was used. One hundred (100) maize producers were selected. Data obtained from maize producers were of primary sources and the data were collected using well-designed and also well-structured questionnaire. The questionnaire was administered to maize producers using well trained enumerators. Data were analyzed using the following statistical and econometrics tools:

Cobb-Douglas Production Function Model

The model is stated thus:

$$Log\ Y = \alpha_0 + \alpha_1 Log\ X_1 + \alpha_2 Log\ X_2 + \alpha_3 Log\ X_3 + \alpha_4 Log\ X_4 + \alpha_5 Log\ X_5 + \alpha_6 Log\ X_6 + U_i \text{.....(1)}$$

Y = Yield of Maize in Kg,

X_1 = Age of Farmers in Years,

X_2 = Farm Size in Hectares,

X_3 = Labour Input in Mandays,

X_4 = Chemical Input in Litres

X_5 = Seed Input in Kg

X_6 = Fertilizer Input in Kg

U_i = Error Term,

$\alpha_1 - \alpha_6$ = Regression Coefficients,

The input price, output price, elasticity of production of the input of interest, and coefficient of variation of maize yields were used to estimate the value of risk parameter. This was used to specifically achieve objective one (i).

Risk Analytical Tool

The formula for calculating risk parameter is stated thus:

$$K(s) = \frac{1}{\theta}\left[1 - \frac{P_i X_i}{P f_i \mu y}\right] \text{... ... ... (2)}$$

Where,

K(s) = Risk Parameter

θ = Coefficient of Variation of Yield

Pi = Factor Price (Fertilizer Price per Kg)

Xi = Input Level of Interest (Fertilizer in Kg/ha)

μy = Mean Yield of Maize

fi = Elasticity of Production of the Input of Choice (Fertilizer Input)

P = Price of Output Maize/Kg

The coefficient of variation of maize yield was calculated as follows:

$$\theta = \frac{\sigma y}{\mu y} \text{... ... . (3)}$$

Where,

σy = Standard Deviation (Units)

μy = Mean Yield (Units)

Maize farmers can be classified as follows:

Risk Preferring = K<0

Risk Neutral = 0≤K<0.4

Risk Averse = 0.4≤K<2.0

This was used to specifically achieve objective one (i).

Multinomial Logit Regression Model

The general multinomial Logit model is stated thus:

$$\Pr(y_i = j) = \frac{\exp(X_i \beta_j)}{1 + \sum_{j=1}^{j} \exp(X_i \beta_j)} \text{... ... . (4)}$$

And to ensure identifiability,

$$\Pr(y_i = 0) = \frac{1}{1 + \sum_{j=1}^{j} \exp(X_i \beta_j)} \text{... ... . (5)}$$

$$Z = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$$

$$+\beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7$$

$$+\beta_8 X_8 + U_i \ldots\ldots\ldots\ldots (6)$$

Y=Dichotomous Response Model (1,Risk Prefering;2,Risk Averse;3,Risk Neutral),

X_1=Age of Maize Farmers in Years,

X_2=Farm Experience in Years,

X_3=Household Size (Units)

X_4=Gender (Dummy,1,Male;0,Otherwise)

X_5=Marital Status (Dummy,1,Married;0,Otherwise)

X_6=Level of Education(Likert,0,Non-Formal;1,Primary;2,Secondary;3,Tertiary)

X_7= Extension Contacts (Number)

X_8=Membership of Cooperative Organizations (Dummy,1,Member;0,Otherwise)

U_i=Error Term,

β_1-β_8=Regression Coefficients,

β_0=Constant Term,

This was used specifically to achieve objective two (ii).

Probit Dichotomous Regression Model

The dichotomous response model is defined as follows:

$$Y = \alpha_0 + \alpha_1 Z_1 + \alpha_2 Z_2 + \alpha_3 Z_3 + \alpha_4 Z_4 + \alpha_5 Z_5 + \alpha_6 Z_6 + \alpha_7 Z_7 + \alpha_8 Z_8 + U_i \ldots\ldots\ldots\ldots (7)$$

Y= Dichotomous Response Model (1,Preference for Crop Insurance Policy;0,Otherwise),

Z_1= Age of Maize Farmers in Years,

Z_2= Farm Size in Hectares,

Z_3= Household Size in Units

Z_4= Gender (Dummy,1,Male;0,Otherwise)

Z_5= Risk Aversion(Dummy,1 High;0,Otherwise)

Z_6= Level of Education(Likert,0,Non-Formal;1,Primary;2,Secondary;3,Tertiary)

Z_7= Access to Extension Services (Dummy,1,Access;0,Otherwise)

Z_8= Memberships of Cooperative Organization (Dummy,1,Member;0,Otherwise)

U_i= Error Term,

α_1-α_8= Regression Coefficients,

α_0= Constant Term,

This was used specifically to achieve objective three (iii).

Henry Garrett Index Ranking Technique

According to this technique, maize farmers were employed to specify the rank for all risk management strategies and crop insurance policy as factors and the results of the ranking were converted into appropriate score value. The percentage score is calculated as follows:

$$\text{Percentage Score} = \frac{100(R_{ij} - 0.5)}{N_j} \ldots\ldots\ldots\ldots (8)$$

Where,

R_{ij} = Rank i^{th} Item j^{th} Individual,

N_j = Number or Item Ranked by j^{th} Individual,

This was used specifically to achieve part of objective four (iv)

Principal Component Analysis

The constraints facing maize farmers was subjected to principal component analysis. This was used specifically to achieve objective five (v).

RESULTS AND DISCUSSION

Attitudes of Maize Farmers towards Risk and Uncertainties

The risk attitudes of maize farmers towards risk and uncertainty was evaluated and the results were presented in Table 1. About 51% of sampled respondents were risk averse maize farmers,28% were risk neutral, while 21% were risk preferring maize farmers. Risk averse maize farmers tried to avoid taking risk, risk preferring maize farmers were open to risky options, while risk neutral maize farmers were indifferent to risky options. Smallholder, smallscale, resource poor, peasant, farmers had low income thereby do not like taking risk. Risk ability are linked to financial ability of maize farmers to take loss or small gain, they can only involve in risky situations when the maize farmers had opportunity of making more profits.

Factors Influencing Risk Attitudes of Maize Farmers

The predictors influencing risk attitudes of maize farmers was examined using multinomial Logit model. The regressors under consideration in the multinomial Logit model were age of maize farmers, farm experience, gender, household size, education level, marital status, extension contacts, and member of cooperatives. The risk preferring maize farmers was used as reference group. The regression coefficients of gender, age, education level marital status, and membership of cooperatives of risk averse maize farmers were positive. This signifies that the probability of being risk averse maize farmers tend to increase with the positive signs of

the predictors. The statistically and significant predictors influencing risk averse attitudes of maize farmers were age, education level at (P< 0.05), and gender at (P < 0.01). The Log Likelihood (value of 96.241 was significant

Table 1. Attitudes of Maize Farmers towards Risk and Uncertainties

Risk Attitudes	Frequency	Percentage
Risk Preferring	21	21.00
Risk Neutral	28	29.00
Risk Averse	51	51.00
Total	**100.00**	**100.00**

Source: Data Computation (2021).

at (P < 0.01) and this confirmed that all the gradients of the coefficients from regression were statistically and significantly different from zero. The Pseudo-R^2 value of 0.5820 confirmed that the model is of good fit and all the gradients of the coefficients from regression were statistically and significantly from zero.

Factors Influencing Maize Farmers Preference for Crop Insurance

The results of Probit dichotomous regression model of factors influencing maize farmers'

preference for crop insurance was shown in Table 3. The predictors under consideration in the Probit regression dichotomous model were age, farm size, gender, risk aversion, level of education, access to agricultural extension services, and membership of cooperative organizations. The results show that farm size, household size, gender, risk aversion, level of education, access to agricultural extension services were statistically and significant regressors influencing preference for crop insurance policy by maize farmers at (P < 0.05). Age of maize farmers was statistically significant predictor influencing preference for crop insurance policy at (P < 0.01). The negative coefficient of age shows that the probability of preference for crop insurance policy decreases as maize farmers get older keeping all other regressors constant. This signifies that older maize farmers are more conservative and risk-averse than younger ones who are receptive to ideas and more innovative. The gender of maize farmers was positive and statistically significant at 5% probability level, male respondents had higher probability of preference for crop insurance policy compared to female counterparts because male farmers are decision makers among the farming households with regards to their access to resources and partaking in agricultural insurance projects. Risk aversion negatively affects the preference for crop insurance policy, this implies lack of trust in the credibility of the insurer and this is likely to affects their insurance preference. Diagnostic statistics showed that the Probit regression dichotomous model had a good fit with Wald chi-square test statistics that was statistically significant at 1% probability level. This shows the regressors variables were relevant in explaining the preference decisions. The diagnostics statistics of Pseudo R^2 value was 0.7213, this is another measure of goodness of fit of the model.

Risk Management Strategies and Crop Insurance Policy Adopted by Maize Farmers

Application of Garrett Index Ranking Technique

The risk management strategies and crop insurance policy adopted by maize farmers was examined by Garrett index ranking technique and was adequately shown in Table 4. Based on the ranks assigned by the maize farmers, the order of importance of risk management strategies and crop insurance policy was identified. To find the most significant factor influencing the maize farmers' preference for risk adaptation strategies and crop insurance policy, Garrett index ranking technique was employed. The technique was calculated as percentage score, and the scale value was estimated by employing Garrett scale conversion Table. The percentage score for

Table 2. Multinomial Logit Results of Factors Influencing Risk Attitudes of Maize Farmers

		Risk Averse		Risk Neutral		Risk Preferring
Factors	**Parameters**	**Coefficient**	**t-value**	**Coefficient**	**t-value**	**Coefficient**
Age of Maize Farmers (X_1)	β_1	0.1724**	2.91	-0.0032	-0.27	- 0.1702
Farm Experience (X_2)	β_2	-0.2410	-1.29	0.0218	0.02	0.0721
Household Size (X_3)	β_3	-0.1201	-1.45	0.0161	0.49	0.0923
Gender (X_4)	β_4	0.3421***	3.91	0.2980	0.42	-0.3198
Marital Status (X_5)	β_5	0.3930	0.79	-0.1287	-1.10	0.4120
Level of Education (X_6)	β_6	0.1923**	2.87	-0.2109	-0.65	-0.0642
Extension Contacts (X_7)	β_7	-0.2208	-1.21	-0.3108	-0.57	0.4509
Membership of Cooperative (X_8)	β_8	0.1132	1.09	0.0236	0.24	-0.4509
Constant	β_0	-3.2109**	-2.65	0.4035	0.34	5.5203
Log Likelihood = 96.241***						
Wald Chi Square=1092.24***						
Pseudo =0.5820						

Source: Data Analysis (2021). *Significant at (P<0.10)., **Significant at (P<0.05), ***Significant at (P<0.01).

each rank from 1 to 12 was estimated. The percentage score evaluated for all the twelve ranks were converted appropriately into scale values using Garrett scale conversion table. The estimated scale values for 1st rank to 12th ranks were 84, 73, 67, 61, 57, 52, 48, 44, 40, 34, 27, and 17 respectively. The *(fx)* (score value) was evaluated for each factor appropriately by multiplying the obtained number of respondents (*f*) with respective calculated scale values (*x*). The total scores were found by adding the score values (*fx*) of each rank for every factor. The mean score was than estimated to know the appropriate order of importance or preference given by the maize farmers for the factors. In Table 4, it is clear that maize farmers were giving more importance to crop diversification (58.67), followed by weather information (53.95), crop insurance (52.29), off-farm activities (51.69), and the least

Table 3. Results of Probit Dichotomous Regression Model of Factors Influencing Maize Farmers Preference for Crop Insurance Policy

Factors	Parameters	Coefficients	P-value
Age (Z_1)	α_1	-0.035***	-0.001
Farm Size ($Z_{2)}$	α_2	0.231**	0.041
Household Size (Z_3)	α_3	-0.142**	-0.032
Gender (Z_4)	α_4	0.301**	0.039
Risk Aversion (Z_5)	α_5	-0.290**	-0.028
Level of Education (Z_6)	α_6	0.430**	0.033
Access to Extension Services (Z_7)	α_7	0.329**	0.021
Memberships of Cooperative Organization (Z_8)	α_8	0.371	0.481
Constant	α_0	0.261	0.642
Wald Chi^2	67.21***		
Pseudo R^2	0.7213		
Prob > Chi^2	0.0000		
Number of Observations	100		

Source: Data Analysis (2021) *Significant at (P<0.10)., **Significant at (P<0.05), ***Significant at (P<0.01).

Table 4. Garrett Index Ranking Technique of Risk Management Strategies and Crop Insurance Policy Adopted by Maize Farmers

	Rank Given by Maize Farmers														
Strategies/ Insurance Policy	**1st 84**	**2nd 73**	**3rd 67**	**4th 61**	**5th 57**	**6th 52**	**7th 48**	**8th 44**	**9th 40**	**10th 34**	**11th 27**	**12th 17**	**Total**	**Mean**	**Rank**
Crop Diversification	12 (1008)	16 (1168)	10 (670)	15 (915)	7 (399)	8 (416)	10 (480)	3 (132)	4 (160)	9 (306)	6 (162)	3 (51)	**5867**	**58.67**	**1st**
Wealth Savings	8 (672)	7 (517)	6 (402)	7 (427)	4 (228)	11 (572)	8 (384)	12 (528)	8 (320)	7 (238)	10 (270)	12 (204)	**4762**	**47.62**	**10th**
Loan Facilities	6 (504)	7 (511)	10 (670)	9 (549)	10 (570)	8 (416)	7 (336)	9 (396)	8 (320)	8 (272)	9 (243)	9 (153)	**4940**	**49.40**	**7th**
Extension Services	6 (504)	7 (511)	9 (603)	11 (671)	8 (456)	11 (572)	7 (336)	4 (176)	8 (320)	8 (272)	10 (270)	11 (187)	**4878**	**48.78**	**8th**
Selling Assets	8 (420)	9 (657)	7 (469)	4 (244)	8 (456)	11 (572)	6 (288)	7 (308)	11 (440)	9 (306)	5 (135)	12 (204)	**4499**	**44.99**	**11th**
Crop Insurance	7 (588)	13 (949)	11 (737)	15 (915)	5 (285)	7 (364)	8 (384)	4 (176)	10 (400)	3 (102)	4 (108)	13 (221)	**5229**	**52.29**	**3rd**
Cooperative Societies	6 (420)	9 (657)	13 (871)	6 (366)	4 (228)	12 (624)	7 (336)	10 (440)	7 (280)	11 (374)	5 (135)	10 (170)	**4901**	**49.01**	**8th**
Insurance Awareness	11 (924)	7 (511)	9 (603)	10 (610)	9 (513)	6 (312)	8 (384)	5 (220)	9 (360)	4 (136)	12 (324)	10 (170)	**5067**	**50.67**	**5th**
Off-Farm Activities	13 (1092)	8 (584)	6 (402)	7 (427)	9 (513)	3 (156)	12 (576)	14 (616)	5 (200)	6 (204)	11 (297)	6 (102)	**5169**	**51.69**	**4th**
Use of Agrochemicals	7 (588)	9 (657)	10 (670)	14 (854)	12 (684)	6 (312)	4 (192)	5 (220)	3 (120)	10 (340)	9 (243)	11 (187)	**5067**	**50.67**	**5th**
Use of Resistant Varieties	8 (672)	9 (657)	5 (335)	6 (366)	7 (399)	9 (468)	6 (288)	11 (484)	13 (520)	9 (306)	7 (189)	10 (170)	**4854**	**48.54**	**9th**
Weather Information	9 (756)	12 (876)	11 (737)	9 (549)	8 (456)	7 (364)	6 (288)	10 (440)	11 (440)	10 (340)	3 (81)	4 (68)	**5395**	**53.95**	**2nd**

Source: Data Computation (2021). fx = Figures in Brackets

importance is given by selling assets (44.99).

Constraints Faced by Maize Farmers: Application of Principal Component Model or Factor Analysis

The constraints faced by maize farmers was subjected to principal component analysis or factor analysis and the result was presented in Table 5. Lack of extension services was ranked 1st with Eigen-value of 1.9834 and this explained 19.34% of all constraints included in the principal component model. Lack of credit facilities was ranked 2nd with Eigen-value of 1.8210 and this explained 15.36% of all constraints included in the model. All the constraints included in the model explained 74.85% of all constraints included in the model. The chi-square value of 3051.39 was significant at 1% probability level. This signifies that the model is of good fit.

Table 5. Principal Component Analysis of Constraints Faced by Maize Farmers

Constraints	Eigen-Value	Difference	Proportion	Cumulative
Lack of Extension Services	1.9834	0.3425	0.1934	0.1934
Lack of Credit Facilities	1.8210	0.2756	0.1536	0.3470
Inadequate Knowledge of Agricultural Insurance	1.7521	0.2664	0.1435	0.4905
High Premium of Agricultural Insurance	1.6342	0.2338	0.1373	0.6278
Lack of Fertilizers	1.5362	0.2201	0.1207	0.7485
Bartlett Test of Sphericity				
KMO	0.723			
Chi Square	3051.39***			
Rho	1.000000			

Source: Computed from Data Analysis (2021). ***-Significant at 1% probability level

facing maize producers include: lack of extension services (1st), lack of credit facilities (2nd), inadequate knowledge of agricultural insurance (3rd), high premium of agricultural insurance (4th) and lack of fertilizers (5th). This research work provides the following recommendations:

- Extension officers should be employed to teach and disseminate research results, new ideas and innovations on risk management strategies and crop insurance among others to maize farmers.
- Credit facilities at low interest rate should be given to maize farmers, and bureaucracy and
- cumbersome administrative procedures in accessing the credit facilities should be removed.
- Fertilizers input, improved seeds input, resistance varieties and agrochemicals should be provided for the maize producers.
- Weather information, risk and insurance awareness should be provided for the maize producers.

CONCLUSION AND RECOMMENDATIONS

This study has basically established that the attitudes of maize farmers to risk and uncertainties can be categorized into risk preferring, risk neutral and risk averse. Risk averse maize farmers tried to avoid taking risk, risk neutral were those that were indifferent to risky options, while risk preferring were maize farmers open to risky options. Age, gender, and level of education were the statistically and significant predictors influencing risk averse maize farmers. Age, farm size, household size, gender, risk aversion, level of education, and access to extension services were the statistically and significant predictors influencing maize farmers' preference for crop insurance policy. Garrett index ranking technique revealed that crop diversification, weather information, and crop insurance were ranked 1st, 2nd, and 3rd among the risk management strategies and crop insurance policy employed by maize farmers respectively. The remaining risk management strategies and crop insurance policy examined by Garrett index ranking technique include: off-farm activities (4th), insurance awareness (5th), use of agrochemicals (5th), loan facilities (7th), cooperative societies (8th), use of resistance varieties (9th), wealth savings (10th), and selling assets(11th). The constraints

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

Authors do not declare any conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are
original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Akinola, B.D (2014). Determinants of Farmers' Adoption of Agricultural Insurance: The Case of Poultry Farmers in Abeokuta of Ogun State, Nigeria, *British Journal of Poultry Sciences,* 3 (2): 36 – 41. http://doi.org/10.5829/idosi.bjps.2014.3.2.83216

Alabi,O.O. and Abdulazeez, I. (2018). Economics of Maize (*Zea mays*) Production in Igabi Local Government Area, Kaduna State, Nigeria. *Journal of Agricultural Faculty of Gaziosmanpasa University* 35 (3): 248 – 257. http://doi.org/10.13002/jafag4434

Amanza,P., Mailumo, S., and Silong, A (2021). The Political Economy of the Maize Value Chain in Nigeria, Agricultural Policy Research in Africa (APRA) Working Paper 60, Brighton: Future Agricultures Consortium 1 – 56.

Aminu, F.O., Balogun, E.O.S and Oke, O.B (2019). Farm Risk and Management Strategies among Arable Crop Farmers in Odogbolu Local Government Area of Ogun State, Nigeria. *Agrosearch* 19(2): 41 – 53. http://doi.org/10.4314/agrosh.v192.4

Obike,K.C., Amusa, T.A., and Olowolafe,H.B (2017). Risk Management and Determinants of Farm Output among Smallscale Poultry Farmers in Ekiti State, Nigeria. *Journal of Tropical Agriculture, Food, Environment and Extension* 16(2): 9 – 16. http://doi.org/10.4314/as.v16i2.2

Ellis, E (2017). Farmers' Willingness to Pay for Crop Insurance: Evidence from Eastern Ghana. *International Journal of Agricultural Management and Development (IJAMAD)*, 7(4): 447– 463. http://doi.org/10.22004/ag.econ.292508

Egwuma, H., Dutse, F., Oladimeji, Y.U., Ojeleye, O.A., Ugbabe, O.O. and, Ahmed, M.A (2019). Demand and Supply Estimation of Maize in Niger State, Nigeria. *Journal of Agriculture and Agricultural Technology* 5(2): 12 – 20.

Ggigbi, T.M and Ndubuokwu, G.O (2022). Determinants of Agricultural Insurance Patronage among Crop Farmers in Delta North Agricultural Zone, Delta State, Nigeria. *Ege Univ Ziraat Fak. Derg.,* 59 (2): 235 – 248. http://doi.org/10.20289/zfgergi.883004

Ngango, J., Nkurunziza, F.,and Ndagijimana,J (2022). Assessing Rural Farmers' Willingness to Pay for Crop Insurance Scheme: Evidence from Rwanda. *Cogent Economics and Finance* 10 (1): 1 – 14 . http://doi.org/10.1080/23322039.2022.2104780

Nwosu, F.O., Oguoma, N.N.O., Lemchi, J.I., Ben-Chendo, G.O., Henri-Ukoha, A., Onyeagocha, S.U.O., and Ibeawuchi, I.I (2012). Output Performance of Food Crop Farmers under the Nigerian Agricultural Insurance Scheme in Imo State, South East Nigeria. *Academia Arena* 2(6): 43 – 47.

Oparinde, L.O., Amos, T.T., Aturamu, O.A.,and Daramola, G.A (2018). Attitudes towards Risk and Risk Combatting Strategies among Maize and Cassava Farmers in Southwest, Nigeria. *Journal of Economics, Management and Trade 21(7): 1 – 12. http://doi:10.97734/JEMT/2018/40755*

Sulewski,P and Kloczko-Gajewska,A (2014). Farmers' Risk Perception, Risk Aversion and Strategies to Cope with Production Risk: An Empirical Study from Poland. *Studies in Agricultural Economics* 116: 140 – 147. http://doi.org/10.7896/j.1414

USDA (2021). Nigeria's Maize Production at Highest Level Since Independence by United States Department of Agriculture, Premium Times March 12, 2022.

Yanuarti, R., Aji, M.M.,and Rondhi, M (2019). Risk Aversion Level Influence on Farmers' Decision to Participate in Crop Insurance: A Review. *Agricultural Economics-Czech* 65 : 481 – 489. http://doi.org/10.17221/93/2019.AGRICECON

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 70-78
DOI: https://doi.org/10.31015/jaefs.2023.1.8

The impact of hematite on the anaerobic digestion of cattle manure

Yasin Odabas[1] • **Yasemin Dilsad Yilmazel**[1]

[1] Department of Environmental Engineering, Faculty of Engineering, Middle East Technical University, Ankara, Türkiye

Abstract

A metal-based conductive material, hematite (Fe_2O_3), was used as an amendment in the anaerobic digestion process to determine the effects on the performance of anaerobic digestion of cattle manure (CM) at mesophilic temperature (35 °C). The first set of experiments (Set 1) was designed to assess whether there is a need to supplement nutrients for the effective digestion of CM. To this purpose, basal medium (BM) composed of macro nutrients, micro nutrients, reducing agent, and buffer was added to the reactors and a biochemical methane production assay was conducted. The presence of BM showed negative impacts on the anaerobic digestion of CM and its absence caused up to 40% higher methane production yield. In Set 2 experiments, the impact of hematite addition on methane production performance was determined. Two different dosages as 20 mM Fe (Fe20) and 50 mM Fe (Fe50) were applied to the batch reactors. Hematite amendments increased methane yield; at Fe20 (131 ± 2.6 mL CH_4/g VS_{added}) the increase was around 8% and at Fe50 (135 ± mL 0.2 CH_4/g VS_{added}) the increase was around 12% as compared to the control. Further, up to 36% increase in the methane production rate was calculated via Modified Gompertz fitting.

Keywords: Anaerobic digestion, Cattle manure, Methane production, Conductive materials, Hematite

Citation: Odabas, Y., Yilmazel, Y.D. (2023). The impact of hematite on the anaerobic digestion of cattle manure. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 70-78

Received: 11 December 2022
Revised: 05 February 2023
Accepted: 09 February 2023
Published Online: 21 February 2023

Correspondence: Yasemin Dilsad Yilmazel
E-mail: dilsad@metu.edu.tr

INTRODUCTION

Conventional anaerobic digestion is an effective technology for the treatment of organic wastes and bioenergy production in the form of biogas (Anukam et al., 2019). During anaerobic digestion, biogas containing 60-70% methane (CH_4) and 30-40% carbon dioxide (CO_2) can be produced (Speece, 1983). Different feedstocks such as animal manure and wastewater treatment plant sludge may be used in anaerobic digestion for methane production. Especially, cattle manure due to its high organic content, and high level of microbial activity has been commonly preferred as a feed in anaerobic digestion (Zheng et al., 2015). Handling of cattle manure via digestion can also decrease its adverse environmental impacts. Although anaerobic digestion is a well-known and effective technology for organic waste disposal and simultaneous renewable energy production, it has some limitations. These limitations can be counted as low methane production rate due to slow reaction kinetics, high sensitivity to inhibitory compounds such as ammonia, volatile fatty acids (VFAs), and unstable operations with changing conditions due to accumulation of VFAs (Park et al., 2018; Yin et al., 2020). The slow processing of wastes is a result of little energy

gained by anaerobic microbes during process, and the slow growth rate of the microorganisms involved in the process (Yin et al., 2020). These drawbacks are important for effective process operation, and they should be properly managed.

During anaerobic digestion, production of methane occurs via a series of reactions; hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Park et al., 2018). Hydrolytic bacteria, acidogenic bacteria, and acetogenic bacteria are responsible for hydrolysis, acidogenesis and acetogenesis. Methanogenic archaea, on the other hand, are responsible for methanogenesis step (Kumar et al., 2021). The syntrophic interactions between bacteria and methanogens are the key to effective process performance and this interaction is based on electron transfer between different microbial communities (Park et al., 2018). Recent studies suggest that bacteria and methanogenic archaea perform direct interspecies electron transfer (DIET) via the use of conductive materials, leading to higher efficiency digestion process (Kutlar et al., 2022; Liu et al., 2012). Studies showed that DIET via conductive materials enables faster electron transfer than electron transfer via intermediates such as acetate and hydrogen as in conventional systems (He et al., 2021). This in turn is related to the faster utilization of feed and enhanced process performance. Further, amendment of conductive materials may lower the impact of inhibitory compounds, and decrease the oxidation-reduction potential of the medium, hence offering a more suitable environment for methanogenic activity (Kutlar et al., 2022). Therefore, this study aims to investigate the effect of conductive material amendment on biomethane production from cattle manure. To this purpose, a metal-based conductive material, hematite (Fe_2O_3) was used in the experiments. The hematite-amended reactor performances were evaluated based on the comparison of lag time, biomethane production rate, and biomethane production yield with a control reactor.

MATERIALS AND METHODS

Waste and Inoculum Characteristics

Cattle manure was taken from the inlet of a full-scale biogas plant located in Ankara, Turkey. The sample was blended for 1 hour for homogenization and then characterization analysis was conducted (Table 1). The inoculum used in this study has been taken from a municipal wastewater treatment plant located in Eskisehir, Turkey. Due to the waiting period, for two different sets of experiments two different samples of inoculum and manure were collected and used in the experiments.

Basal Medium and Conductive Material

To provide nutrients and other necessary compounds such as reducing agents a cocktail named basal medium (BM) was prepared. The composition of BM used in the experiments is as follows (concentrations are given in parenthesis as mg/L): NH_4Cl (1200), $MgSO_4.7H_2O$ (400), KCl (400), $Na_2S.9H_2O$ (300), $CaCl_2.2H_2O$ (50), $(NH_4)_2HPO_4$ (80), $FeCl_2.4H_2O$ (40), $CoCl_2.6H_2O$ (10), KI (10), $MnCl_2.4H_2O$ (0.5), $CuCl_2.2H_2O$ (0.5), $ZnCl_2$ (0.5), $AlCl_3.6H_2O$ (0.5), $NaMoO_4.2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2.6H_2O$ (0.5), $Na_2WO_4.2H_2O$ (0.54), Na_2SeO_3 (0.5), cysteine (10), $NaHCO_3$ (6000) (Demirer et al., 2000). In this cocktail, cysteine acts as a reducing agent, and bicarbonate solution works as a buffer, while the others provide the macronutrients and micronutrients.

In the experiments, hematite (Fe_2O_3) was used as a metal-based conductive material and was used at two different concentrations as 20 mM Fe and 50 mM Fe. The particle size distribution of the hematite sample was determined through sieving and given in Figure 1. The particle size was mostly around 2.5 mm. Before use, the solids content of the hematite sample was measured and the results are as follows: 99.2 ± 0.0% of TS, 0.4 ± 0.0 VS corresponding to 0.4% of VS/TS.

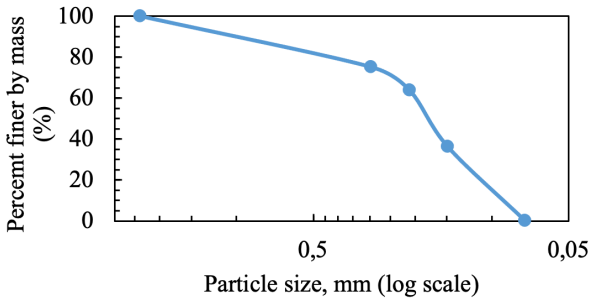


Figure 1. Particle Size Distribution of Hematite used in the Experiments.

Analytical Methods

For TS (Method 2540B), VS (Method 2540E), and chemical oxygen demand (COD) standard methods were used (Standard Methods for the Examination of Water and Wastewater, 1999). For COD measurements of manure sample open reflux method (Method 5220 B) was used and for inoculum closed reflux method (Method 5220C) was used (Standard Methods for the Examination of Water and Wastewater, 1999). For nitrogen (Hach Method 8038) and phosphorus (Hach Method 8178) measurements, colorimetric methods were used via a spectrophotometer (Hach DR9200, USA). pH measurements were conducted with a pH meter (St300, OHAUS, USA). Biogas production in the reactors was measured periodically during the operation via a liquid displacement device. The composition of biogas produced in the reactors was determined by a gas chromatography device (Trace GC Ultra, Thermo Scientific) equipped with a thermal conductivity detector (TCD) and columns connected series in (CP-Moliseve 5A and CP-Porabond Q). The temperature of the oven, injector and detector were

Table 1. Waste and inoculum characteristics.

Parameter	Set 1		Set 2	
	Cattle manure	Inoculum	Cattle manure	Inoculum
Density, (g/mL)	0.997	0.997	0.881	0.974
pH	7.8	7.6	7.8	7.5
Total solids (TS), (%)	12.2± 0.1	4.7 ± 0.0	12.2 ± 0.1	3.3 ± 0.0
Volatile Solids (VS), (%)	9.2 ± 0.1	1.8 ± 0.0	9.5 ± 0.1	1.8 ± 0.0
VS/TS (%)	76.0 ± 0.7	39 ± 0.5	77.6 ± 0.0	52.7 ± 0.4
Chemical oxygen demand (COD), (mg/L)	88,000± 11,000	30,500 ± 1,050	151,743 ± 6,446	30,027 ± 610
Nitrogen, (mg NH_4-N/L)	4,380 ± 390	nd	1,897 ± 116.8	nd
Phosphorus, (mg PO_4-P/L)	nd	nd	35.3 ± 1.6	nd

nd: not determined

35 ^{0}C, 50 ^{0}C, and 80 ^{0}C, respectively. The carrier gas was helium at a constant pressure of 75 kPa.

Daily produced methane was calculated from methane content and total produced biogas (Filer et al., 2019) using the following Equation (1):

$$V_{(CH4)} = \left(\frac{\%\,CH4,t}{100} * Vbiogas + \frac{\%CH4,t - \%CH4,t-1}{100}\right) * Vheadspace \quad (1)$$

where V_{CH4}, $V_{biogas,}$ and $V_{headspace}$ represent daily produced methane volume (mL), daily produced total gas (mL), and volume of reactor headspace (mL), respectively. $\%CH_{4,t}$ and $\%CH_{4,t-1}$ are the methane percentages of total biogas production on the corresponding day and the previous day, respectively.

Experimental Design

There were two sets in this study. In the first set, our objectives were two-fold: to determine (i) the impact of BM on the anaerobic digestibility of cattle manure, and (ii) the effect of initial COD on digestion performance. Two different initial COD concentrations representing higher (~ 30,000 mg/L) and lower (~20,000 mg/L) COD concentrations were adjusted. After setting up the reactors initial CODs were measured and in the reactors with higher COD, the COD concentrations ranged between 30,240 mg COD/L and 31,240 mg COD/L, and in the reactors with lower COD, the initial COD concentrations ranged between 21,760 mg COD/L and 22,240 mg COD/L. These additions established different food-to-microorganisms (F/M) ratios (in mg/L VS basis) in the reactors. F/M ratios of HCOD reactors were around 1.9 and LCOD reactors were around 1. The experimental design for Set 1 is shown in Table 2.

When needed, 10 mL of BM was added into the reactors according to the experimental design to determine the effect of BM on AD performance. Blank reactors having only inoculum in the absence of BM (B1) and the presence of BM (B2) were also operated to find out the background methane production from the inoculum.

In the second set, we run experiments to investigate the effect of hematite amendment on the performance of anaerobic digestion without any addition of BM. Two different dosages of hematite; 20 mM Fe containing hematite (Fe20) and 50 mM Fe containing hematite (Fe50) were used in the experiments. The experimental design of Set 2 is given in Table 3. For the determination of the performance of anaerobic digestion of cattle manure and comparing it with the hematite-amended reactors, we set the control without any hematite (AD). Also, we set a blank reactor (B) having only inoculum as another control to determine the methane production coming only from the inoculum.

Table 2. Experimental Design of Set 1.

Reactor	Substrate	BM	Initial COD (mg/L)	Initial VS (mg/L)	F/M ratio
B1 w/o BM	-	-	-	-	-
B2 w/ BM	-	+	-	-	-
AD1, HCOD w/o BM	+	-	30,240 ± 1,020	21,950 ± 317	1.9
AD2, HCOD w/ BM	+	+	31,200 ± 880	23,650 ± 450	1.9
AD3, LCOD w/o BM	+	-	21,760 ± 640	16,500 ± 167	1.0
AD4, LCOD w/ BM	+	+	22,240 ± 720	17,067 ± 567	1.0

HCOD: higher COD; LCOD: lower COD; BM: basal medium; F/M: food to microorganisms ratio

Table 3. Experimental Design of Set 2.

Reactor	Substrate	Hematite	Initial COD (mg/L)	Initial VS (mg/L)	F/M ratio
Blank	-	-	-	-	
Control	+	-	52,400 ± 640	32,066 ± 505	1.2
Fe20	+	+ (20 mM Fe)	49,200 ± 940	31,964 ± 209	1.2
Fe50	+	+ (50 mM Fe)	48,400 ± 820	31,580 ± 104	1.1

Reactor Operation

Set 1 experiments were conducted in 110 mL serum bottles with an active volume of 60 mL. All reactors were inoculated with 30 mL of AD seed. Then, 20 mL of cattle manure was added to the reactors. All reactors were covered with aluminum foil and incubated at 35±1 °C in the temperature-controlled room. In Set 1, all reactors were operated in triplicate without mixing.

In Set 2, the reactor volumes were increased slightly in comparison to Set 1. In Set 2, 300 mL serum borosilicate bottles were used with a working volume of 150 mL. The initial COD of the reactors was aimed to be around 50 g/L and initial COD measurements ranged between 48,400-52,400 mg COD/L with an F/M ratio of approximately 1. Similar to Set 1, all reactors were operated in triplicate. In Set 2, the reactors were mixed with a shaker at 150 rpm to prevent the settlement of hematite particles.

When reactors were filled according to the experimental design before the incubation, all reactors were sparged with 70% nitrogen (N_2) and 30% carbon dioxide (CO_2) for 3 mins to maintain anaerobic conditions. After sparging, the reactors were immediately sealed with rubber stoppers that are tied with plastic cable. For the removal of oxygen in the headspace and providing an anaerobic environment, the headspaces of the reactors were purged with the same gas for 2 mins.

During the incubation period, produced biogas amount and its composition were monitored periodically. When cumulative methane production as compared to previous measurement was less than 10% for two times in a row, the operation of Set 1 reactors was stopped. After the completion of the batch test, all reactors were stored at 4 °C until the final analysis of composition was complete. TS, VS, and COD analysis for the reactor effluents were conducted. For the comparison of the reactor performances, cumulative methane productions, methane yields (based on the amount of added VS), and organic removals were calculated. In Set 2, after the reactor operation, pH, conductivity, and ORP measurements of the effluents were conducted. Also, the final phosphorus and ammonium concentrations of the effluents were analyzed.

Modeling of Biomethane Production

Modified Gompertz fitting to cumulative methane production data was conducted for comparing the performances of hematite-added reactors and conventional AD in Set 2 (Zwietering et al., 1990). The kinetic parameters such as specific methane production potential (mL, B_t), maximum methane production potential (mL, B_0), methane production rate (mL CH_4/day, R_m), and lag time for the reactor (day, λ) were determined using the modified Gompertz model provided in Equation (2) (Zwietering et al., 1990):

$$\mathbf{B_{(t)}} = B_0 * \exp\{[\frac{Rm*e}{B0} * (\lambda - t) + 1]\}, t \geq 0 \qquad \mathbf{(2)}$$

In Equation (2), t is incubation time (day) and e is 2.718.

RESULTS AND DISCUSSION

Impact of Basal Medium

In terms of cumulative methane production, the highest production was observed in AD1 w/o BM (141 ± 5 mL CH_4) which is 25% higher than the production in AD2 w/ BM (113 ± 6 mL CH_4) among HCOD reactors (Figure 2). Similarly, for LCOD reactors, AD3 w/o BM (79 ± 8 mL CH_4) produced 39% higher cumulative methane than AD4 w/ BM (57 ± 4 mL CH_4). AD1 and AD2 reactors produced 78% and 98% higher methane as compared to AD3 and AD4, respectively. This is due to higher COD levels in AD1 and AD2 in comparison to AD3 and AD4.

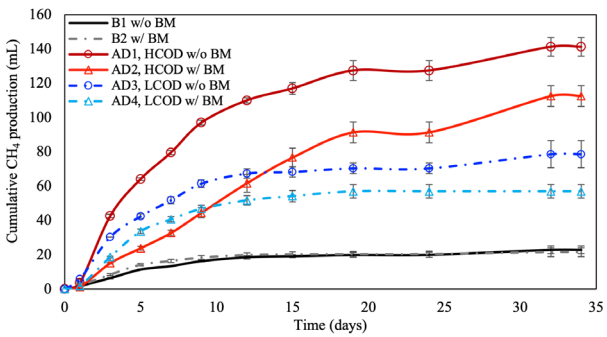


Figure 2. Cumulative Methane Production in Set 1 Reactors. (Error bars may be smaller than the symbols).

VS removals in the reactors were similar. VS removals were 34% and 37% for HCOD and LCOD reactors, respectively, without showing any significant change due to BM addition (Figure 3). Among HCOD reactors, COD removals were 44% and 40% in AD1 and AD2, respectively. For the reactors with LCOD, the removal of COD did not change with the addition of BM, and it was 36% for AD3 and AD4.

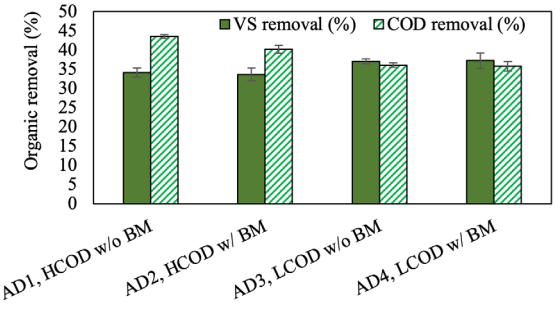


Figure 3. Organic Removal in Set 1 Reactors. (Error bars may be smaller than the lines).

Methane yields were also evaluated based on the added amount of VS and COD (Figure 4). HCOD reactors, AD1 (107 ± 4 mL CH_4/g VS_{added}) showed 35% higher yield as compared to AD2 (79 ± 4 mL CH_4/g VS_{added}). Similarly, for LCOD, the absence of BM in AD3 (79 mL ± 8 CH_4/g VS_{added}) resulted in 41% increase in methane yield over AD4 (56 ± 4 mL CH_4/g VS_{added}) with BM addition. In addition to this, the application of HCOD resulted in higher methane yield in AD1 and AD2 as compared to AD3 and AD4. 35% higher methane yield was observed in AD1 in comparison to AD3 which is due to higher organic content. When BM present reactors were compared a similar result was attained; 43% higher methane yield was obtained in AD2 as compared to AD4. Methane yields based on added COD had also similar trends. The absence of BM increased the yield 30% in AD1 (78 mL ± 3 CH_4/g COD_{added}) over AD2 (60 ± 3 mL CH_4/g COD_{added}) for HCOD reactors and 40% enhancement was observed in AD3 (60 ± 6 mL CH_4/g COD_{added}) without BM addition as compared to AD4 (43 ± 3 mL CH_4/g COD_{added}) with BM among LCOD reactors.

Methane yields suggested that the presence of BM on anaerobic digestion of cattle manure causes inhibition in both LCOD and HCOD reactors. This can be attributed to the nutrient content of cattle manure. Similarly, it was reported that the nutrient in cattle manure was already sufficient for anaerobic microbial growth without the need for an extra addition and BM addition resulted in lower performance (Güngör-Demirci & Demirer, 2004). In other words, since available nutrients in cattle manure is sufficient for anaerobic digestion, the digestion is inhibited with the extra nutrient addition via BM. In terms of the amount of organic loading, the HCOD reactors showed higher methane productions and methane yields than the LCOD reactors, and this may result from an amount of higher available carbon source for microbial growth and methane production.

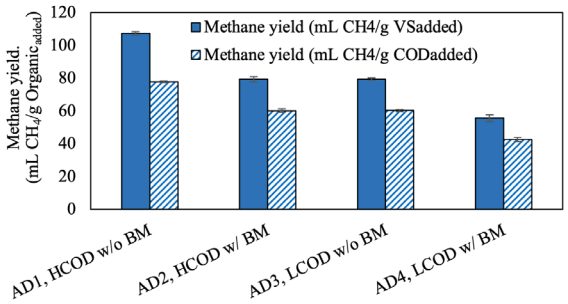


Figure 4. Methane Yield in Set 1 Reactors. (Error bars may be smaller than the lines).

As given in Table 4, a wide range (89-267 mL CH_4/g VS_{added}) in terms of methane yield of cattle manure digestion was reported in the literature. The methane yields attained in HCOD reactors of this study are within this range. There

Table 4. Comparison of Methane Yields with Other Studies where Cattle Manure was used as Feed.

Inoculum	T (°C)	Operation	Organic Load	Methane Yield* (mL/g VS_{added})	Reference
AD seed from WWTP	36	Batch	32,904 mg/L initial COD	266	(Huang et al., 2016)
Laboratory-scale anaerobic digester	37	Batch	6% initial TS	89	(Zheng et al., 2015)
Cattle manure anaerobic digester	37	Batch		150	(Song & Zhang, 2015)
Mixed anaerobic culture	35	Batch	a) 12,000 mg/L initial COD b) 53,500 mg/L initial COD	a) (155) b) (195)	(Güngör-Demirci & Demirer, 2004)
Dairy farm			7% initial TS	124	(Rosenberg & Kornelius, 2017)
Laboratory-scale anaerobic digester	35	Batch	15% initial VS	231	(Wei et al., 2019)
Digested slurry	35	Batch	15% initial TS	251 (210)	(Li et al., 2011)
AD seed from WWTP	35	Batch	a) 22,000 mg/L initial COD b) 30,720 mg/L initial COD	a) 79 (60) b) 107 (78)	This study

AD: anaerobic digester; WWTP: wastewater treatment plant
*Number in parenthesis show methane yield based on added COD: mL/g COD_{added}

can be several reasons for obtaining different methane yield in different studies, such as the lignin content of manure used, the initial organic loading, F/M ratio, and the use of different inoculums.

Impact of Hematite

The amendment of hematite slightly increased cumulative methane production in comparison to control reactor (Figure 5). There was a slight difference in the cumulative methane production of different dosage reactors (Fe20 and Fe50). Fe20 reactors produced a total of 626 ± 13 mL of CH_4 and Fe50 reactors produced 641 ± 1 mL of CH_4 corresponding to 7% and 10% increase over control reactor, respectively (Figure 5).

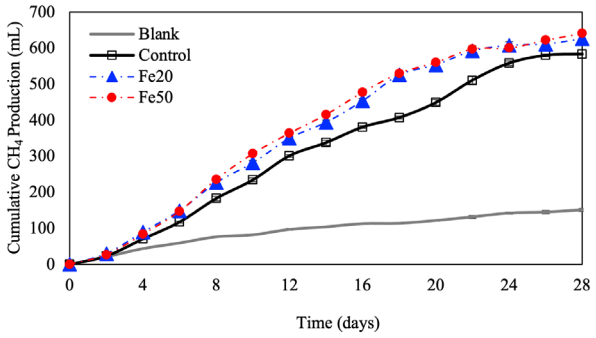


Figure 5. Cumulative Methane Production in Set 2 Reactors. (Error bars may be smaller than the symbols).

Through the addition of hematite, there was no significant change in VS or COD removal as compared to the control was not observed (Figure 6). This is similar to other studies in the literature. As reported in the literature, the amendment of hematite did not enhance the organic removal in anaerobic digestion of swine manure (Lu et al., 2019). A similar trend was observed with the application of another iron-based conductive material, magnetite (Fe_3O_4) (Yin et al., 2017). There was no significant change in organic removal via magnetite application over the control (Yin et al., 2017).

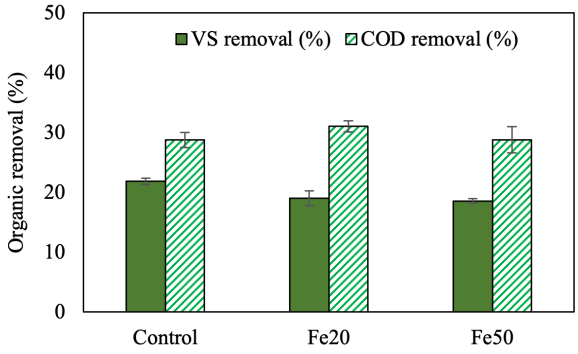


Figure 6. Organic Removal in Set 2 Reactors. (Error bars may be smaller than the lines).

Methane production yield based on added VS and added COD were also calculated (Figure 7). Fe20 (131 ± 2.6 mL CH_4/g VS_{added}) and Fe50 (135 ± mL 0.2 CH_4/g VS_{added}) enhanced methane yield by 8% and 12% as compared to the control, respectively (Figure 7). In another recent work, authors reported that the addition of hematite improved the methane production yield by 7% during anaerobic digestion of swine manure (Lu et al., 2019). Similarly, Ye et al., (2018) observed 36% increase in methane yield via hematite application on anaerobic digestion of activated sludge. In our work, although there is a 2.5 times difference in dosage values of Fe20 and Fe50, the enhancement in methane yield in Fe50 as compared to Fe20 was not as significant.

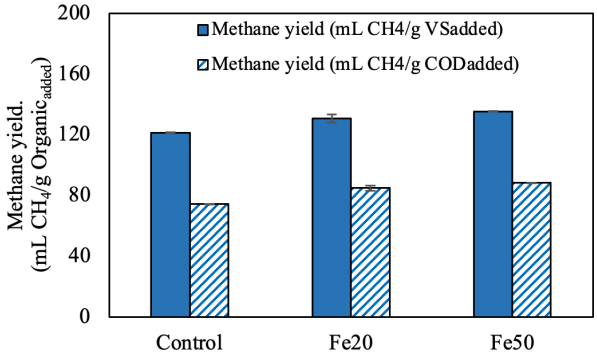


Figure 7. Methane Yields in Set 2 Reactors. (Error bars may be smaller than the lines).

For quantification of the change in the methane production rate and the lag time, modified Gompertz model was fitted to the cumulative methane production graphs of Set 2 reactors (Figure 8). Based on this fitting the increase in methane production rate (R_m) and the decrease in the lag time for the production of methane were calculated (Table 5). The application of hematite enhanced methane production rate as given in Table 5. Fe20 (35.9 ± 0.7 mL CH_4/day) and Fe50 (38.2 ± 0.6 mL CH_4/day) improved the rate 26% and 34% over the control, respectively (Table 5). Similarly, it was reported that the application of hematite enhanced methane production rate by 34% on AD of swine manure (Lu et al., 2019). On the other hand, there was no improvement in lag time with the application of hematite over the control.

Other studies using hematite for the enhancement of conventional anaerobic digestion are summarized in Table 6. There was only one study conducted with animal manure and the authors reported 11% increase in methane yield via hematite addition to AD reactors fed with swine manure (Lu et al.,2019). Comparison of enhancements in this study with others in the literature show that our results are consistent with the literature. Yet, it should be highlighted that in none of the studies cattle manure was used as a feed during hematite amendment. However, because of the presence of

different microbial communities in different complex wastes an experimental study is required to investigate the impact of conductive material amendment.

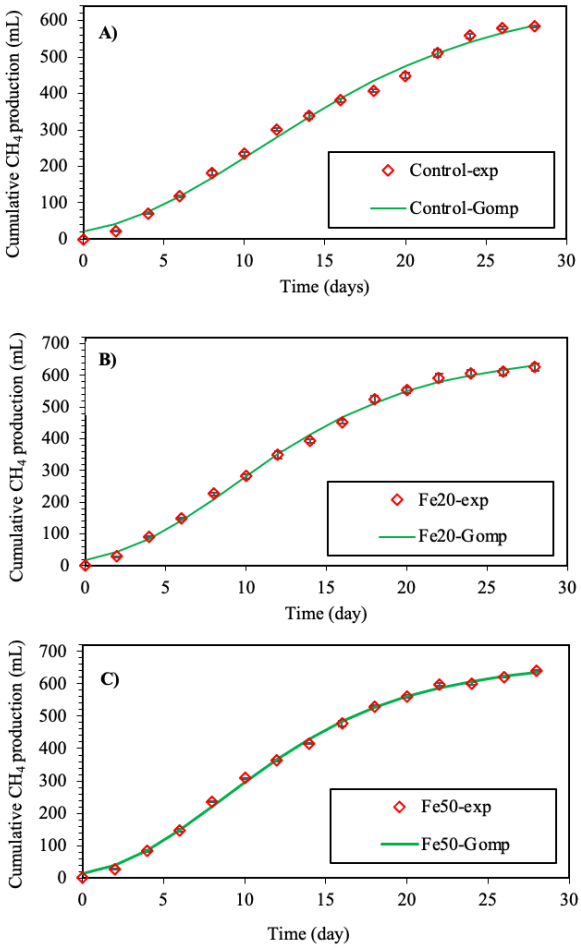


Figure 8. Cumulative Methane Production Curves of Set 2 Reactors fitted with Modified Gompertz.

Ammonia concentration is an important parameter for anaerobic digestion since the release of ammonia during protein degradation can cause inhibition of methanogenic activity (Rasapoor et al., 2020). In the literature, various ammonia concentrations were reported to cause inhibition in anaerobic digesters and this inhibitory level significantly depends on the feed and reactor conditions (Yenigün & Demirel, 2013). For example, it was reported that with unacclimated inoculum total ammonia concentrations of 1700 – 1800 mg/L were inhibitory, yet with acclimated inoculum when cattle manure was used as feed the inhibitory total ammonia concentration was raised to 6000 mg/L (Yenigün & Demirel, 2013). Nutrient (N and P) concentrations for each reactor of Set 2 were measured at the end of the operation (Figure 9). In Set 2 reactors ammonium nitrogen (NH_4-N) concentrations were around 1500 mg/L and pH in Set 2 reactors was measured as 7.8 in each reactor (Figure 10). pH level being below 8 when considered along with moderate NH_4-N concentrations it is concluded that there may only be a slight inhibition, if any.

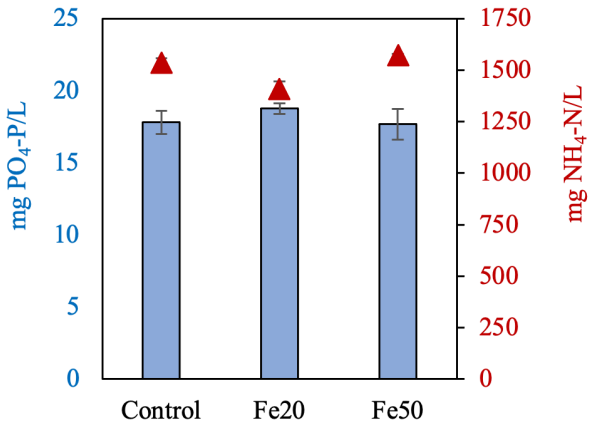


Figure 9. Nitrogen and Phosphorus Concentrations in Set 2 reactors. (Triangles show NH_4-N concentrations. Error bars may be smaller than the symbols).

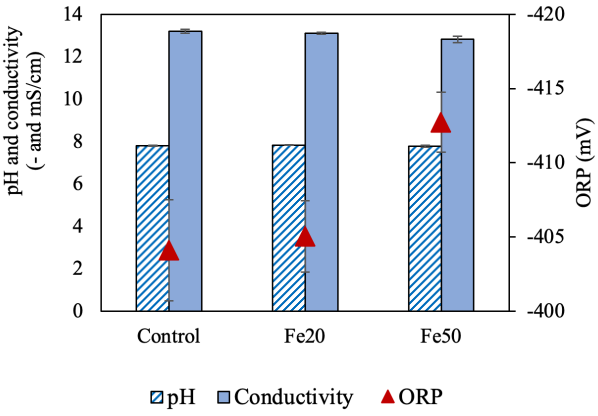


Figure 10. pH, conductivity and ORP of Set 2 reactors. (Error bars may be smaller than the lines).

The final pH, ORP, and conductivity of the reactors are shown in Figure 10. As reported in the literature, these

Table 5. Kinetic parameters calculated from the fitting with the modified Gompertz model in Set 2

Reactor	P (mL)	R_m (mL CH_4/day)*	λ (day)	R^2
Control	674.9 ± 6.8	28.5 ± 0.5	2.2 ± 0.1	0.9939
Fe20	674.2 ± 14.1	35.9 ± 0.7 (26%)	2.1 ± 0.1	0.9973
Fe50	666.3 ± 4.3	38.2 ± 0.6 (34%)	2.2 ± 0.1	0.9982

*The number in the parenthesis indicates the enhancement in methane production rate as compared to the control.

Table 6. Comparison of Enhancements in Different Studies where Hematite was applied during Anaerobic Digestion.

Dosage (mM Fe)	Reactor	Inoculum	Substrate	Impovements as compared to control	Reference
20	Batch	Paddy soil	Acetate	Acceleration of methanogenesis and decrease in lag time	Kato et al., 2011
25	Batch	Paddy soil	Acetate	110% increase in cumulative methane production	Zhou et al., 2013
25	Batch	Paddy soil	Benzoate	25% increase in the methane production rate	Zhuang et al., 2015
187.5 Batch Inoculum from swine farm Swine Manure 11% increase in methane yield					Lu et al., 2019
250	Batch	Laboratory-scale UASB reactor	Activated Sludge	36% increase in methane yield	Ye et al., 2018
a) 20 b) 50	Batch	AD seed from WWTP	Cattle Manure	a) 10% increase in methane yield 26% increase in methane production rate b) 12% increase in methane yield 34% increase in methane production rate	This study

UASB: upflow anaerobic sludge blanket; AD: anaerobic digester; WWTP: wastewater treatment plant

pH values are between the optimum pH range which is 7.0-8.0 (Uçkun Kiran et al., 2016). Final pH values were around 7.8, which is approximately the neutral pH range indicating no significant acid accumulation.

Conductivity has also been measured in the reactor effluents (solid bars, Figure 10). The addition of hematite did not significantly change the conductivity levels as compared to the control. We also conducted the final ORP measurement, which is an important parameter for the microbial activity of methanogens. Methanogenesis ideally occurs at ORP range of -200 mV to -400 mV (Martins et al., 2018). ORP values in the reactors having hematite were -405 ± 2 mV and -413± 2 mV in Fe20 and Fe50, respectively, which are very close to ORP value in the control (-404 ± 3 mV).

CONCLUSION

In this study, the impacts of supplementation of an all-inclusive nutrient cocktail, BM, and a metal-based conductive material, hematite, on methane production from cattle manure were investigated

- At VS concentrations around 20 g/L the supplementation of BM has adverse effects on anaerobic digestion of cattle manure. Methane production yield was 35 – 41 % higher in the absence of BM.
- Addition of hematite is beneficial during anaerobic digestion of cattle manure, and up to 12% increase in the methane production yield and around 34% increase in the methane production rate were attained via supplementation of hematite at a dosage of 50 mM Fe.

These results are promising for effective anaerobic digestion of cattle manure and may be used for increasing the performance of a full-scale biogas plants.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Yasin Odabas carried out experiments, data analysis and writing the original draft. Yasemin Dilsad Yilmazel designed the study, supervised, wrote, reviewed and edited the original draft. All authors read and approved the final manuscript. All authors verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

Partial funding received from the Scientific and Technological Research Council of Turkey (TUBITAK) (Grant no: 218M854) and Bilim Akademisi for the BAGEP award.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

The authors thank the Science Academy for BAGEP award.

REFERENCES

Anukam, A., Mohammadi, A., Naqvi, M., & Granström, K. (2019). A review of the chemistry of anaerobic digestion: Methods of accelerating and optimizing process efficiency. Processes, 7 (8), 1–19. https://doi.org/10.3390/PR7080504

Demirer, G. N., Duran, M., Ergüder, T. H., Güven, E., Ugurlu, Ö., & Tezel, U. (2000). Anaerobic treatability and biogas production potential studies of different agro-industrial wastewaters in Turkey. Biodegradation, 11, 401–405. https://doi.org/10.1023/A:1011659705369

Filer, J., Ding, H. H., & Chang, S. (2019). Biochemical methane potential (BMP) assay method for anaerobic digestion research. Water, 11(5), 921, 1-29. https://doi.org/10.3390/w11050921

Güngör-Demirci, G., & Demirer, G. N. (2004). Effect of initial COD concentration, nutrient addition, temperature and microbial acclimation on anaerobic treatability of broiler and cattle manure. Bioresource Technology, 93(2), 109–117. https://doi.org/10.1016/j.biortech.2003.10.019

He, X., Guo, Z., Lu, J., & Zhang, P. (2021). Carbon-based conductive materials accelerated methane production in anaerobic digestion of waste fat, oil and grease. Bioresource Technology, 329. https://doi.org/10.1016/j.biortech.2021.124871

Huang, X., Yun, S., Zhu, J., Du, T., Zhang, C., & Li, X. (2016). Mesophilic anaerobic co-digestion of aloe peel waste with dairy manure in the batch digester: Focusing on mixing ratios and digestate stability. Bioresource Technology, 218, 62–68. https://doi.org/10.1016/j.biortech.2016.06.070

Kato, S., Hashimoto, K., & Watanabe, K. (2012). Methanogenesis facilitated by electric syntrophy via (semi)conductive iron-oxide minerals. Environmental Microbiology, 14(7), 1646–1654. https://doi.org/10.1111/j.1462-2920.2011.02611.x

Kumar, V., Nabaterega, R., Khoei, S., & Eskicioglu, C. (2021). Insight into interactions between syntrophic bacteria and archaea in anaerobic digestion amended with conductive materials. Renewable and Sustainable Energy Reviews, 144, 110965. https://doi.org/10.1016/j.rser.2021.110965

Kutlar, F. E., Tunca, B., & Yilmazel, Y. D. (2022). Carbon-based conductive materials enhance biomethane recovery from organic wastes: A review of the impacts on anaerobic treatment. Chemosphere, 290. https://doi.org/10.1016/j.chemosphere.2021.133247

Li, J., Kumar Jha, A., He, J., Ban, Q., Chang, S., & Wang, P. (2011). Assessment of the effects of dry anaerobic co-digestion of cow dung with waste water sludge on biogas yield and biodegradability. International Journal of the Physical Sciences, 6(15), 3723–3732. https://doi.org/10.5897/IJPS11.753

Liu, F., Rotaru, A. E., Shrestha, P. M., Malvankar, N. S., Nevin, K. P., & Lovley, D. R. (2012). Promoting direct interspecies electron transfer with activated carbon. Energy and Environmental Science, 5(10), 8982–8989. https://doi.org/10.1039/c2ee22459c

Lu, T., Zhang, J., Wei, Y., & Shen, P. (2019). Effects of ferric oxide on the microbial community and functioning during anaerobic digestion of swine manure. Bioresource Technology, 287, 121393 https://doi.org/10.1016/j.biortech.2019.121393

Martins, G., Salvador, A. F., Pereira, L., & Alves, M. M. (2018). Methane Production and Conductive Materials: A Critical Review. Environmental Science and Technology, 52 (18), 10241–10253. https://doi.org/10.1021/acs.est.8b01913

Park, J. H., Kang, H. J., Park, K. H., & Park, H. D. (2018). Direct interspecies electron transfer via conductive materials: A perspective for anaerobic digestion applications. Bioresource Technology, 254,300–311. https://doi.org/10.1016/j.biortech.2018.01.095

Rasapoor, M., Young, B., Asadov, A., Brar, R., Sarmah, A. K., Zhuang, W. Q., & Baroutian, S. (2020). Effects of biochar and activated carbon on biogas generation: A thermogravimetric and chemical analysis approach. Energy Conversion and Management, 203, 112221. https://doi.org/10.1016/j.enconman.2019.112221

Rosenberg, L., & Kornelius, G. (2017). Experimental investigation of biogas production from feedlot cattle manure. Journal of Energy in Southern Africa, 28(4), 1–8. https://doi.org/10.17159/2413-3051/2017/v28i4a1753

Song, Z., & Zhang, C. (2015). Anaerobic codigestion of pretreated wheat straw with cattle manure and analysis of the microbial community. Bioresource Technology, 186, 128–135. https://doi.org/10.1016/j.biortech.2015.03.028

Speece, R. E. (1983). Anaerobic biotechnology for industrial wastewater treatment. Environmental Science and Technology, 17(9), 416A-427A. https://doi.org/10.1021/es00115a001

Standard Methods for the Examination of Water and Wastewater. (1999).

Uçkun Kiran, E., Stamatelatou, K., Antonopoulou, G., & Lyberatos, G. (2016). Production of biogas via anaerobic digestion. In Handbook of Biofuels Production: Processes and Technologies: Second Edition (pp. 259–301). Elsevier Inc. https://doi.org/10.1016/B978-0-08-100455-5.00010-2

Wei, L., Qin, K., Ding, J., Xue, M., Yang, C., Jiang, J., & Zhao, Q. (2019). Optimization of the co-digestion of sewage sludge, maize straw and cow manure: microbial responses and effect of fractional organic characteristics. Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-38829-8

Ye, J., Hu, A., Ren, G., Chen, M., Tang, J., Zhang, P., Zhou, S., & He, Z. (2018). Enhancing sludge methanogenesis with improved redox activity of extracellular polymeric substances by hematite in red mud. Water Research, 134, 54–62. https://doi.org/10.1016/j.watres.2018.01.062

Yenigün, O., & Demirel, B. (2013). Ammonia inhibition in anaerobic digestion: A review. In Process Biochemistry, 48(5-6), 901–911. https://doi.org/10.1016/j.procbio.2013.04.012

Yin, Q., Gu, M., & Wu, G. (2020). Inhibition mitigation of methanogenesis processes by conductive materials: A critical review. Bioresource Technology, 317, 123977. https://doi.org/10.1016/j.biortech.2020.123977

Yin, Q., Miao, J., Li, B., & Wu, G. (2017). Enhancing electron transfer by ferroferric oxide during the anaerobic treatment of synthetic wastewater with mixed organic carbon. International Biodeterioration and Biodegradation, 119, 104–110. https://doi.org/10.1016/j.ibiod.2016.09.023

Zheng, Z., Liu, J., Yuan, X., Wang, X., Zhu, W., Yang, F., & Cui, Z. (2015). Effect of dairy manure to switchgrass co-digestion ratio on methane production and the bacterial community in batch anaerobic digestion. Applied Energy, 151, 249–257. https://doi.org/10.1016/j.apenergy.2015.04.078

Zhou, S., Xu, J., Yang, G., & Zhuang, L. (2014). Methanogenesis affected by the co-occurrence of iron(III) oxides and humic substances. FEMS Microbiology Ecology, 88(1), 107–120. https://doi.org/10.1111/1574-6941.12274

Zhuang, L., Tang, J., Wang, Y., Hu, M., & Zhou, S. (2015). Conductive iron oxide minerals accelerate syntrophic cooperation in methanogenic benzoate degradation. Journal of Hazardous Materials, 293, 37–45. https://doi.org/10.1016/j.jhazmat.2015.03.039

Zwietering, M. H., Jongenburger, I., Rombouts, F. M., van ' K., & Riet, T. (1990). Modeling of the Bacterial Growth Curve. Applied and Envrionmental Microbiology, 56(6): 1875–1881. 10.1128/aem.56.6.1875-1881.1990

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 79-87
DOI: https://doi.org/10.31015/jaefs.2023.1.9

Valorization of shalgam juice plant waste for the production of carotenoids by *Rhodotorula glutinis*

Abdulkadir Keskin[1] • **Melis Fırat**[1] • **Ayşe Ezgi Ünlü**[1]

[1] Department of Chemical Engineering, Faculty of Engineering, Ankara University, Ankara, Türkiye

Citation: Keskin, A., Fırat, M., Unlu, A.E. (2023). Valorization of shalgam juice plant waste for the production of carotenoids by Rhodotorula glutinis. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 79-87

Received: 24 October 2022
Revised: 29 January 2023
Accepted: 01 February 2023
Published Online: 19 February 2023

Correspondence: Ayşe Ezgi Ünlü
E-mail: aeunlu@eng.ankara.edu.tr

Abstract

Food waste is an emerging global problem that should not be underestimated. One of the most abundant wastes in Türkiye and neighbour countries is the waste of shalgam juice plants (WSJP). In this study, WSJP was used as a growth medium for *Rhodotorula glutinis*. The effects of initial medium pH (3.4-5.4), carbon (0-60 g/L glycerol) and nitrogen sources (0-1 g/L urea) were investigated on biomass, medium pH, reducing sugar concentration, total lipid and carotenoid contents, as well as carotenoid composition (torulene, torularhodin, β-carotene). When crude waste extract (no additional nutrient) was used as growth medium (initial medium pH 3.4, 6.14 g/L sugar), biomass was relatively low (1.47±0.055 g/L), due to acidic pH and insufficient nutrients. However, these stress conditions increased the production of total lipid and carotenoid contents by *R. glutinis*. The highest lipid and carotenoid contents were obtained as 0.14±0.0004 g/g dry cell weight (dcw) and 1221.57±0.59 µg/g dcw, respectively, under these circumstances. As additional stress factor fermentation medium -crude WSJP extract- was illuminated for 72 h. This increased lipid content by 1.7-fold, while showing low impact on carotenoid content. Interestingly, illumination changed the carotenoid composition by decreasing torulene and β-carotene percentages, but increasing torularhodin percentage. On the other hand, tuning the initial pH to an ambient value (5.4) and the addition of carbon and nitrogen sources stimulated cell growth (4.67±0.07 g/L). This study presents the first time use of WSJP extract as a growth medium, without any additional nutrition, moreover, the simultaneous production of high-value added carotenoids.

Keywords: *Rhodotorula glutinis,* Waste, Shalgam juice, Lipid, Carotenoid

INTRODUCTION

Today, food waste is one of the major problems in the world. The amount of food waste has reached to almost billions of tones annually (De Souza Mesquita et al., 2019). In addition to the economic cost of the waste management, environmental pollution also creates a serious problem. Regarding this issue, worldwide meetings and conferences have been organized to take the attention of authorized organizations (Bröring et al., 2020; Hanedar et al., 2021). As a result of the increasing consciousness, 'zero waste' have gradually become the primary aim of the processes to prevent waste and ensure sustainability.

Fermented beverages are generally preferred due to various beneficial effects on human health. Especially the presence of microorganisms such as probiotics,

together with the healthful ingredients, have positive effects on the gastrointestinal system (Marsh et al., 2014). Shalgam juice (SJ) is one of the most popular non-alcoholic traditional beverages in Turkiye. Due to the interaction with the contiguous countries and exportation of the beverages, SJ has also gained attention around Middle East and Balkans region (Keskin & Güneş, 2021).

SJ is mainly produced by fermentation of two vegetables; black carrot (Daucus carota) and turnip (Brassica rapa). The other ingredients are wheat, sourdough, salt and water (Turker et al., 2004). However, some processes prefer to produce this drink using one of the vegetables, that is mostly black carrot. In 2020, the production of SJ in Turkiye was reported as 2,599 tones and showed an increasing trend every year (Omrak, 2021). Considering an average SJ production process, this value corresponds to almost 520 tones of fermented black carrot waste, that is quite high (Coşkun, 2017). The fermented vegetables are disposed to the environment as a waste of the process.

Black carrot contains several antioxidant molecules such as vitamin, C, vitamin E, carotenoids and phenolic components and the characteristic purple-black color is due to its high anthocyanin content (Wrolstad, 2004). Black carrot also contains fermentable sugars such as sucrose, glucose and fructose (Kammerer et al., 2004). The rich composition of fresh black carrot makes it attractive for researchers in terms of extraction of different components such as anthocyanins (Agcam et al., 2017; Gizir et al., 2008; Nistor et al., 2021), phytoceuticals (Kumar et al., 2019), pectin (Sucheta et al., 2020) and pectin methylesterase (Ünal & Bellur, 2009). On the other hand, it is mainly used for pigment production in industry (Kumar et al., 2019).

Carotenoids are natural isoprenoid pigments that can be synthesized by photosynthetic organisms and some of non-photosynthetic microorganisms (Moise et al., 2014; Rodriguez-Concepcion et al., 2018). Humans and animals are not capable of synthesizing carotenoids, therefore these molecules should be uptaken do their provitamin A activity that is crucial for human health (Maiani et al., 2009). Besides the significant systemic roles of carotenoids in human body, the lack of vitamin A in humans was proved to cause premature death (Britton, 1995). Carotenoids also serve as antioxidants that protect cells from free radicals (Maiani et al., 2009). Additionally, there are many studies that show the lowered risk of many chronic diseases as a result of the consumption of carotenoids in diets (Mayne, 1996). Related with the awareness of the benefits of carotenoids, not only in human health, but also as additives in different industries such as textile, pharmaceutical, and cosmeceutical products (Hernández-Almanza et al., 2016; Mussagy et al., 2022), global market of carotenoids has grown significantly. The annual growth rate of the market was predicted as 5.7% between 2017-2022 with a market value of 2.0 billion dollars (Rapoport et al., 2021).

The abundant sources of carotenoids are colorful fruits and vegetables such as carrot, orange, mango, bell pepper, etc. They contain mainly, β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin (Maiani et al., 2009). Other sources are filamentous fungi and pigmented yeasts, such as *Rhodotorula glutinis.*

Rhodotorula glutinis is an oleaginous red yeast that is capable of producing carotenoids that gives its unique color and is also capable of accumulating considerable amount of lipid inside (Hernández-Almanza et al., 2016). Besides the well-established carotenoid β-carotene, *Rhodotorula glutinis* can also synthesize torularhodin and torulene, which can only be synthesized by yeast and fungi (Kot et al., 2018). These carotenoids have the potential to be used in several different fields such as food and feed, medical and pharmaceutical industries (Kot et al., 2018).

In the literature, there are numerous studies on the varolization of black carrot pomace as waste of marmalade or juice production. The majority of these studies targets the extraction of anthocyanins or bioactive compounds as the ultimate product (Agcam et al., 2017, 2021; Kumar et al., 2019). However, there are no studies on the valorization of the waste of shalgam juice plant (WSJP) that mainly differs from marmalade or juice production in regard of the fermentation step. Our preliminary experiments on WSJP showed that fermented black carrot could still be evaluated due to the bioactive compounds conserved in the waste.

In this study our aim is the simultaneous valorization of WSJP and production of carotenoids by *R. glutinis*. With this aim, the effects of different parameters such as initial medium pH, carbon and nitrogen sources, and illumination were investigated that are known as stimulators of carotenogenesis (Gu et al., 1997; Johnson & Lewis, 1979; Orosa et al., 2005).

MATERIALS AND METHODS

Materials

Rhodotorula glutinis (70398) was purchased from DSM, Braunschweig, Germany. Yeast extract (70161), malt extract (70167), peptone (70171), agar (05039) and potassium-sodium tartrate (60410) were obtained from Fluka. Glucose (A1349,5000) was purchased from Applichem, sodium sulfide (13471) from Riedel de Haen. Urea (108488), chloroform (1.022445.2500) and hexane (1.04391.2500) were purchased from Merck. The following chemicals were obtained from Sigma-Aldrich: glycerol (G-5516), DNS (dinitrosalicylic acid) (D-0550), sodium hydroxide pellet (06203), Folin & Ciocalteu's phenol reagent (9252), β-carotene (C4582) and methanol (24229). All other chemicals used were at least analytical grade.

Waste of Shalgam Juice Plant

Waste of shalgam juice plant (WSJP) was supplied from Yeni Kavaklıdere Company (Ankara, Turkiye). Solid waste was grinded by blender (Braun MQ9078X). WSJ was extracted by distilled water at 50°C, 700 rpm for 90 min. Reducing sugar content of the WSJP extract was 6.15 g/L and pH value was 3.4. Supernatant was stored at -30°C until further use.

Fermentation of *Rhodotorula glutinis*

Solid growth medium contained 10 g/L glucose, 5 g/L peptone, 3 g/L yeast extract, 3 g/L malt extract and 20 g/L agar in WSJP extract. The cells were inoculated to the solid growth medium and cultivated at 30°C for 24 h and transferred to the 100 mL of liquid fermentation medium. The liquid medium basically consisted of WSJP extract, urea (0-1 g/L) and glycerol (0-60 g/L), and the cultivations were carried out at 150 rpm and 30°C for 72 h. Then, cells were separated by centrifugation at 7200xg for 10 min (Hettich Zentrifugen Rotina 35 R), washed twice, and lyophilized at -50°C and 0.04 mbar (Hetosicc) and used for further analysis. The effect of illumination was investigated using white light (Osram L 15 W/25, Germany).

Dry Cell Weight

An aliquat was withdrawn from the liquid medium, centrifuged at 7200xg for 10 min at +4°C, the cells were washed twice and dried for 3 h at 80°C (Zhichang- ZRD 5110, Forced-Air Drying Oven) and expressed in dry cell weight (dcw).

Reducing Sugar Concentration

Reducing sugar concentration was determined by Dinitrosalicylic acid (DNS) method (Miller, 1959). 750 µL DNS solution (10 g/L DNS, 0.5 g/L sodium sulfite, 10 g/L sodium hydroxide) was added to 750 µL sample, vortexed and incubated at 90°C for 10 min. After addition of 250 µL of potassium-sodium tartrate solution (40 g/L) absorbance was measured 575 nm (UV-VIS spectrophotometer, Shimadzu 1601, Japan) and reducing sugar concentration was calculated in glucose equivalents.

Total Lipid and Total Carotenoid Content

Chloroform (6.25 mL) and methanol (12.5 mL) were added to a flask containing 0.5 g of lyophilized cells and stirred for 1h. Then, NaCl (0.5%, 6.25 mL) and chloroform (6.25 mL) were added to the flask and stirred for an additional 1 h. The phases were separated by centrifugation (7200xg, 10 min) and chloroform phase was separated and evaporated (Heidolph, Hei-VAP Advantage). The residue containing lipid and carotenoids was determined gravimetrically yielding total lipid amount (Bligh & Dyer, 1959). The residue, then was dissolved in hexane and filtered. Total carotenoid amount was measured in equivalents of β-carotene at a wavelength of 450 nm in a UV-VIS spectrophotometer (Shimadzu 1601, Japan).

Carotenoid Composition

Carotenoid composition was determined by HPLC (Waters Alliance) at 450 nm (UV-VIS Dual Absorbance Detector Waters 2487), using C18 column (XTerra RP18, Waters, 150 mm x 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile, methanol, and chloroform (47:47:6) with a flow rate of 1.0 mL/min. Analysis were performed at 30°C with a sample volume of 50 µl. β-carotene was identified based on the retention time of the external standard.

Thin-Layer Chromatography (TLC)

Torularhodin and torulene solutions were obtained by thin-layer chromatography (TLC) (Kot et al., 2017). The spots, which were carotenoids, were identified using the retention factor coefficients according to the method published (Kot et al., 2017). As a result of many runs, the spots corresponding to torularhodin and torulene were scraped separately into hexane and solutions were filtered through a PVDF filter before injection to HPLC.

Statistics

All cultivations were repeated at least two times and all analyses were repeated at least three times and their average values were presented. The error bars were prepared using Excel 2010.

RESULTS AND DISCUSSION

Effect of Initial Medium pH

The initial pH value of WSJP extract was measured as 3.4 due to the acidic nature of the shalgam juice production process. In order to investigate the effect of the initial medium pH on the growth of *R. glutinis*, and additionally to increase the relatively low pH to ambient values, initial pH was adjusted to 4.4 and 5.4 using 1 M NaOH. The change in pH, biomass and reducing sugar concentrations were measured with time. The results showed that medium pH increased within 24 h of fermentation for all media tested and remained almost constant till 48 h and decreased at 72 h (Figure 1). The pH values of the two media, pH 5.4 and 4.4, were almost equalized after 24 h and showed almost the same profile. On the other hand, the variation of the medium of initial pH 3.4 medium remained lower than the others for 72h.

The change in the biomass with time at different initial pH values are presented at Figure 2. The highest biomass was obtained at pH 5.4 as 1.87±0.055 g/L, followed by pH 4.4 as 1.73±0.06 g/L. The lowest cell concentration was obtained at pH 3.4, as 1.46±0.055 g/L.

Relatively low amount of sugar in the WSJP extract (6.15 g/L) is considered to be the major reason of the low cell concentrations. When the change in reducing sugar concentrations are evaluated (Figure 3), they were found to be compatible with the growth of the cells. Sugar was

consumed higher at the initial pH values of 4.4 and 5.4 than pH 3.4. Considering the decrease in reducing sugar concentration with time, fermentation media was ended after 72 h, in order to maintain the cells at the stationary phase, to produce the target antioxidant components (Mata-Gómez et al., 2014).

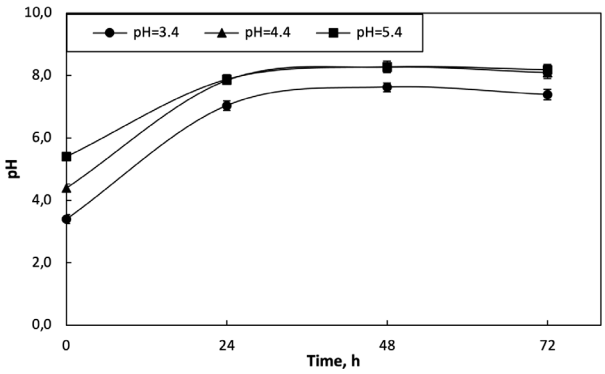


Figure 1. The variation of pH with time at different initial pH values (WSJP extract, 150 rpm, 30°C, 100 mL)

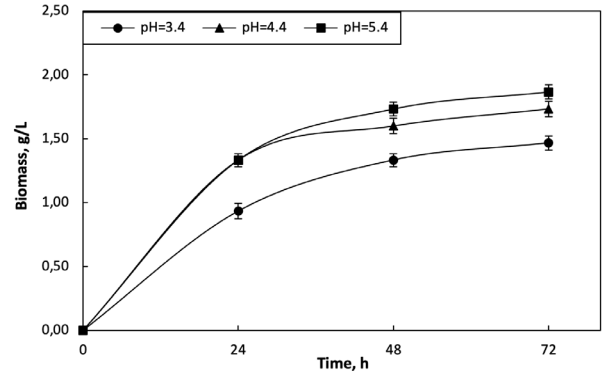


Figure 2. The variation of biomass with time at different initial pH values (WSJP extract, 150 rpm, 30°C, 100 mL)

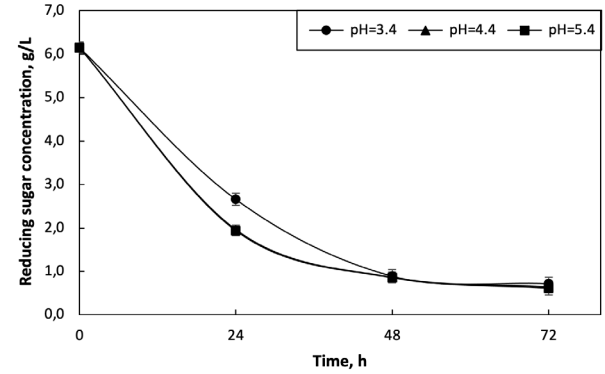


Figure 3. The variation of reducing sugar concentration with time at different initial pH values (WSJP extract, 150 rpm, 30°C, 100 mL)

Carotenoids synthesized by *R. glutinis* were extracted simultaneously with the extraction of lipids and solubilized in lipid phase. According to the results (Table 1), the highest lipid and carotenoid contents were obtained at pH 3.4, as 0.14±0.0007 g/g dcw and 1221±0.68 µg/g dcw, respectively. These results showed that lipid and carotenoid productions were high when the cell growth was low. It is well-known phenomenon that carotenoids are antioxidant molecules that are primarily produced under stress conditions and stress conditions limit the growth of the cells (Goiris et al., 2015; Mata-Gómez et al., 2014; Young & Lowe, 2018).

Table 1. The change in total lipid and total carotenoid contents at different initial pH values

pH	Total lipid content (g/g dcw)	Total carotenoid content (µg/g dcw)
3.4	0.14±0.0007	1221.57±0.68
4.4	0.11±0.0008	448.90±0.65
5.4	0.10±0.0007	587.24±0.72

Torulene and torularhodin are significant carotenoids produced by *R. glutinis*, besides β-carotene. In order to determine the distribution of the three components, HPLC analysis was performed and the results are presented in Figure 4. According to the results, carotenoid profile was found to be the same for all initial pH values and β-carotene was found to be the most abundant component. Evaluating the results of the effect of initial pH value experiments, pH 3.4 was selected as the optimum pH for the production of target carotenoids.

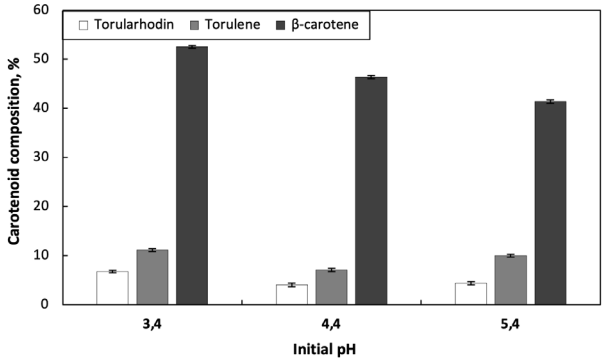


Figure 4. Carotenoid composition of *R. glutinis* cells grown at different initial pH values

Effect of Nitrogen Source

In order to observe the effect of nitrogen source, different amounts of urea were added to the WSJP extract. Addition of urea did not affect the initial medium pH, however, affected the time-course pH values of the fermentation media. As presented in Figure 5, pH values of 0.5 g/L and 1.0 g/L urea containing media were found to be higher

than that of 'no urea' medium. Similarly, higher biomass was obtained when additional nitrogen source was utilized (Figure 5). The highest cell concentration was 2.13±0.05 g/L at 1 g/L urea, followed by 1.73±0.06 g/L at 0.5 g/L urea concentration. The change in the growth of cells were found to be compatible with the decrease in the reducing sugar concentrations (Figure 6). The higher the growth rate, the higher the consumption rate of the reducing sugar was obtained, as expected (Figure 7).

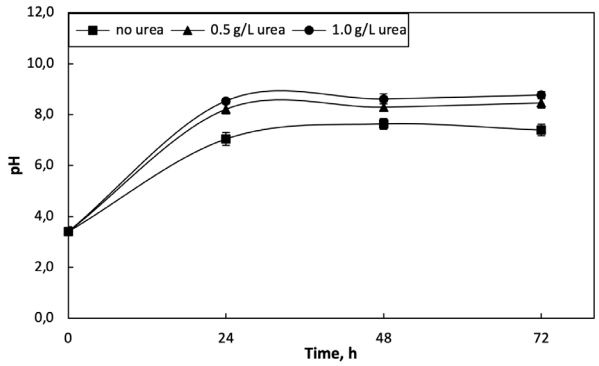


Figure 5. The variation of pH with time at for different urea concentrations (WSJP extract, 150 rpm, 30°C, 100 mL)

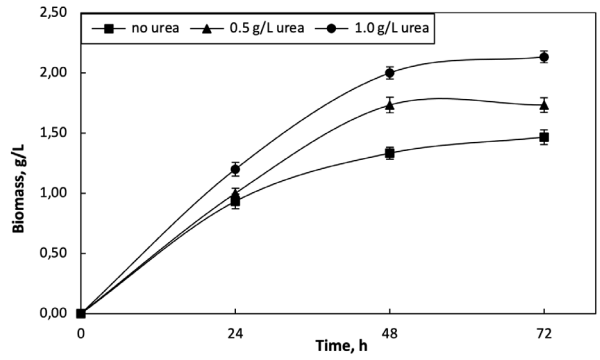


Figure 6. The variation of biomass with time at for different urea concentrations (WSJP extract, 150 rpm, 30°C, 100 mL)

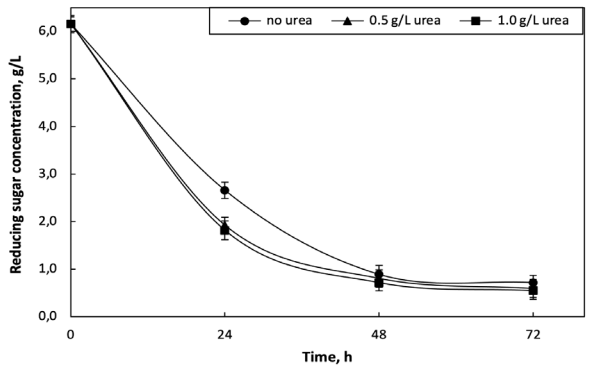


Figure 7. The variation of reducing sugar concentration with time for different urea concentrations (WSJP extract, 150 rpm, 30°C, 100 mL)

The highest total lipid and total carotenoid contents were achieved when no nitrogen source was added to the media, as 0.14±0.0006 g/g dcw and 1221.57±0.63µg/g dcw, respectively (Table 2). When urea-containing media were compared, 1 g/L of urea provided higher carotenoid content (587.24±0.71 µg/g dcw) than 0.5 g/L (448.9±0.58 µg/g dcw). When carotenoid profiles are evaluated, β-carotene was found to be the most abundant component at all media tested (Figure 8). The profiles were found to be similar for 0 and 0.5 g/L urea containing media while they were found to change at 1 g/L urea containing medium. The relative amount of torularhodin exceeded torulene. This showed that nitrogen source had a considerable effect on the composition of carotenoids. This effect of nitrogen source was also presented before in the literature (Mussagy et al., 2022).

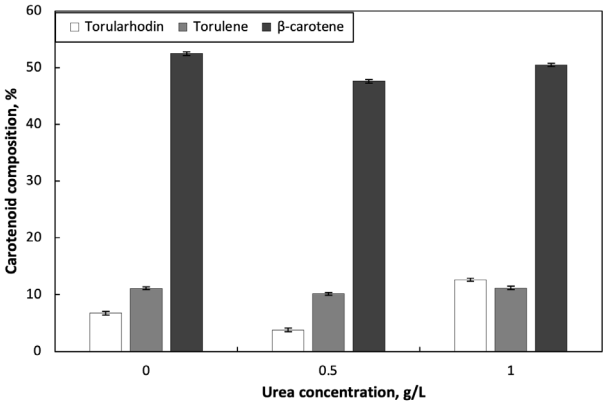


Figure 8. Carotenoid composition of *R. glutinis* cells grown at different urea concentrations (150 rpm, 30°C, 100 mL)

Effect of Carbon Source

To investigate the effect of an additional carbon source on WSJP extract, different concentrations of glycerol were added to the fermentation media. Glycerol was selected as the additional carbon source for being the side-product of biodiesel production process. Therefore, utilization of glycerol also contributed to valorization of another waste. The initial pH values of 'no glycerol' and 30 g/L glycerol containing media remained constant at pH 3.4, while an increase was observed 60 g/L glycerol containing medium. Interestingly, the variation of pH was found to be significantly different for glycerol containing media (Figure 9). pH increased very slowly during the first 24 h. This could be explained by the utilization of glycerol by *R. glutinis*, as the major carbon source in the medium, by releasing catabolism products that alter the pH profile.

On the other hand, biomass increased significantly in glycerol containing media (Figure 10). The final cell concentration increased to 4.67±0.07 g/L at 60 g/L glycerol, that is almost 3-fold when compared to 'no glycerol' medium.

Table 2. The change in total lipid and total carotenoid contents at different urea concentrations (150 rpm, 30°C, 100 mL)

Urea concentration (g/L)	Total lipid content (g/g dcw)	Total carotenoid content (µg/g dcw)
0	0.14±0.0006	1221.57±0.63
0.5	0.12±0.0006	448.90±0.58
1	0.10±0.0005	587.24±0.71

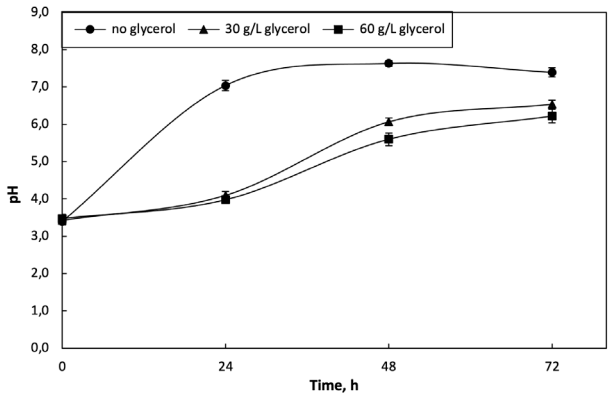


Figure 9. The variation of pH with time at for different glycerol concentrations (150 rpm, 30°C, 100 mL)

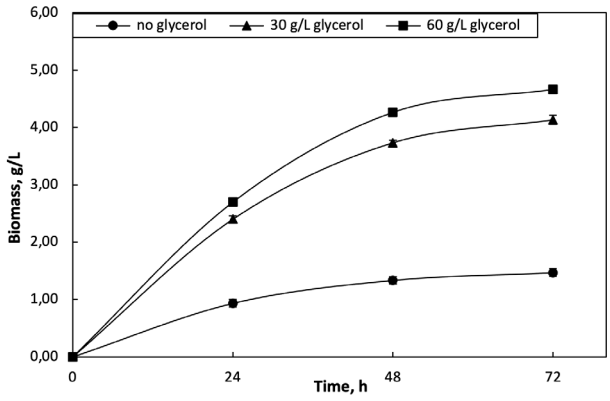


Figure 10. The variation of biomass with time for different glycerol concentrations (150 rpm, 30°C, 100 mL)

Reducing sugar concentrations decreased at the same rate for all three media in the first 24 h and then differed according to the glycerol concentrations (Figure 11). On the other hand, the increase of biomass for glycerol containing media in 24 h (Figure 10) illustrated that *R. glutinis* consumed both glycerol and reducing sugar. Reducing sugar concentrations at 48 h and 72 h (Figure 11) showed that reducing sugar consumption decreased with increasing glycerol concentration.

In contrast to the cell growth, total lipid and total carotenoid contents were found to be the highest without glycerol (Table 3). Total carotenoid content showed almost a 16-fold decrease when 60 g/L of glycerol used. This result supported the fact of induction of the production of antioxidant molecules at stress conditions, ie., insufficient carbon source.

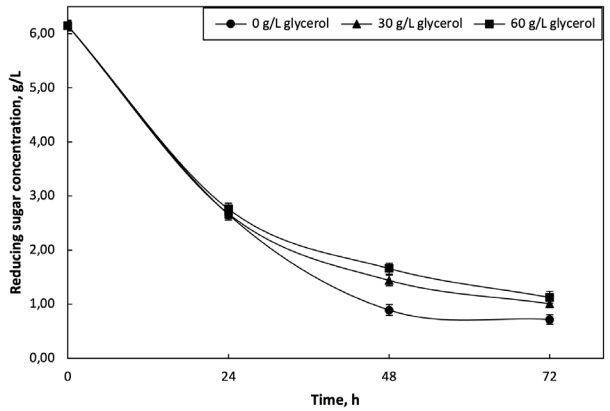


Figure 11. The variation of reducing sugar concentration with time for different glycerol concentrations (150 rpm, 30°C, 100 mL)

When carotenoid profiles are evaluated (Figure 12) the percentage of torularhodin was found to increase significantly, from 8% to 30%, at 60 g/L of glycerol. This showed the considerable effect of glycerol concentration on the carotenoid composition. This result is consistent with the carotenoid profiles obtained for different urea concentrations (Figure 8) and also with the literature. The change in the composition of carotenoids produced by *R. glutinis* with different carbon and nitrogen sources was reported in different studies (Buzzini & Martini, 2000; Peng et al., 2021). Nevertheless, since total carotenoid content was quite low as 75.89±0.71 µg/g dcw when 60 g/L glycerol was used, further experiments were conducted without additional carbon source.

Table 3. The change in total lipid and total carotenoid contents at different glycerol concentrations (150 rpm, 30°C, 100 mL)

Glycerol concentration (g/L)	Total lipid content (g/g dcw)	Total carotenoid content (µg/g dcw)
0	0.14±0.0004	1221.57±0.59
30	0.22±0.0007	209.60±0.65
60	0.12±0.0005	75.89±0.71

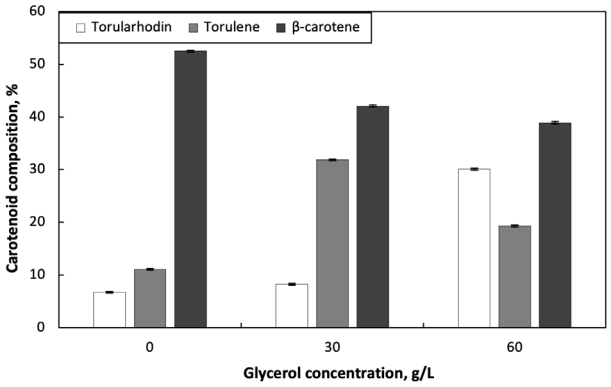


Figure 12. Carotenoid composition of *R. glutinis* cells grown at different glycerol concentrations (150 rpm, 30°C, 100 mL)

Effect of Illumination

Carotenoid production is reported to be enhanced by illumination for different microorganisms. This is associated with the promoted activity of the enzymes that take place in carotenogenesis (Bhosale, 2004). Therefore, to enhance the production of carotenoids, fermentation medium of WSJP extract was subjected to illumination for 72 h. According to the results, illumination decreased the cell growth during fermentation and caused nearly 9% of decrease in final biomass concentration, in accordance with the literature (Bhosale & Gadre, 2002) (Figure 13). The change in reducing sugar concentration with time was found to be similar for illuminated and non-illuminated media (Figure 13). Similarly, the variation of pH values of illuminated and non-illuminated media with time showed almost the same values (Table 4). On the other hand, total lipid content was found to increase from 0.14±0.0004 g/g dcw to 0.24±0.0005 g/g dcw after 72 h of illumination (Table 5), showing the stress effect on *R. glutinis* cells. Despite the minor change in the total carotenoid content (Table 5), carotenoid composition of *R. glutinis* was significantly affected by illumination. Torulene and β-carotene percentages was found to decrease while torularhodin percentage increased from 6.74% to 19.96% (Figure 14).

Table 4. The effect of illumination on the variation of pH with time (WSJP extract, 150 rpm, 30°C, 100 mL)

	pH			
	Incubation time, h			
Illumination	**0**	**24**	**48**	**72**
No illumination	3.40±0.04	7.04±0.07	7.63±0.07	7.39±0.05
72 h illumination	3.40±.0.04	7.08±0.05	7.63±0.06	7.76±0.06

Table 5. The effect of illumination on total lipid and total carotenoid contents (WSJP extract, 150 rpm, 30°C, 100 mL, 72 h)

Illumination	Total lipid content (g/g dcw)	Total carotenoid content (µg/g dcw)
No illumination	0.14±0.0004	1221.57±0.59
72 h illumination	0.24±0.0005	1260.30±0.62

When the effect of initial medium pH, carbon and nitrogen sources on the total carotenoid content are evaluated, the highest total carotenoid amount was obtained at initial pH value of 3.4 in the absence of urea and glycerol, therefore, further experiments were conducted using WSJP extract.

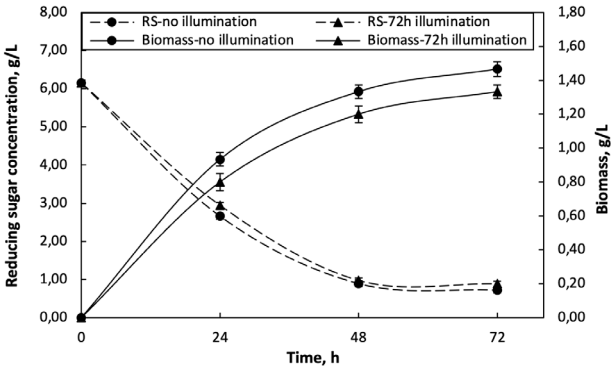


Figure 13. The effect of illumination on the variation of biomass and reducing sugar concentration RS: reducing sugar (WSJP extract, 150 rpm, 30°C, 100 mL)

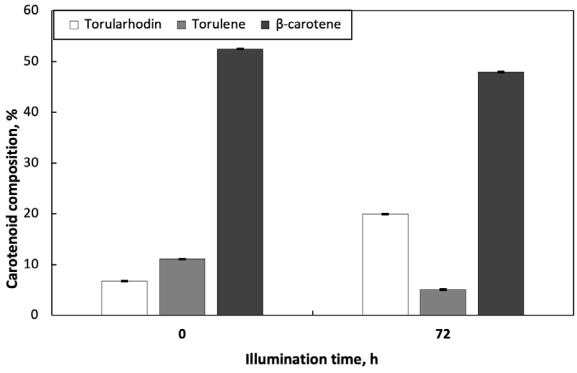


Figure 14. Carotenoid composition of *R. glutinis* cells under different illumination time (WSJP extract, 150 rpm, 30°C, 100 mL)

CONCLUSION

In this study we aimed to evaluate the waste of shalgam juice plant, to produce carotenoids, that are high-value-added components. The results showed that biomass reached to higher values when initial pH was tuned to 5.4 and additional nitrogen and carbon sources were added to the fermentation medium. On the other hand, lipid and carotenoid synthesis was induced when cell growth was hindered. The highest total carotenoid and lipid contents were achieved when crude WSJP extract was used. This is an advantage in terms of the cost of the fermentation medium. The waste can directly be extracted and used as the growth medium. On the other hand, illumination had a positive effect on total lipid and total carotenoid contents and additionally, increased the relative amount of torularhodin significantly. This is an important result, since torularhodin is a rare member of carotenoids that can only be synthesized by yeast and fungi. Nevertheless, even with no light, carotenoids were synthesized by *R. glutinis*. Regarding the promising results of this study, the process may be scaled up using a bioreactor. In addition to the investigated parameters, the effect of dissolved oxygen concentration may be investigated on the production of carotenoids which is a significant stress effect on the microorganisms. Moreover, pH-stat or chemostat operation strategies may be performed and the effect on the total lipid and carotenoid contents, as well as their compositions may be presented.

To our knowledge, this is the fist study in the literature, that presents a process for the valorization of the waste of shalgam juice plant and the simultaneous production of carotenoids as high-value-added components.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that they have no conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Agcam, E., Akyıldız, A. & Balasubramaniam, V. M. (2017). Optimization of anthocyanins extraction from black carrot pomace with thermosonication. *Food Chemistry*, *237*, 461–470. https://doi.org/10.1016/j.foodchem.2017.05.098

Agcam, E., Akyıldız, A., Kamat, S. & Balasubramaniam, V. M. (2021). Bioactive Compounds Extraction from the Black Carrot Pomace with Assistance of High Pressure Processing: An Optimization Study. *Waste and Biomass Valorization*, *12*(11), 5959–5977. https://doi.org/10.1007/s12649-021-01431-z

Bhosale, P. (2004). Environmental and cultural stimulants in the production of carotenoids from microorganisms. *Applied Microbiology and Biotechnology*, *63*(4), 351–361. https://doi.org/10.1007/s00253-003-1441-1

Bhosale, P. & Gadre, R. V. (2002). Manipulation of temperature and illumination conditions for enhanced β-carotene production by mutant 32 of Rhodotorula glutinis. *Letters in Applied Microbiology*, *34*(5), 349–353. https://doi.org/10.1046/j.1472-765X.2002.01095.x

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917.

Britton, G. (1995). Structure and properties of carotenoids in relation to function. *FASEB Journal*, *9*, 1551–1558.

Bröring, S., Laibach, N. & Wustmans, M. (2020). Innovation types in the bioeconomy. *Journal of Cleaner Production*, *266*, 121939. https://doi.org/10.1016/j.jclepro.2020.121939

Buzzini, P. & Martini, A. (2000). Production of carotenoids by strains of Rhodotorula glutinis cultured in raw materials of agro-industrial origin. *Bioresource Technology*, *71*(1), 41–44. https://doi.org/10.1016/S0960-8524(99)00056-5

Coşkun, F. (2017). A Traditional Turkish Fermented Non-Alcoholic Beverage, "Shalgam." *Beverages*, *3*(49), 1–13.

De Souza Mesquita, L. M., Ventura, S. P. M., Braga, A. R. C., Pisani, L. P., Dias, A. C. R. V. & De Rosso, V. V. (2019). Ionic liquid-high performance extractive approach to recover carotenoids from: Bactris gasipaes fruits. *Green Chemistry*, *21*(9), 2380–2391. https://doi.org/10.1039/c8gc03283a

Gizir, A. M., Turker, N. & Artuvan, E. (2008). Pressurized acidified water extraction of black carrot [Daucus carota ssp. sativus var. atrorubens Alef.] anthocyanins. *European Food Research and Technology*, *226*(3), 363–370. https://doi.org/10.1007/s00217-006-0546-z

Goiris, K., Van Colen, W., Wilches, I., León-Tamariz, F., De Cooman, L. & Muylaert, K. (2015). Impact of nutrient stress on antioxidant production in three species of microalgae. *Algal Research*, *7*, 51–57. https://doi.org/10.1016/j.algal.2014.12.002

Gu, W. L., An, G. H. & Johnson, E. A. (1997). Ethanol increases carotenoid production in Phaffia rhodozyma. *Journal of Industrial Microbiology and Biotechnology*, *19*, 114–117.

Hanedar, A., Gül, B., Güneş, E., Kaykıoğlu, G. & Güneş, Y. (2021). Waste management and zero waste practices in educational institutions. *Environmental Research & Technology*, *4*(2), 126–133.

Hernández-Almanza, A., Montañez, J., Martínez, G., Aguilar-Jiménez, A., Contreras-Esquivel, J. C. & Aguilar, C. N. (2016). Lycopene: Progress in microbial production. *Trends in Food Science and Technology*, *56*, 142–148. https://doi.org/10.1016/j.tifs.2016.08.013

Johnson, E. A. & Lewis, M. J. (1979). Astaxanthin formation by the yeast Phaffia rhodozyma. *Journal of General Microbiololgy*, *115*, 173–183.

Kammerer, D., Carle, R. & Schieber, A. (2004). Quantification of anthocyanins in black carrot extracts (Daucus carota ssp. sativus var. atrorubens Alef.) and evaluation of their color properties. *European Food Research and Technology*, *219*, 479–486. https://doi.org/10.1007/s00217-004-0976-4

Keskin, B. & Güneş, E. (2021). Social and cultural aspects of traditional drinks: A review on traditional Turkish drinks. *International Journal of Gastronomy and Food Science*, *25*(July), 100382. https://doi.org/10.1016/j.ijgfs.2021.100382

Kot, A. M., Błazejak, S., Gientka, I., Kieliszek, M. & Bryś, J. (2018). Torulene and torularhodin: "New" fungal carotenoids for industry? *Microbial Cell Factories*, *17*(49), 1–14. https://doi.org/10.1186/s12934-018-0893-z

Kot, A. M., Błażejak, S., Kurcz, A., Bryś, J., Gientka, I., Bzducha-Wróbel, A., Maliszewska, M. & Reczek, L. (2017). Effect of initial pH of medium with potato wastewater and glycerol on protein, lipid and carotenoid biosynthesis by Rhodotorula glutinis. *Electronic Journal of Biotechnology*, *27*, 25–31. https://doi.org/10.1016/j.ejbt.2017.01.007

Kumar, M., Dahuja, A., Sachdev, A., Kaur, C., Varghese, E., Saha, S. & Sairam, K. V. S. S. (2019). Valorisation of black carrot pomace: microwave assisted extraction of bioactive phytoceuticals and antioxidant activity using Box–Behnken design. *Journal of Food Science and Technology*, *56*(2), 995–1007. https://doi.org/10.1007/s13197-018-03566-9

Maiani, G., Castón, M. J. P., Catasta, G., Toti, E., Cambrodón, I. G., Bysted, A., Granado-Lorencio, F., Olmedilla-Alonso, B., Knuthsen, P., Valoti, M., Böhm, V., Mayer-Miebach, E., Behsnilian, D. & Schlemmer, U. (2009). Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Molecular Nutrition and Food Research*, *53*(SUPPL. 2), 194–218. https://doi.org/10.1002/mnfr.200800053

Marsh, A. J., Hill, C., Ross, R. P. & Cotter, P. D. (2014). Fermented beverages with health-promoting potential: Past and future perspectives. *Trends in Food Science and Technology*, *38*(2), 113–124. https://doi.org/10.1016/j.tifs.2014.05.002

Mata-Gómez, L. C., Montañez, J. C., Méndez-Zavala, A. & Aguilar, C. N. (2014). Biotechnological production of carotenoids by yeasts: An overview. *Microbial Cell Factories*, *13*(12), 1–11. https://doi.org/10.1186/1475-2859-13-12

Mayne, S. T. (1996). Beta-carotene, carotenoids, and disease prevention in humans. *FASEB Journal*, *10*, 690–701.

Miller, G. L. (1959). Modified DNS method for reducing sugars. *Analytical Chemistry*, *31*(3), 426–428.

Moise, A. R., Al-Babili, S. & Wurtzel, E. T. (2014). Mechanistic aspects of carotenoid biosynthesis. *Chemical Reviews*, *114*(1), 164–193. https://doi.org/10.1021/cr400106y

Mussagy, C. U., Gonzalez-Miquel, M., Santos-Ebinuma, V. C. & Pereira, J. F. B. (2022). Microbial torularhodin–a comprehensive review. *Critical Reviews in Biotechnology*, *0*(0), 1–19. https://doi.org/10.1080/07388551.2022.2041540

Nistor, M., Diaconeasa, Z., Frond, A. D., Stirbu, I., Socaciu, C., Pintea, A. & Rugina, D. (2021). Comparative efficiency of different solvents for the anthocyanins extraction from chokeberries and black carrots, to preserve their antioxidant activity. *Chemical Papers*, *75*(2), 813–822. https://doi.org/10.1007/s11696-020-01344-6

Omrak, H. (2021). *Bitkisel Üretim*. Türk Tarım Dergisi. (in Turkish).

Orosa, M., Franqueira, D., Cid, A. & Abalde, J. (2005). Analysis and enhancement of astaxanthin accumulation in Haematococcus pluvialis. *Bioresource Technology*, *95*(3), 373–378.

Peng, T., Fakankun, I. & Levin, D. B. (2021). Accumulation of neutral lipids and carotenoids of Rhodotorula diobovata and Rhodosporidium babjevae cultivated under nitrogen-limited conditions with glycerol as a sole carbon source. *FEMS Microbiology Letters*, *368*(18), 1–7. https://doi.org/10.1093/femsle/fnab126

Rapoport, A., Guzhova, I., Bernetti, L., Buzzini, P., Kieliszek, M. & Kot, A. M. (2021). Carotenoids and some other pigments from fungi and yeasts. *Metabolites*, *11*(2), 1–17. https://doi.org/10.3390/metabo11020092

Rodriguez-Concepcion, M., Avalos, J., Bonet, M. L., Boronat, A., Gomez-Gomez, L., Hornero-Mendez, D., Limon, M. C., Meléndez-Martínez, A. J., Olmedilla-Alonso, B., Palou, A., Ribot, J., Rodrigo, M. J., Zacarias, L. & Zhu, C. (2018). A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. *Progress in Lipid Research*, *70*(February), 62–93. https://doi.org/10.1016/j.plipres.2018.04.004

Sucheta, Misra, N. N. & Yadav, S. K. (2020). Extraction of pectin from black carrot pomace using intermittent microwave, ultrasound and conventional heating: Kinetics, characterization and process economics. *Food Hydrocolloids*, *102*(December 2019), 105592. https://doi.org/10.1016/j.foodhyd.2019.105592

Turker, N., Aksay, S. & Ekiz, H. I. (2004). Effect of storage temperature on the stability of anthocyanins of a fermented black carrot (Daucus carota var. L.) beverage: Shalgam. *Journal of Agricultural and Food Chemistry*, *52*(12), 3807–3813. https://doi.org/10.1021/jf049863s

Ünal, M. Ü. & Bellur, E. (2009). Extraction and characterisation of pectin methylesterase from black carrot (Daucus carota L.). *Food Chemistry*, *116*(4), 836–840. https://doi.org/10.1016/j.foodchem.2009.03.031

Wrolstad, R. E. (2004). Anthocyanin Pigments-Bioactivity and Coloring Properties. *Food Chemistry and Toxicology*, *69*(5), 419–425.

Young, A. J. & Lowe, G. L. (2018). Carotenoids—antioxidant properties. *Antioxidants*, *7*(2), 10–13. https://doi.org/10.3390/antiox7020028

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 88-100
DOI: https://doi.org/10.31015/jaefs.2023.1.10

Production and characterization of crude proteases from Halobacillus salinus strain DZ28 newly isolated from salt lake sediments in Algeria and their use as detergent bioadditives

Samir Medjekal[1] • **Mouloud Ghadbane**[2] • **Hani Belhadj**[3] • **Laid Benderradji**[2]

[1] Department of Biochemistry and Microbiology, Faculty of Science, University Mohamed Boudiaf of M'sila, Po. Box 166, Ichbilia, M'sila 28000, Algeria

[2] Department of Life Sciences and Nature, Faculty of Science, University Mohamed Boudiaf of M'sila, Po. Box 166, Ichbilia, M'sila 28000, Algeria

[3] Laboratory of Applied Microbiology, Faculty of Life Sciences and Nature, University Ferhat Abbas of Sétif, Campus El Baz, Sétif 19000, Algeria

Citation: Medjekal, S., Ghadbane, M., Belhadj, H., Benderradji, L. (2023). Production and characterization of crude proteases from Halobacillus salinus strain DZ28 newly isolated from salt lake sediments in Algeria and their use as detergent bioadditives. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 88-100

Received: 22 April 2022
Revised: 20 September 2022
Accepted: 24 September 2022
Published Online: 17 February 2023

Correspondence: Samir Medjekal
E-mail: samir.medjekal@univ-msila.dz

Abstract

New Algerian Gram-positive, rod-shaped, endospore-forming, salt-philic bacteria (DZ28 strain) that overproduce extracellular alkaline proteases have been isolated from salt lake deposits in Lake Oubeira, El Taref. Strain DZ28 was assigned as Halobacillus salinus DZ28 on the basis of phenotypic properties and 16S rDNA gene sequencing (ripotyping). The maximum protease activity registered after 36 hours of incubation in optimized medium at 30° C was 19,000 U / ml in a shaking bottle culture at 160 rpm. The crude extract protease showed optimal activity at 60 ° C temperature and pH 12. It is actively inhibited by PMSF and DIFP, indicating that it belongs to the serine protease family. Interestingly, the crude extract protease was not only very stable to nonionic surfactants and oxidants, but also showed high stability and compatibility with some commercial detergents. It retaining more than 100% of its initial activity after pre-incubation for 1 h at 40°C with ISIS, followed by Pril (98%), Tide (95%) and Dixan (90%). More curiously, the wash overall performance evaluation discovered that it may dispose of blood-stains remove at 40°C for 1 h with low supplementation (500 U/mL). This is the first report of a protease from Halobacillus salinus and has potential as a promising candidate for future applications as a bioadditive for detergent formulations.

Keywords: *Halobacillus salinus*, Protease, Process optimization, Laundry detergent, Wash performance

INTRODUCTION

Proteases denote an enzyme group with catalytic activity involving in various processes such as digestion, cell growth and apoptosis, blood coagulation, as well as protein catabolism and so others (Chew et al., 2019; Gurumallesh et al., 2019). Based on data from Allied Market Research, entitled, "Enzymes Market, by Type, Source, Reaction Type, and Application: Global Opportunity Analysis and Industry Forecast, 2018-2024", at the beginning of 2017 the global enzymes market was evaluated at $7,082 million. Hence, it recorded compound annual growth rate (CAGR) of 5.7% from 2018 to 2024 and it is estimated to reach $10,519 million in 2024. More than 60 % of commercially available enzymes worldwide are proteases (Gurumallesh et al., 2019). As the first commercial protease produced by Bacillus licheniformis in the 60s of the last century is an alkaline protease, researches focused more and more of such Bacillus derived-biomolecules with high catalytic activity and stability whilst keeping low production cost (dos Santos Aguilar and Sato, 2018; Raval et al., 2014).

Alkaline proteases enzymes are largely used by the industry (Maghsoodi et

al., 2013; Annamalai et al., 2014; Liu and Kokare, 2017;) in particular as additives in detergent formulations (Chew et al., 2019), registering 89% of the entire selling (Baweja et al., 2017). Of particular interest, proteases from extremophiles gained more attention over their classical strains counterparts regarding their promising performances and broad applicability. These enzymes are usually further benefits namely high activity in non-physiological conditions, such as extreme pH or temperature, intensive calcium chelating agents, and detergents solvent-tolerance, substrate selectivity and stability. Due to these unique properties, thermozymes are of tremendous importance for industrial applications and, accordingly, screening for novel biocatalysts from extremophiles represents a valuable alternative to elaborative engineering procedures for the optimization of available enzymes from mesophiles (Mechri et al., 2017a,b; Mechri et al., 2019a,b).

As a result of growing interest in proteolytic enzymes application by different industries like laundry detergents, wastewater treatment and tanning factories, researchers are looking into the diversity of salt lake deposits and salt resistant microorganism with prospetive biotechnology applications. Bacillus strains are often preferred as major sources for commercial alkaline proteases due to their exceptional ability to secrete large amounts of highly active enzymes, that are more stable at high temperature and pH (Nazari and Mehrabi, 2019). Bacillus licheniformis, Bacillus subtilis, Bacillus amyloliquefaciens, and Bacillus pumilus were traditionally the extra wide utilized species in industry operating with alkaline proteases (Baweja et al., 2016; Habicher et al., 2019). As far as we know, the protease capacity of Bacillus atrophaeus, which has been registered as a bioactive substance in surfactants, has not been described so far. Emphasizing the extremophile origins of Bacillus atrophaeus, its contribution to protease synthesis and improvement of detergent formulation and commercially available (Rahem et al., 2021). In this context, the present study reports, for the first time, on the optimization and biochemical characterization of the detergent-stable crude extract alkaline protease from Halobacillus salinus isolated from the saline Lake Oubeïra at the Province of El-Tarf (Algeria). It also provides basic information on the potential use of this crude enzyme as a prospective candidate for future applications in detergent formulations.

MATERIALS AND METHODS

Strain isolation and culture conditions

Five sediments samples were collected from of the Oubeïra salt lake (Algeria, El Taref Province (GPS coordinates: latitude: 36 ° 50`38.246 "N, longitude: 8 ° 23 '14 .695" E)) using a sediment gripper. All of the samples were kept at 4°C until use. Once brought to the laboratory, samples were heat-treated (20 min at 90°C) to kill vegetative cells and used to inoculate skimmed milk agar plates (SMAP). The protease enzymatic potential for strain isolates was evaluated. These microbial strains were screened for the qualitative, quantitative protease activity and their ability to hydrolyze casein. Initial screening of protease activity was carried out using a plate assay in a SMAP medium. The solid medium contains in g/L: peptone 5, yeast extract 3, skimmed milk 250 mL, and agar, 20 at pH 7. The culture plates were incubated overnight at 30°C and colonies surrounded by clear halos, upon colonies were selected as putative protease producers. Among these isolates, a strain designed as DZ28, resulted in a large clear zone of hydrolysis and a high proteolytic potency (4,000 U/mL) in a casein-based initial liquid medium was selected for further experimental study.

A first growth medium for protease production at pH 7 comprised (in g/L): casein, 10; yeast extract, 1; CaCl2, 0.5; K2HPO4, 0.25; and KH2PO4, 0.25. In each time, one factor was altered to investigate individually the impacts of the different parameters on protease production. Various carbon sources (casein, gelatin, glucose, fructose, galactose, sucrose, and maltose) were individually selected then added to the production broth (100 ml) at a concentration of 5 g. Used / L. The impacts of the finest carbon source (casein) concentration on protease production were evaluated on adding up 5, 10, 15, 20, and 25 g / l casein to the production broth undergoing the overhead conditions. Different organic matter were investigated (yeast peptone, yeast extract, beef extract, and soya peptone) and inorganic compounds (ammonium chloride, ammonium sulphate, NaNO3, NH4Cl; (NH4)2SO4, and sodium nitrate) nitrogen sources, at a concentration of 2 g/L, were also studied in a medium containing casein at 10 g/L as the only carbon and energy source. The ideal source of organic nitrogen for protease synthesis was found to be yeast extract and their various levels were evaluated (2, 4, 6, 8, and 10 g / L) to recognize the ideal concentration needed for maximum protease activity. As reported by Jaouadi et al. (2009) an optimum growth medium for protease production at pH 7 composed of (g / l): casein, 10 g; yeast extract, 4 g; K2HPO4, 1.5 g; KH2PO4, 1.5 g; CaCl2, 1 g; 1% (v / v) and trace element. At 120 ° C for 20 minutes the medium was autoclaved and Incubations were accomplished in a 1000 ml Erlenmeyer flask with a functioning size of 100 ml at 30 ° C for 36 hours in a rotary shaker (160 rpm). To estimate bacterial growth, optical density was measured at 600 nm and transformed to cell dry weight (g / l) founded on the biomass vs. cell dry weight control. The cell-free supernatant was collected after centrifugation 8000 g, 40 minutes at 4 ° C and be as a source of protease in subsequent additional experiments.

Bacterial strain identification

Analytical profiling index (API) strip examinations and 16S rDNA gene sequencing (ribotyping) were carried out to identify the genus that's the strain appertains. The API 50 CH strip (bioMérieux, SA, Marcyl`Etoile, France)

was utilized to investigate the phenotypic, biochemical and physiological properties of the DZ28 strain in accordance with the manufacturer's guidance. The 16S rDNA gene was amplified by PCR using forward primer 5'AGAGTTTGATCCTGGCTCAG3' and reverse primer 5' GGTTACCTTGTTACGACTT3'. Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) was used to purify Genomic DNA of strain DZ28 then utilized like a template for PCR amplifications (30 cycles, 94°C for 40 s denaturation, 64°C for 45 s primer annealing, and 72°C for 90 s extension). Amplified ~1.5 kb PCR products were cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA), conducting to pDZ28-16S plasmid (this study). The E. coli DH5α (Invitrogen, Carlsbad, CA, USA) was utilized like host strain. Every recombinant clones of E. coli were grown in Luria-Bertani (LB) medium with the addition of ampicillin (100 µg/mL), IPTG (0.4 mM), and X-gal (360 µg/mL) for screening. Every DNA purification, DNA electrophoresis, ligation, restriction, and transformation were carried out according to previously described method (Sambrook et al. 1989).

DNA sequencing and bioinformatics analysis

The nucleotide sequence of the cloned 16S rDNA gene was determined on both strands using the BigDye Terminator Cycle Sequence Ready Reaction Kit and the automated DNA sequencer ABIPRISM® 3100 AvantGeneticAnalyzer (Applied Biosystems, Foster City, CA, USA). The obtained sequence was compared with sequences available in the public sequence databases and with the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) (Kim et al., 2012), a web-based tool for the identification of prokaryotes based on 16S rDNA gene sequences from type strains. Phylogenetic and molecular evolutionary genetic analysis was performed using MEGA software v. 4.1 Implementation. Distances and clusters were calculated using the neighbor-joining method. Alignment of multiple nucleotide sequences was performed using the software program BioEdit version 7.0.2 and the program Clustal W2 available on the servers of the European Bioinformatics Institute. (http://www.ebi.ac.uk/Tools/msa/clustalw2/)(Rahem et al., 2021).

Protease activity assays

Protease activity was investigated using Hammerstein casein (Merck, Darmstadt, Germany) as a substrate, as well as elsewhere (Jaouadi et al. 2012). Unless otherwise stated, to an appropriately diluted enzyme solution (0.5 ml), add 2.5 ml of 100 mM disodium hydrogen phosphate-NaOH buffer at pH 12, add 1 mM CaCl2 (buffer A) containing 10 g / l of casein, and add. Mixed and incubated. 15 minutes at 60 ° C. The reaction was stopped by adding 2.5 ml of 20% trichloroacetic acid (TCA). The mixture was left at room temperature for 30 minutes and undigested protein was removed by centrifugation at 14,000 rpm for 20 minutes. Next, 0.5 ml of clear supernatant was mixed with 2.5 ml of 500 mM Na2CO3 and 0.5 ml of FolinCiocalteus phenolic reagent and incubated for 30 minutes at room temperature. The absorbance of the resulting supernatant was measured at 660 nm with respect to the blank. Protease activity was measured spectrophotometrically by detecting tyrosine released during protease hydrolysis. Protease activity present in detergent solutions has been described elsewhere using N,N-dimethylated casein (DMC) and 2,4,6-trinitrobenzene sulfonic acid (TNBSA) as substrates. Measured at 450 nm by the method of (Jaouadi et al., 2009) and evaluated as a color indicator.

Biochemical characterization of the crude protease DZ28

Effects of some inhibitors, reducing agents, and metal ions on enzyme stability

Various inhibitors and divalent metal ions have been added to study the need for crude protease inhibitors, reducing agents, and metal ions. The experiment was carried out as described above using casein as a substrate after incubation at 40 ° C. for 1 hour.

Effects of different pH on enzyme activity and stability

Crude protease DZ28 activity was measured at a pH range of 2 to 13 at 60°C using casein as a substrate. The effect of pH on the crude protease DZ28 activity was calculated with measuring constantly the enzyme activity at 60°C over the pH range of 2–13 using the following buffers at 100 mM, supplemented with 1 mM CaCl2: glycine-HCl (pH 2-5), MES (pH 5-6), HEPES (pH 6-8), Tris-HCl (pH 8-9), glycine-NaOH (pH 9-11), bicarbonate-NaOH (pH 11-11.5), Na2HPO4-NaOH (pH 11.5-12), and sodium phosphate dibasic-NaOH (pH 12-13). Its pH stability was determined by preincubating at 40 ° C for 6 hours with different buffers at different pH values. Aliquots were removed and residual enzyme activity was measured at pH 12 and 60 ° C.

Effects of different temperature on enzyme activity and stability

To measure the temperature effect of the crude protease DZ28, enzyme activity was measured at various temperatures in the range of 40-100 ° C. The thermal stability of the crude protease DZ28 i was determined after enzyme incubation at different temperatures (50, 60, and 70°C) and pH 12 for 6 h with and without 1 mM CaCl2 and measuring the residual enzyme activity at specific time. The unheated crude protease was 100% and was considered as a control.

Effect(s) of some polyols and/or calcium on enzyme thermal stability

The effect of the addition of several polyols [PEG 1000, PEG 1500, PEG 6000, glycerin, sorbitol, mannitol, and xylitol] with a final concentration of 100 g / L on the thermal

stability of the crude protease DZ28 was investigated after preincubation at 70°C for 6 hours. Residual activity was determined at pH 12 and 60 ° C compared to two controls performed in the absence of polyol (I) and unincubated (NI). The effect of calcium (1 mM) alone and / or mannitol (100 g / L) on the thermal stability of the crude protease DZ28 after 6 hours of preincubation at 70 ° C was also investigated. The residual activity of the enzyme without additives was assumed to be 100% and was considered as a control.

Substrate specificity determination

The substrate specificity profile of the crude protease DZ28 was measured on natural (gelatin, casein, albumin, ovalbumin and keratin) and modified (albumin azure, azocasein and keratin azure) protein substrates. Enzyme activity was previously determined elsewhere in each substrate according to standard conditions (Jaouadi et al., 2013).

Performance evaluation of the crude protease DZ28

Effect of detergent additives and compatibility of enzyme with laundry detergents

Crude protease DZ28 was pre-incubated for 6 h at 40°C to study the effect of bleaches [hydrogen peroxide (H2O2), sodium perborate], surfactants [SDS, linear alkylbenzenesulfonate (LAS), sulfobetaine], non-ionic surfactants (Tween 20, Tween 40, Tween 80, and Triton X-100), anti-redeposition agents (Na2CO3, STPP, TAED, and Na2CMC) and other detergent additives on the enzyme stability. Residual activity was performed at 60 ° C and pH 12. The effect of H2O2 on the activity and stability of each enzyme was also monitored with 100 mM NaOH borate buffer under the above conditions. Crude enzyme activity (without any additive) was set to 100%.

Stability and compatibility of enzyme with laundry detergents

The stability and compatibility of the crude protease DZ28 with some currently commercially available liquid and solid detergents was investigated. Detergents liquid list included Dipex and Ecovax (Klin Productions, Sfax, Tunisia), Class (EJM, Sfax, Tunisia), Omino Bianco, Fairy, ISIS, and Pril (Henkel, Algeria), and Skip (Unilever, France). The solid detergents used were Ariel, Persil, and Tide (Procter & Gamble, Switzerland), OMO (Unilever, France), Dixan, Axion, and Nadhif (Henkel, Tunisia), and Det (Sodet, Sfax, Tunisia). In order to research their balance and compatibility, the above referred to industrial detergents had been diluted in tap water to acquire a final concentration of 7 mg/mL (to simulate washing conditions) (Vojcic et al. 2015). The endogenous proteases found in those laundry detergents had been inactivated with the aid of heating the diluted detergents for 1 h at 65°C according to Banik et al., (2004). A 500 U/mL of every crude protease DZ28 become shake-incubated with every laundry detergent for 1 h at 40°C, and residual activity become decided at finest pH and temperature of every used enzyme using DMC as a substrate. The enzyme activity of a control (with none detergent), incubated under similar conditions, was taken as 100%.

Removal of protein stains from cotton fabrics

A new cotton cloth (8 cm x 10 cm) was stained with blood and used to simulate wash conditions and measure the efficiency of the crude protease DZ28 as a detergent bio-additive compared to the commercial protease Alcalase ™. The stained pieces of cloth were shaken (200 rpm) with tap water and a commercially available isis detergent (7 mg / ml), and varied in a 1 liter beaker with a total volume of 100 ml at 40 ° C for 30 minutes. After the treatment, a piece of cloth was taken out, rinsed with water, dried, and visually observed to examine the effect of removing the enzyme stain. An untreated blood stain cloth was used as a control.

Statistical analysis

All measurements were performed on three independent replicas and enzyme-free control experiments were performed under the same conditions. Experimental results were expressed as repeat mean and standard deviation (mean ± SD).

Nucleotide sequences accession number

The nucleotide sequence data of 16S rDNA (1501 bp) gene from Halobacillus salinus DZ28 strain reported in this paper has been submitted to the GenBank/ENA/EMBL databases under accession number: MZ156960.

RESULTS AND DISCUSSION

Screening of protease-producing strains

In the current study, seven salt-tolerant bacterial strains are protease producers based on the pattern of clear zone formation in protein-containing media from 45 bacteria isolated from the deposits of Lake Oubeira (El Taref, Algeria). Using the ratio of clear zone diameter (on skim milk agar plate) to colony diameter served as an indicator for selecting strains with high protease production capacity. Seven isolates (DZ19, DZ25, DZ28, DZ33, DZ35, and DZ44) with the highest proportions (> 3.5 mm) were tested for protease production in liquid cultures. Of the seven strains, DZ28 showed the highest ratio of 4.3 mm and the highest extracellular protease activity (about 4,000 U / mL) after culturing in non-optimized medium for 36 hours, so all subsequent strains retained in the study.

Identification of the strain DZ28

A new isolated bacterium (termed DZ28) was recognized utilizing some molecular and catabolic methods. According to the Bergey's Manual of Systematic Bacteriology methods described in (Yabuuchi 2001), morphological, biochemical and physiological properties

happen in the DZ28 isolate in the form of bacilli, aerobic, gram-positive and rod-shaped. It was shown to be endospore formation oxidase, catalase positive, motile, colonies were round to slightly irregular and smooth. Carbohydrate degradation profiles of the isolate were additionally investigated using the API 50CH gallery test. Our results indicate that besides to monosaccharides, this strain is citrate, malate, glucose, glycerol, D-trehalose, ribose, D-xylose, D-galactose, D-fructose, D-mannitol, D-maltose, D-sucrose, D-turanose, D-tagatose, gluconate and lacate. It was shown to have metabolized L-aspartate and L-glutamate. Lactose, sorbitol, glycogen, L-xylose, xylitol, drixose, darabinose, starch, adonitol, sorbose, erythritol, inulin, D-arabitol, L-arabitol, capric acid, adipic acid, phenylacetic acid, propionic acid and glycine are not metabolized like energy origins. For that reason, the whole results acquired concerning the biochemical and physiological properties and phenotype of this isolate completely established that DZ28 strain belongs to the genus Halobacillus (Table 1).

A phylogenetic tree based on the 16S rDNA gene Fig. 1 reveal that the novel isolates clustered with members of the genus related Bacillus, the nearest neighbor being Halobacillus salinus HSL-3T with an average similarity of 98.60% (accession no.: AF500003). Based on the results obtained in the course of the present study, we suggest the assignment of this isolate (accession no. MZ156960) as Halobacillus salinus strain DZ28.

Table 1. Phenotypic, physiologic, and biochemical characteristics of the DZ28 isolate.

Characterization features of the strain DZ28		
Phenotypic characteristics	Colony density	Translucent
	Colony morphology	Smooth circular to slightly irregular
	Cell shape	Ellipsoidal
	Cell arrangement	Single/pairs
	Motile	+
	Gram	+
Physiological characteristics	Temperature range (°C)	10–45 (30)
	pH range	6–9 (8)
	NaCl range (%)	2–20 (10)
Biochemical characteristics	Catalase	+
	Oxidase	+
	Nitrate reduction	-
	Sporulation	+
	ß-galactosidase	+
	Arginine Dihydrolase	-
	Lysine Decarboxylase	-
	Ornithine Decarboxylase	-
	Citrate	-
	H_2S	-
	Urease	-
	Tryptophane Desaminase	-
	Indol	-
	Voges-Proskauer	+
	Aesculin	+
	Casein	+
	ONPG	+
	Gelatin	-
	D-Glucose	+
	D-Mannitol	+
	D-Fructose	+
	D-Maltose	+
	D-Raffinose	+
	D-Sucrose	+
	D-Melibiose	+
	D-Galactose	+
	D-Trehalose	+

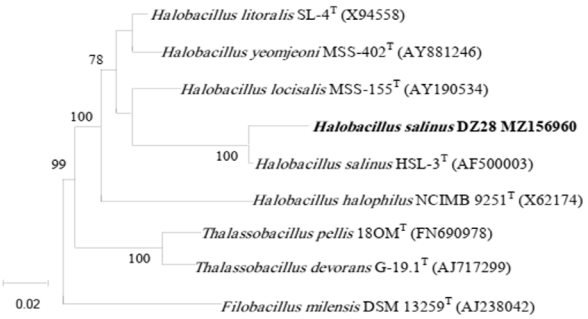


Figure 1. A phylogenetic tree derived from 16S rDNA gene sequence specifying the position of the DZ28 strain (accession number MZ156960) within the radiation of the genus Halobacillus. Bar, 0.02 nt substitutions per base. The node number (> 50%) designates support for internal branches in the tree acquired by bootstrap analysis (percentage of 1000 bootstraps). The NCBI accession number is shown in parentheses.

Protease production by strain DZ28

No defined medium has been established for the best production of proteases from different microbial sources. Each strain has its own special conditions for maximum enzyme production (Zhu and Zhang, 2019). Protease production was tested in the initial medium, containing 5 g/L of different carbohydrates. Results conclude that, the best carbon source for protease production was casein (4,000 U/mL) followed by gelatin (2,500 U/mL). However, enzyme production was significantly low (570 and 500 U/mL) when strain DZ28 was grown on glucose and sucrose, respectively, and was nearly the same as that of the control without carbon source. Since casein was the best carbon source, the effect of its various concentrations on protease production was studied. The optimum concentration of casein for protease production was 10 g/L (10,300 U/mL). Different workers have used different organic nitrogen sources (simple or complex), inorganic nitrogen sources and amino acids for enhancing protease production. The best nitrogen source for protease production was yeast extract, 12,500 U/mL; followed by beef extract, 11,230 U/mL and (NH4)2SO4, 10,250 U/mL. Protease production was significantly lower with NH4Cl, 855 U/mL and NaNO3, 1,060 U/mL as nitrogen sources. Based on these observations, yeast extract was selected and its various concentrations were tested for the protease production. Maximum protease activity was achieved at a concentration of 4 g/L (14,900 U/mL), giving about 19.22-fold enzyme activity, compared to the medium without nitrogen sources (775 U/mL). In the medium containing (in g/L): casein, 10; meat extract, 4; CaCl2, 1; K2HPO4, 1.5; and KH2PO4, 1.5; the addition of trace elements at 2% (v/v) significantly improved protease production by 1.27-folds, reaching 19,000 U/mL. Considering the overall modifications, this last optimized medium was retained for all further studies. Under this particular condition, the enzyme production started after a 4 h lag phase and then increased exponentially and concomitantly with the increase on cellular growth and reached the maximum within 36 h of cultivation Fig. 2.

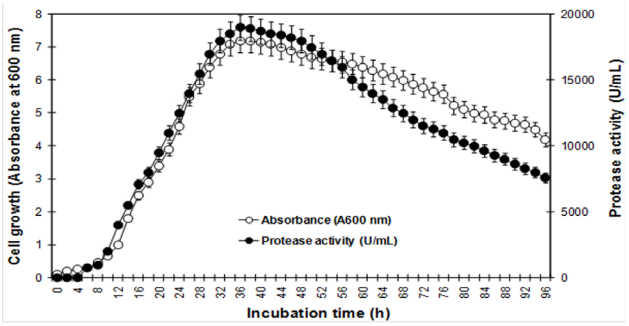


Figure 2. Proteases production kinetics from the DZ28 strain. Switches gradually in cell proliferation (○) of the DZ28 strain and production (•) of the crude protease DZ28. Cell generation was managed after calculating absorbance at 600 nm then converted to dry weight (g / l) of cells. The results shown as mean (n = 3) ± standard deviation.

Biochemical characterization of the crude protease DZ28

Effects of some inhibitors, reducing agents, and metal ions on enzyme stability

As may be seen in Table 2 two well-known inhibitors of serine proteases; PMSF and DIFF actively inhibited crude enzyme activity. However, further inhibitors such as TPCK, TLCK, benzamidine and SBTI tested in this experiment resulted in no inhibitory effect against enzyme activity. Moreover, thiol reagents (NEM, iodoacetamid and DTNB) result in small-scale impact on enzyme activity. Based on these results, it can be concluded that our crude extracellular enzymes appertain to serine proteases families. In the existence of 2 mM EDTA and 10 mM EDTA like metalloprotease inhibitors, proteases have been found to keep 84% and 94% of their activity, respectively, indicating the non-existence of metal cofactors. The enzyme activity was conserved even after large-scale dialysis when the enzyme was opposed to buffer A containing 2 mM EGTA (results not presented). Insensitiveness against chelators is a beneficial quality with an important implication especially during detergent preparation since these agents were very useful like water softeners and spot eliminators (Rekik et al., 2019). As a matter of fact, moving of calcium out-off the powerful binding locations was related with a significant depletion in thermal stability since the serine protease family encloses two calcium binding sites (Lee and Jang, 2001).

Table 2. Effects of various inhibitors and reducing agents on the crude protease DZ28 stability. Protease activity measured in the absence of any inhibitor or reducing agent was taken as control (100%). Residual activity was measured at pH 12 at 60°C.

Inhibitor/reducing agent	Concentration	Residual protease activity (%) [a]
None	–	100 ± 2.5
PMSF	5 mM	0 ± 0.0
DIFP	5 mM	0 ± 0.0
SBTI	1 mg/mL	99 ± 2.5
TLCK	1 mM	97 ± 2.5
TPCK	1 mM	92 ± 2.2
Benzamidine	10 mM	101 ± 2.5
LD-DTT	10 mM	96 ± 2.4
2-ME	5 mM	95 ± 2.3
DTNB	5 mM	94 ± 2.3
NEM	2 mM	92 ± 2.2
Iodoacetamide	5 mM	82 ± 2.0
Leupeptin	50 µg/mL	93 ± 2.1
Pepstatin A	2 µg/mL	99 ± 2.5
EDTA	10 mM	94 ± 2.3
EGTA	2 mM	84 ± 2.1

[a] Values represent means of a three replicates, and ± standard errors are reported.

PMSF: phenylmethanesulfonyl fluoride; DIFP: diiodopropyl fluorophosphates; SBTI: soybean trypsin inhibitor; TPCK: *Na-p*-tosyl-L-phenylalanine chloromethyl ketone; TLCK: *Na-p*-tosyl-L-lysine chloromethyl ketone; LD-DTT: LD-dithiothreitol; 2-ME: 2-mercaptoethanol; DTNB: 5,5′-dithio-bis-2-nitro benzoic acid; NEM: *N*-ethylmalemide; EDTA: ethylene-diaminetetraacetic acid; EGTA: ethylene glycol-bis (b-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

The impacts of some metal ions on the extracted protease DZ28 stability were further studied (Table 3). Sequentially, crude protease activity was ameliorated by 145, 166, and 180%, following MgCl2, MnCl2, and CaCl2 at 1 mM addition in comparison with controls. Based on this results our enzyme is in need of Mn2+, Mg2+ and Ca2+ for its adequate action. In our experiment, adding Co2+ and Cu2+ have a positive impact on the extracted crude enzyme and a slight activity elevation was recorded. However, the enzyme was totally inhibited by Hg2+, Cd2 and Ni2+, and relatively within Fe2+. Including enzymes, ions recognized as toxic metal may bind to different organic ligands and will be toxic and responsible of proteins denaturation. Elevated crude protease activity using Mg2+, Mn2+, and Ca2+ may be explained by the protective results on the enzyme opposed to thermic denaturation by metal ions and consequently relax there principal character on keeping good activity at high level temperature degrees like was described by (Hadjidj et al., 2018).

Apparently, Ca2+ has a positive effect on the activity and stability of the crude protease DZ28 within preservation of its structure from autolysis. The data obtained are broadly consistent with the major trends of Bouacem et al describing the impacts of EDTA and Ca2+ on protease activity (Bouacem et al., 2015).

Table 3. Effects of some metal ions on the crude protease DZ28stability. The non-treated enzyme to which 2 mM EGTA were added was considered as 100%. Residual activity was measured at pH 12 at 60°C.

Metal ions at 1 mM	Origin	Residual protease activity (%) [a]
None	–	100 ± 2.5
Ca^{2+}	$CaCl_2$	180 ± 4.5
Mn^{2+}	$MnSO_4$	166 ± 4.1
Mg^{2+}	$MgSO_4$	145 ± 3.8
Cu^{2+}	$CuSO_4$	101 ± 2.5
Zn^{2+}	$ZnSO_4$	90 ± 2.1
Co^{2+}	$CoSO_4$	102 ± 2.5
Fe^{2+}	$FeSO_4$	75 ± 1.9
Ni^{2+}	$NiCl_2$	0 ± 0.0
Hg^{2+}	$HgCl_2$	0 ± 0.0
Cd^{2+}	$CdCl_2$	0 ± 0.0

[a] Values represent means of a three replicates, and ± standard errors are reported.

Effects of different pH and temperature on enzyme activity and stability

As shown in Figure 3a the crude protease DZ28 was active over a large pH range (2-13) and was optimal at pH 12. Respectively, a relative activity was observed 60% and 50% at pH 6.5 and 13. In our study, the effect of pH on enzyme stability profile exhibited that the extracted enzyme was very active in a large pH range from 8-13. Figure 3b. The crude protease DZ28 half-lives at different pH; 7, 8, 9, 10, 11 and 12 were 345, 300, 255, 210, 165 and 120 minutes, respectively. In comparison with the major and large commercial detergents enzymes our values were further competent as well as Alcalase ™ extrated from Bacillus licheniformis, with large activity between 8-9 pH values (Beg and Gupta, 2003) and the produced Savinase ™ from Bacillus lentus, with large activity between pH value of 8-10 (Beg and Gupta, 2003). These results further validate the good potentiality of the crude protease DZ28 in later manufacturing utilizations which requires enzymatic strength above large pH range (9-11) (Gupta et al., 2002). Furthermore, our crude protease DZ28 was ideally operative at 50 ° C in the presence of 1 mM Ca2+ At pH 12 and in the absence of CaCl2 and at 60 ° C applying casein like a substrate, (Fig. 3C). The extracted protease DZ28 half-lives away of additives were 255, 180 and 90 minutes at 50, 60 and 70 ° C (Fig. 3D). As appear in figure. 3D the crude protease DZ28 half-life rises to 300, 225, and 135 minutes at 50, 60, and 70 ° C in company of 1 mM CaCl2. As a matter of fact, Ca2+ cations were

reported in previous studies to enhance the stability and the activity of Aeribacillus pallidus VP3 proteases (Mechri et al., 2017a). The thermic stability and thermic activity of the extracted protease were high up than some previous reported other proteases extracted from Bacillus strain (Benkiar et al., 2013; Rekik et al., 2019).

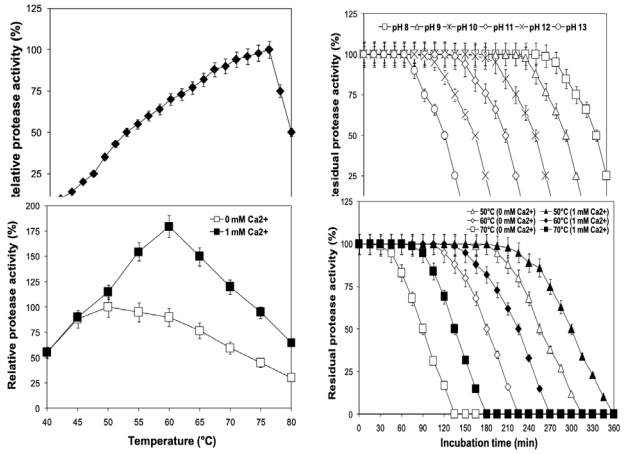


Figure 3. Effect of different pH values on (a) activity and (b) stability of the crude protease DZ28. The activity of the enzyme at pH 12 was supposed to be 100%. The buffers used for pH activity and stability are shown in Section 2. Effects of (C) thermoactivity and (D) thermostability of the crude protease DZ28. The enzyme was pre incubated at various temperatures of 50, 60, and 70 ° C in the absence or presence of 1 mM CaCl2. Remaining protease activity was measured from 0 to 6 hours at 15 minute intervals. The activity of the non-heated enzyme was assumed to be 100%.

Combined effect(s) of some polyols and/or calcium on the enzyme thermal stability

As shown in Figure 4a, addition of some polyols appears to improve the thermic stability of the enzyme in comparison to standard. As matter of fact, residual activities were 90, 70, 66, 60, 55, 41 and 35% in the company of mannitol, sorbitol, glycerol, PEG 1000, PEG 150, PEG 6000 and Xylitol respectively, 6 hours after the incubation at 70 ° C. Away from polyols the activity was 50% after incubation for 50-6 hours. The results shown in Figure 4a indicated that the elevated levels of enzyme activities were registered in the presence of mannitol like additive at a concentration of 100 g/L. In accordance to some reports in the literature, the thermic stabilities of the alkaline proteases of Bacillus safensis RH12 (Rekik et al., 2019) and Lysinibacillus fusiformis C250R (Mechri et al., 2017b) were improved in the presence of glycerol, mannitol, and some polyethylene glycol.

Data shown in Fig. 4b indicate that the addition of mannitol or CaCl2 relatively enhance the thermostability. Take note of, addition of mannitol only was better to CaCl2 solo, with half-lives all over 180 and 135 minutes, appropriately. Nevertheless, the presence of both additives ameliorated the half-lives at 70 ° C to be 225 minutes, designating a positive blend effect of mannitol and CaCl2 on the thermic stability of the extracted DZ28 strain protease. The addition of polyols causes changes in enzyme's micoenvironment which can boost and elevate their thermal stability (Steuer et al., 2009). The hydroxyl groups of polyols stand on their protective effect (Omrane Benmrad et al. 2019). In actual fact, polyols addition protects against enzyme molecules denaturation by elevating the hydrophobic interactivities inside the protein fragments that come further resistant to thermic inactivation (Joo and Chang, 2005).

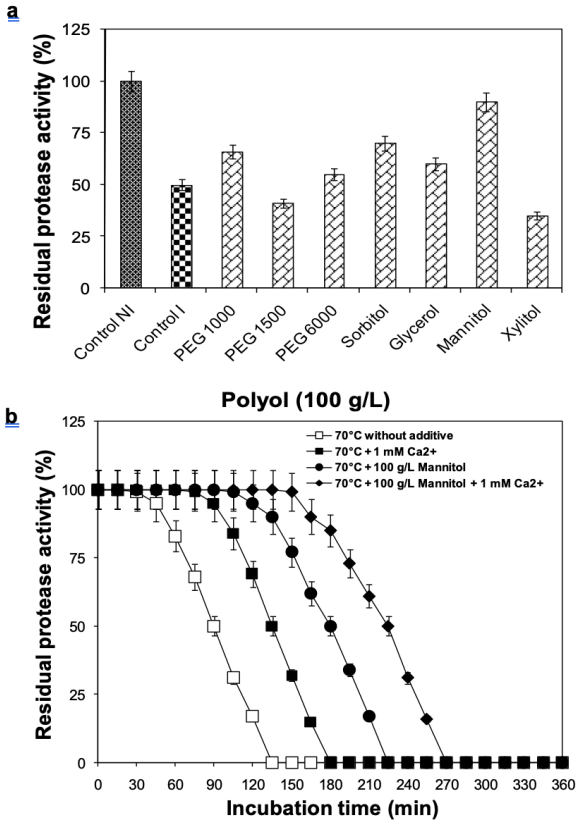


Figure 4(a). The effect of some polyols on the thermal stability of proteases. The crude protease DZ28 was heated to 70 ° C for 6 hours in the absence or presence of various polyols (10%). The remaining enzyme activity was determined under optimal conditions. The vertical bar indicates the standard error of the mean (n = 3). Fig. 4. (b) Combined effect of mannitol and calcium on the thermal stability of the crude protease DZ28 at 70 ° C. The enzyme was preincubated in the absence (□) or presence of additives: 1 mM Ca2+ (■); 100 g / L mannitol (•); and 1 mM Ca2+ and 100 g /L mannitol (♦). Remaining protease activity was determined from 0 to 360 minutes at 15 minute intervals. The activity of the non-heated enzyme was considered to be 100%. Each point represents the mean (n = 3) ± standard deviation.

Substrate specificity profile

As shown in Table 4, natural substrates were degraded on different degrees by the extracted proteases. More effectively the extracted protease of the DZ28 strain was in case of casein more than ovalbumin, albumin, keratin and gelatin. These results are in good agreement with other studies which have shown that protease extracted from Aeribacillus pallidus VP3, degraded preferably, casein followed by other substrate such as keratin, ovalbumin, gelatin, and BSA, which were slightly degraded by the extracted crude enzyme (Mechri et al. 2017a). Furthermore, alkaline protease manufactured by Brevibacillus brevis US575 demonstrated large range of activity against multiple substrates lake keratin, come after gelatin, casein, myoglobin, elastin and albumin (Jaouadi et al., 2013). Once the crude proteases from the DZ28 strain were examined against varied proteins substrates, elevated amounts of hydrolytic activity was registered with large number of substrates and the highest specificity were recorded with azocasein and albumin.

Performance evaluation of the crude protease from strain DZ28

Effects of some additive on enzyme stability

The crude protease DZ28 activity and stability, extracted from medium grown in the existence of anti redeposition agents and 1% perfume, mainly cationic (TTAB and CTAB),10% Na2•CMC, and zwitterionic (Zwittergent 3-12 and CHAPS) detergents agent, overall measurement results are summarized in (Table 5). Various commercially convenient modifiers or anionic surfactants, nonionic surfactants, and bleaching factors to 15% Tween 20 and powerful anionic surfactants, mainly LAS and SDS, were tested as opposed to Alcalase ™ 1 and 40 or Triton X100 by incubating at 40 °C for 6 hours the extracted protease DZ28 (Table 5). Our crude enzyme DZ28 kept 250 and 125% of its native action in comparison to 144 and 75% of Alcalase ™ following dealing with 15% hydrogen peroxide and 5% sodium perborate, as a consequence after bleaching, however the crude protease stay highly strong. Such protease activity and stability were fascinating because to our knowledge, extremely little wild-type proteases notified to be invariable in opposition to detergents, bleaches and oxidants. According to Jaouadi et al. (2010) Streptomyces p the AB1 strain stability was notably higher with the existence of 1.5% SDS, 15% H2O2 and 5% Tween 40. Furthermore the two proteases (BM1 and BM2) shared good quality in the existence of 1% Triton X100 and manifested solidity in the existence of 1% H2O2 and 0.1% SDS (Gupta et al., 1999; Haddar et al., 2009). Bleach balance has additionally been accomplished with the aid of using protein engineering (Radha and Gunasekaran, 2008). Our data presents several solutions to detergent formulations with cleaning bio-additives using the potential of crude proteases from the DZ28 strain.

Stability and compatibility of crude enzyme with commercial laundry detergents

As follows from the Fig. 5a, in comparison to Alcalase™, our crude protease DZ28 was exceptionally strong as much as the stable washing detergents and the commercial product tested at a concentration of 7 mg/mL, their starting activity was retained 100% in presence of ISIS and Pril (vs 90% within Alcalase™) and 98, 97, and 95 with OMO, Ariel, and Dixan, respectively (vs 90, 100, and 88% for Alcalase™, respectively). Additionally, fig. 5a shown the high stability of the crude protease DZ28 exceeding 90% in Det (as opposed 66% of Alcalase ™) despite 6 hours of incubating at 40 ° C as illustrated. Nevertheless, a low stability of the crude protease DZ28 was recorded in the existence of Axion and Persil (77%), while on the contrary Alcalase ™ maintained 100% and 94% of the initial action of Axion and Persil, respectively. It has been reported that Proteases VM10 and SSR1 and protease SSR1 (Venugopal and Saramma, 2006; Singh et al., 2001) retained at most 42% and 37% of their initial action, respectively, during incubation in the existence of Ariel below the identical conditions. Our observations, furthermore hold-up the effectiveness of the extracted crude protease DZ28 in some hereafter manufacturing implementations like a cleaning bio-additive for detergent preparations.

Stains removal from cotton fabrics

In the aim to evaluate the crude proteases DZ28 and Alcalase ™ for their ability to remove blood stains performances, several pieces of stained cotton cloth

Table 4. Substrate specificity profile of the crude protease DZ28.

Substrate	Concentration		Absorbance (nm)[a]	Relative protease activity (%)[a]
Natural protein	Casein	30 g/L	600	100 ± 2.5
	Albumin	30 g/L	600	90 ± 2.1
	Gelatin	30 g/L	600	50 ± 1.2
	Ovalbumin	30 g/L	600	38 ± 1.0
	Keratin	30 g/L	600	20 ± 0.8
Modified protein	Azo-casein	25 g/L	440	100 ± 2.5
	Albumin azure	25 g/L	440	87 ± 2.3
	Keratin azure	25 g/L	440	23 ± 0.9

[a] Values represent the means of three replicates, and ± standard errors are reported.

Table 5. Effect of some detergents on the crude protease DZ28 stability. The crude protease DZ28 and Alcalase™ were pre-incubated with each detergent additive for 6 h at 40°C and the residual activity were measured under the each assay standard conditions of each used enzyme. The activity is expressed as a percentage of the activity level in the absence of additives

Detergent	Concentration	Residual protease activity (%) [a]	
		Crude protease DZ28	Alcalase™
None	–	100 ± 2.5	100 ± 2.5
Tween 20	10% (v/v)	135 ± 3.6	142 ± 3.8
	15%	144 ± 3.9	137 ± 3.6
Tween 40	10% (v/v)	140 ± 3.8	129 ± 3.3
	15%	130 ± 3.4	110 ± 2.7
Tween 80	10% (v/v)	136 ± 3.4	108 ± 2.7
	15%	128 ± 3.3	106 ± 2.6
Triton X-100	10% (v/v)	180 ± 4.5	130 ± 3.2
	15%	165 ± 4.1	120 ± 3.2
SDS	1% (w/v)	91 ± 2.3	80 ± 2.0
	5%	72 ± 2.1	61 ± 1.5
LAS	1% (w/v)	99 ± 2.5	96 ± 2.3
	5%	93 ± 2.2	73 ± 1.9
Sulfobetaine	50 mM	102 ± 2.5	105 ± 2.6
H_2O_2*	10% (v/v)	250 ± 6.9	155 ± 3.6
	15%	190 ± 5.1	144 ± 3.5
Sodium perborate	2% (w/v)	125 ± 2.9	89 ± 2.1
	5%	105 ± 2.6	75 ± 2.1
TAED	10% (w/v)	134 ± 3.6	114 ± 2.7
$Na_2{\cdot}CO_3$	50 mM	98 ± 2.5	121 ± 3.2
$Na_2{\cdot}CMC$	10% (w/v)	101 ± 2.5	101 ± 2.5
STPP	25 mM	96 ± 2.4	92 ± 2.2
Zwittergent 3-12	15 mM	111 ± 2.7	113 ± 2.6
CHAPS	25 mM	98 ± 2.5	125 ± 3.2
CTAB	25 mM	104 ± 2.6	110 ± 2.7
TTAB	25 mM	107 ± 2.7	106 ± 2.6
Zeolithe	1% (w/v)	109 ± 2.7	90 ± 2.2
Perfume	1% (v/v)	141 ± 3.8	97 ± 2.5

were incubated under different conditions. As presented in Fig. 5b, the only limited cleaning performance was registered with ISIS detergent. When the commercial protease Alcalase ™ or the crude protease DZ28 were added on, the washing agent appears to ameliorate the cleaning procedure, like indicated by the quick elimination of blood stains in opposition to the solely washing agent. As a matter of fact, the crude protease DZ28 further help the proteinaceous substances elimination more easily compared the Alcalase ™ enzyme actually used. Besides, the mixture of crude protease DZ28 and solid ISIS detergent offered total stain elimination (Figure 5b). An important implication of these findings is that crude protease DZ28 may have the usefulness in future industrial applications as a cleaning bio-additive for detergent formulations. The data obtained are broadly consistent with the major tendencies reported the usefulness of alkaline proteases in improving the elimination of blood stains from cotton cloth, such as proteases extracted from Bacillus pumilus CBS (Jaouadi et al., 2008), Caldicoprobacter guelmensis D2C22T (Bouacem et al. 2015), and Bacillus licheniformis K7A (Hadjidj et al., 2018). As well, the oil-contaminated metagenomic sludge library protease Pro2T21opti seems to be more effective at alkaline pH range (pH 8-11) and low temperatures (30 ° C) (Gong et al., 2017). For all these reasons and others, we believe that the crude protease DZ28 is further successful.

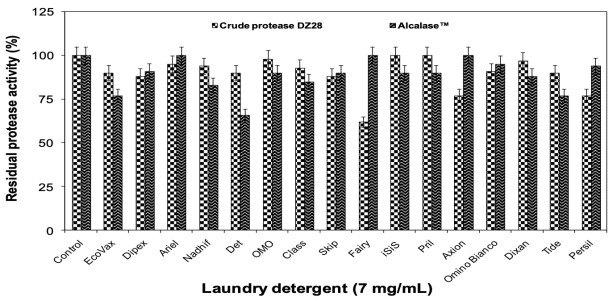


Figure 5a. Stability of crude proteases DZ28 and Alcalase ™ in the presence of liquid and solid detergents. The enzyme activity of the control sample, which contained no additives and was incubated under similar conditions, was considered to be 100%. Each point represents the average of three independent experiments. The vertical bar indicates the standard error of the mean (n = 3). One unit of protease activity was defined as the amount of enzyme required to catalyze the release of 1 µmole peptide bond from the DMC under the experimental conditions used.

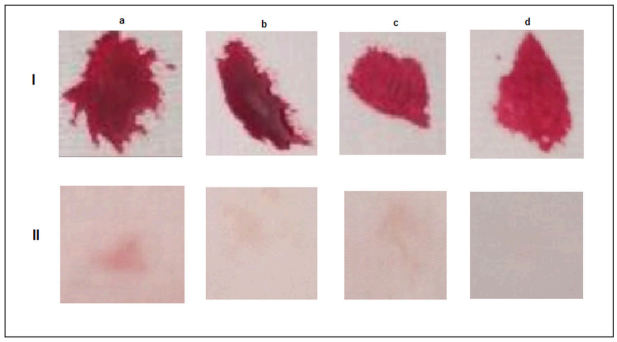


Figure 5b. Cleaning performance analysis test of our crude protease in the presence of the commercial detergent ISIS. (a) A cloth soiled with blood washed with tap water. (b) Blood stain cloth washed with ISIS detergent (7 mg / ml), (c) Blood stain cloth washed with ISIS containing Alcalase ™ 2.5 L (commercially available enzyme, 500 U / ml), (d) Blood stain cloth, washed with ISIS added with crude protease DZ28 (500 U/ml). I: untreated cloths (control) and II: treated cloths.

CONCLUSION

In summary, this study assesses the potential of the detergent-stable crude alkaline protease DZ28 by examining its interesting biochemical properties. This crude protease has exhibited some encouraging characteristics for biotechnologies and their applications that vary from other Bacillus proteases. Furthermore, to our knowledge, this is the first study to deal with the protease activity of Halobacillus salinus strain DZ28, isolated from an Algerian lake sediments. The results documented in our experiment indicate that by optimization and characterization of crude enzymes, stability at high pH values in the presence of specific metal ions, detergents, and detergent additives, this crude enzyme have some industrially useful properties. As a matter of fact, this enzyme may be suitable for a diversity of industrial uses, such as detergent synthesis, besides its potential applications in the laundry industry.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that they have no conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Annamalai, N., Rajeswari, M. V., Sahu, S.K., & Balasubramanian, T. (2014). Purification and characterization of solvent stable, alkaline protease from Bacillus firmus CAS 7 by microbial conversion of marine wastes and molecular mechanism underlying solvent stability. Process. Biochem, 49 (6): 1012-1019. https://doi.org/10.1016/j.procbio.2014.03.007

Banik, R. M., & Prakash, M. (2004). Laundry detergent compatibility of the alkaline protease from Bacillus cereus. Microbiol. Res, 159 (2): 135-140. https://doi.org/10.1016/j.micres.2004.01.002

Baweja, M., Nain, L., Kawarabayasi, Y., & Shukla, P. (2016). Current Technological Improvements in Enzymes toward Their Biotechnological Applications. Front. Microbiol, 7: 965. https://doi.org/10.3389/fmicb.2016.00965

Baweja, M., Singh, P. K., Sadaf, A., Tiwari, R., Nain, L., Khare, S. K., & Shukla, P. (2017). Cost effective characterization process and molecular dynamic simulation of detergent compatible alkaline protease from Bacillus pumilus strain MP27. Process. Biochem, 58: 199-203. https://doi.org/10.1016/j.procbio.2017.04.024

Beg, Q., & Gupta, R. (2003). Purification and characterization of an oxidation stable, thiol-dependent serine alkaline protease from Bacillus mojavensis. Enzyme. Microb. Technol, 32: 294-304. https://doi.org/10.1016/S0141-0229(02)00293-4

Benkiar, A., Nadia, Z. J., Badis, A., Rebzani, F., Soraya, B. T., Rekik, H., Naili, B., Ferradji, F. Z., Bejar, S., & Jaouadi, B. (2013). Biochemical and molecular characterization of a thermo- and detergent-stable alkaline serine keratinolytic protease from Bacillus circulans strain DZ100 for detergent formulations and feather-biodegradation process. Int. Biodeterior. Biodegrad, 83: 129-138. https://doi.

org/10.1016/j.ibiod.2013.05.014

Bouacem, K., Bouanane-Darenfed, A., Laribi-Habchi, H., Elhoul, M. B., Hmida-Sayari, A., Hacene, H., Ollivier, B., Fardeau, M. L., Jaouadi, B., & Bejar, S. (2015). Biochemical characterization of a detergent-stable serine alkaline protease from Caldicoprobacter guelmensis. Int. J. Biol. Macromol, 81: 299-307. https://doi.org/10.1016/j.ijbiomac. 2015.08. 011

Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem, 72: 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Chew, L. Y., Toh, G.T., & Ismail, A. (2019). Chapter 15 - Application of Proteases for the Production of Bioactive Peptides. In: Enzymes in Food Biotechnology, (Ed.) M. Kuddus, Academic Press, pp. 247-261.

Dos Santos, Aguilar, J. G., & Sato, H.H. (2018). Microbial proteases: production and application in obtaining protein hydrolysates. Food. Res. Int, 103: 253-262. https://doi.org/10.1016/j.foodres.2017.10.044

Gong, B. L., Mao, R. Q., Xiao, Y., Jia, M. L., Zhong, X. L., Liu, Y., Xu, P. L., & Li, G. (2017). Improvement of enzyme activity and soluble expression of an alkaline protease isolated from oil-polluted mud flat metagenome by random mutagenesis. Enzyme. Microb. Technol, 106: 97-105. https://doi.org/10.1016/j.enzmictec.2017.06.015

Gupta, R., Beg, Q. K., & Lorenz, P. (2002). Bacterial alkaline proteases: molecular approaches and industrial applications. Appl. Microbiol. Biotechnol, 59(1): 15-32. https://doi.org/10.1007/s00253-002-0975-y

Gupta, R., Gupta, K., Saxena, R., & Khan, S. (1999). Bleach-stable alkaline protease from Bacillus sp. Biotechnol. Lett, 21(21): 135-138. https://doi.org/10.1023/A:1005478117918

Gurumallesh, P., Alagu, K., Ramakrishnan, B., & Muthusamy, S. (2019). A systematic reconsideration on proteases. Int. J. Biol. Macromol, 128: 254-267. https://doi.org/10.1016/j.ijbiomac.2019.01.081

Habicher, T., John, A., Scholl, N., Daub, A., Klein, T., Philip, P., & Buchs, J. (2019). Introducing substrate limitations to overcome catabolite repression in a protease producing Bacillus licheniformis strain using membrane-based fed-batch shake flasks. Biotechnol. Bioeng, 116(6):1326-1340. https://doi.org/10.1002/bit.26948

Haddar, A., Agrebi, R., Bougatef, A., Hmidet, N., Sellami-Kamoun, A., & Nasri, M. (2009). Two detergent stable alkaline serine-proteases from Bacillus mojavensis A21: Purification, characterization and potential application as a laundry detergent additive. Bioresour. Technol, 100(13): 3366-3373. https://doi.org/10.1016/j.biortech.2009.01.061

Hadjidj, R., Badis, A., Mechri, S., Eddouaouda, K., Khelouia, L., Annane, R., El Hattab, M., Jaouadi, B. (2018). Purification, biochemical, and molecular characterization of novel protease from Bacillus licheniformis strain K7A. Int. J. Biol. Macromol, 114: 1033-1048. https://doi.org/10.1016/j.ijbiomac.2018.03.167

Jaouadi, B., Abdelmalek, B., Fodil, D., Ferradji, F. Z., Rekik, H., Zarai, N., & Bejar, S. (2010). Purification and characterization of a thermostable keratinolytic serine alkaline proteinase from Streptomyces sp. strain AB1 with high stability in organic solvents. Bioresour. Technol, 101(21): 8361-9. https://doi.org/10.1016/j.biortech.2010.05.066

Jaouadi, B., Ellouz-Chaabouni, S., Ben Ali, M., Ben Messaoud, E., Naili, B., Dhouib, A., & Bejar, S. (2009). Excellent laundry detergent compatibility and high dehairing ability of the Bacillus pumilus CBS alkaline proteinase (SAPB). Biotechnol. Bioprocess. Eng, 14: 503-512. https://doi.org/10.1007/s12257-008-0244-8

Jaouadi, B., Ellouz-Chaabouni, S., Rhimi, M., & Bejar, S. (2008). Biochemical and molecular characterization of a detergent-stable serine alkaline protease from Bacillus pumilus CBS with high catalytic efficiency. Biochim, 90(9): 1291-305. https://doi.org/10.1016/j.biochi.2008.03.004

Jaouadi, N. Z., Rekik, H., Badis, A., Trabelsi, S., Belhoul, M., Yahiaoui, A. B., Ben Aicha, H., Toumi, A., Bejar, S., & Jaouadi, B. (2013). Biochemical and molecular characterization of a serine keratinase from Brevibacillus brevis US575 with promising keratin-biodegradation and hide-dehairing activities. PLoS One, 8(10): e76722. https://doi.org/10.1371/journal.pone.0076722

Jaouadi, N. Z., Jaouadi, B., Aghajari, N., Bejar, S. (2012). The overexpression of the SAPB of Bacillus pumilus CBS and mutated sapB-L31I/T33S/N99Y alkaline proteases in Bacillus subtilis DB430: new attractive properties for the mutant enzyme. Bioresour. Technol, 105: 142-51. https://doi.org/10.1016/j.biortech.2011.11.115

Joo, H. S., & Chang, C.S. (2005). Production of protease from a new alkalophilic Bacillus sp. I-312 grown on soybean meal: optimization and some properties. Process. Biochem, 40(3-4): 1263-1270. https://doi.org/10.1016/j.procbio.2004.05.010

Kim, O.S., & Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., Won, S., Chun, J., (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. International Journal of Systematic and Evolutionary Microbiology, 62: 716–721.

Lee, S., & Jang, D.J. (2001). Progressive rearrangement of subtilisin Carlsberg into orderly and inflexible conformation with Ca (2+) binding. Biophys. J, 81(5): 2972-2988. https://doi.org/10.1016/S0006-3495(01)75937-1

Liu, X., Kokare, C. (2017). Microbial enzymes of use in industry. In: Biotechnology of Microbial Enzymes. Elsevier, pp. 267-298.

Maghsoodi, V., Kazemi, A., Nahid, P., Yaghmaei, S., & Sabzevari, M.A. (2013). Alkaline protease production by immobilized cells using Bacillus licheniformis. Sci. Iran, 20(3): 607-610. https://doi.org/10.1016/j.scient.2013.01.007

Mechri, S,, Berrouina, M. B. E., Benmrad, M. O., Jaouadi, N. Z., Rekik, H., Moujehed, E., Chebbi, A., Sayadi, S., Chamkha, M., & Bejar, S. (2017a). Characterization of a novel protease from Aeribacillus pallidus strain VP3 with potential biotechnological interest. Int J Biol Macromol, 94: 221-232. https://doi.org/10.1016/j.ijbiomac.2016.09.112

Mechri, S., Kriaa, M., Ben Elhoul Berrouina, M., Omrane Benmrad, M., Zaraî Jaouadi, N., Rekik, H., Bouacem, K., Bouanane-Darenfed, A., Chebbi, A., Sayadi, S., Chamkha, M., Bejar, S., & Jaouadi, B. (2017b). Optimized production and characterization of a detergent-stable protease from Lysinibacillus fusiformis C250R. Int. J. Biol. Macromol, 101: 383-397. https://doi.org/10.1016/j.ijbiomac.2017.03.051

Mechri, S., Bouacem, K., Jabeur, F., Mohamed, S., Addou, N. A., Dab, A., Bouraoui, A., Bouanane-Darenfed, A., Bejar, S., Hacène, H., Baciou, L., Lederer, F., & Jaouadi, B. (2019a). Purification and biochemical characterization of a

novel thermostable and halotolerant subtilisin SAPN, a serine protease from Melghiribacillus thermohalophilus Nari2AT for chitin extraction from crab and shrimp shell by-products. Extremophiles, 23(5): 529-547. https://doi.org/10.1007/s00792-019-01105-8

Mechri, S., Bouacem, K., Zaraî Jaouadi, N., Rekik, H., Ben Elhoul, M., Omrane Benmrad, M., Hacene, H., Bejar, S., Bouanane-Darenfed, A., & Jaouadi, B. (2019b). Identification of a novel protease from the thermophilic Anoxybacillus kamchatkensis M1V and its application as laundry detergent additive. Extremophiles, 23: 687-706. https://doi.org/10.1007/s00792-019-01123-6

Nazari, L., & Mehrabi, M. (2019). Purification and characterization of an extracellular thermotolerant alkaliphilic serine protease secreted from newly isolated Bacillus sp. DEM07 from a hot spring in Dehloran, Iran. Biocatal. Agric. Biotechnol, 18: 101053. https://doi.org/10.1016/j.bcab.2019.101053

Omrane Benmrad, M., Mechri, S., Zaraî Jaouadi, N., Ben Elhoul, M., Rekik, H., Sayadi, S, Bejar, S., Kechaou, N., Jaouadi, B. (2019). Purification and biochemical characterization of a novel thermostable protease from the oyster mushroom Pleurotus sajor-caju strain CTM10057 with industrial interest. BMC Biotechnol, 19(1): 43. https://doi.org/10.1186/s12896-019-0536-4

Radha, S., & Gunasekaran, P. (2008). Sustained expression of keratinase gene under PxylA and PamyL promoters in the recombinant Bacillus megaterium MS941. Bioresour. Technol, 99(13): 5528-5537. https://doi.org/10.1016/j.biortech.2007.10.052

Rahem, F.Z., Badis, A., Zenati, B., Mechri, S., Hadjidj, R., Rekik, H., Eddouaouda, K., Annane, R., & Jaouadi, B. (2021). Characterization of a novel serine alkaline protease from Bacillus atrophaeus NIJ as a thermophilic hydrocarbonoclastic strain and its application in laundry detergent formulations. Algerian J. Env. Sc. Technology, 7: (1) 1707-1724.

Raval, V. H., Pillai, S., Rawal, C. M., & Singh, S. P. (2014). Biochemical and structural characterization of a detergent-stable serine alkaline protease from seawater haloalkaliphilic bacteria. Process. Biochem, 49(6): 955-962. https://doi.org/10.1016/j.procbio.2014.03.014

Rekik, H., Zaraî Jaouadi, N., Gargouri, F., Bejar, W., Frikha, F., Jmal, N., Bejar, S., & Jaouadi, B. (2019). Production, purification and biochemical characterization of a novel detergent-stable serine alkaline protease from Bacillus safensis strain RH12. Int. J. Biol. Macromol, 121: 1227-1239. https://doi.org/10.1016/j.ijbiomac.2018.10.139

Sambrook, J., Fritsch, E., & Maniatis, T. (1989). Molecular cloning: A laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Singh, J., Batra, N., Sobti, R. C. (2001). Serine alkaline protease from a newly isolated Bacillus sp. SSR1. Process Biochem, 36(8-9): 781-785. https://doi.org/10.1016/S0032-9592(00)00275-2

Steuer, C., Heinonen, K. H., Kattner, L., & Klein, C. D. (2009). Optimization of assay conditions for dengue virus protease: effect of various polyols and nonionic detergents. J. Biomol. Screen, 14(9): 1102-8. https://doi.org/10.1177/1087057109344115

Takagi, H., Maeda, T., Ohtsu, I., Tsai, Y. C., & Nakamori, S. (1996). Restriction of substrate specificity of subtilisin E by introduction of a side chain into a conserved glycine residue. FEBS letters, 395(2-3): 127-132. https://doi.org/10.1016/0014-5793(96)01014-9

Venugopal, M., & Saramma, A. V. (2006). Characterization of alkaline protease from Vibrio fluvialis strain VM10 isolated from a mangrove sediment sample and its application as a laundry detergent additive. Process. Biochem, 41(6): 1239-1243. https://doi.org/10.1016/j.procbio.2005.12.025

Vojcic, L., Pitzler, C., Körfer, G., Jakob, F., Ronny, M., Maurer, K. H., & Schwaneberg, U. (2015). Advances in protease engineering for laundry detergents. New. Biotechnol, 32(6): 629-634. https://doi.org/10.1016/j.nbt.2014.12.010

Yabuuchi, E. (2001). Current topics on classification and nomenclature of bacteria. 7. Taxonomic outline of Archeae and bacteria in the Second Edition of Bergey's Manual of Systematic Bacteriology. Kansenshogaku Zasshi, 75(8): 653-655. https://doi.org/10.11150/kansenshogakuzasshi1970.75.653

Zhu, X., & Zhang, H. (2019) .Optimization of [CnPy]Cl (n=2,4,6) ionic liquid aqueous two-phase system extraction of papain using response surface methodology with box-behnken design. Process. Biochem, 77: 113-121. https://doi.org/10.1016/j.procbio.2018.11.016

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 101-106
DOI: https://doi.org/10.31015/jaefs.2023.1.11

Regeneration of safflower genotypes through callus mediated organogenesis using cotyledonary node explants

Süleyman Avcı[1] • **Mehmet Demir Kaya**[1]

[1] Department of Field Crops, Faculty of Agriculture, Eskişehir Osmangazi University, Eskişehir, Türkiye

Abstract

In this study, the cotyledon nodes of five safflower genotypes (Balcı, Linas, inbred lines 24, 25, and 55) were cultured for shoot regeneration via organogenesis in MS medium involving different TDZ (0.1, 0.5, and 1 mg L^{-1}) and NAA (0, 0.2, and 0.5 mg L^{-1}) doses. The highest rate of shoot forming calli was obtained from genotype 25 in all NAA and TDZ combinations, and there was no statistical difference between genotypes 24 and 25. The number of shoots per callus was found to be low in genotypes with a high rate of shoot forming calli. The maximum shoot number was obtained from the cultivar Linas on medium containing 1 mg L^{-1} TDZ, with 9.6 shoots/per callus and this value was followed by cultivar Balcı cultured at the same dose with 6.7 shoots/per callus. The rooting of safflower genotypes differed depending on the NAA content of the medium. Better rooting was achieved on medium with 2 mg L^{-1} NAA for Balcı, 1 mg L^{-1} NAA for Linas, and 0.1 mg L^{-1} NAA for genotypes 25 and 55. On the other hand, genotype 24 indicated rooting only on medium with 2 mg L^{-1} NAA, but it was very low. As a result; regeneration of safflower genotypes via callus-mediated organogenesis from cotyledonary explants was varied depending on TDZ and NAA doses, and many shoots were induced in Linas cultivar at 1 mg L^{-1} TDZ. However, the rooting of the regenerated shoots was quite low at different NAA doses.

Keywords: Safflower, Cotyledonary node, In vitro regeneration, TDZ, NAA

Citation: Avcı, S., Kaya, M.D. (2023). Regeneration of safflower genotypes through callus mediated organogenesis using cotyledonary node explants. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 101-106

Received: 04 November 2022
Revised: 22 January 2023
Accepted: 26 January 2023
Published Online: 22 February 2023

Correspondence: Süleyman Avcı
E-mail: savci@ogu.edu.tr

PUBLISHING

Copyright Author(s)
Available online at www.jaefs.com

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a mostly self-pollinated annual plant and is used both as a nutrition and energy crop (Knowles, 1969; Bérvillé et al., 2005). Safflower oil is important for human nutrition due to its rich unsaturated fatty acids such as oleic and linoleic acid (Liu et al., 2016; Katkade et al., 2018). On the other hand, increasing energy demand worldwide and concerns about environmental protection rise the interest in bioenergy crops such as safflower. It has a production potential in very different climates and soil conditions (Abd El-Lattief, 2012; La Bella et al., 2019).

The main goal of safflower breeding programs is to develop new varieties with high seed yield, oil content and quality, wide adaptability, and resistance to diseases and insects (Babaoglu and Guzel, 2015). Plant tissue culture applications are widely used as contributor methods in conventional breeding programs and gene transfer manipulations, however, the efficient use of these techniques in the improvement of plants requires a high-frequency shoot regeneration from tissues and cells. On the other hand, plant regeneration is specific for

each genotype and is affected by the nutrient media, hormones and environmental factors.

Safflower regeneration is quite difficult and there are specific effects on safflower regeneration of genotype, seedling age, explant type, media components, plant growth regulators and other additives (Fan and Guo, 2013). A higher regeneration rate was obtained in studies using varieties originating from India (Vijaya Kumar et al., 2008; Sri Shilpa et al., 2010) and Australia (Belide et al., 2011) compared to studies using varieties originating from China (Yang et al., 2009), Turkey (Başalma et al., 2008) and Iran (Motamedi et al., 2011). In addition, mostly cotyledon and hypocotyl segments were preferred as explant in these studies and callus-based shoot bud regeneration was achieved (Mandal and Gupta, 2001; Walia et al., 2005 Yang et al., 2009). On the orher hand, the use of cotyledonary nodes for *Agrobacterim*-mediated transformation resulted in genotype-independent regeneration in MS medium supplemented with BAP (6-Benzylaminopurine), NAA (Naphthalene acetic acid), and ascorbic acid in safflower (Patial et al., 2016).

In this study, there are 2 registered safflower cultivars (Balcı and Linas) and 3 hopeful inbred lines (24, 25, and 55) selected for winter hardiness, and their regeneration potential has not been determined by using cotyledonary nodes. The aim of this study was to reveal the effect of TDZ and NAA combinations on direct shoot regeneration from cotyledonary nodes in these safflower genotypes.

MATERIALS AND METHODS

Seeds of five safflower genotypes, 2 commercial cultivars (Balcı and Linas) and 3 inbred lines (24, 25 and 55) selected for winter hardiness, were used to establish callus mediated shoot regeneration and their names, flower colors and spininess are indicated in Table 1. Two plant growth regulators, 1-Naphthaleneacetic acid [(NAA), (Sigma-Aldrich product no: N0640)] and Thidiazuron [(TDZ), (Sigma-Aldrich product no: 45686)] were used for shoot bud regeneration of safflower genotypes. NAA was also used in induction of rooting. Seed surface sterilization was performed with mercuric chloride (PubChem CID: 24085) as described below.

The design of experiment was a completely randomized involving three factors with three replications. First of these factors was safflower genotypes, second was doses of NAA (0, 0.2, and 0.5 mg L^{-1}) and third was doses of TDZ (0.1, 0.5 and 1 mg L^{-1}).

Table 1. Flower colors and spininess of safflower genotypes.

Genotypes	Flower colors	Spininess
Balcı	Yellow	Spiny
Linas	Red	Spiny
Line 25	Yellow	Very few spines
Line 55	Orange/Red	Spiny
Line 24	Yellow	Few spines

First of all, the seeds were washed with tap water for 10 minutes and then were treated with 70% alcohol for 2 minutes and then with 0.1% of mercuric chloride ($HgCl_2$) for 5 minutes. Finally, these seeds were rinsed 5 times with sterile distilled water and were planted on 60 × 15 mm petri dishes including 10 ml of ¼ MS media (Murashige and Skoog, 1962) with 0.75 % sucrose.

These seeds were cultured at 25 °C in the dark condition and 5-6 days after germination (Fig. 1a) , the cotyledon nodes without primary leaves were moved to the full-strength MS medium including 3 % sucrose in different combinations of TDZ and NAA by cutting half of the cotyledon leaves (Fig. 1b) to induce shoot bud regeneration.

These cultures were initially maintained for 2 weeks at 25 °C in darkness and then incubated at the same temperature under 16/8 hours (day/night) at 50-60 % humidity. After 4-6 weeks from the beginning of the culture, the rates of shoot-forming callus (RSFC) were determined and these calli were moved to MS medium comprising 0.5 mg L^{-1} of kinetin with 3 % sucrose to induce shoot elongation. The number of shoots per callus (NSPC) was determined in these cultures two weeks later. Developed shoots were planted to half-strength MS medium involving 0.1, 0.5, 1, and 2 mg L^{-1} of NAA for root induction.

The data were analyzed by using JMP 14 statistical package program. Arcsin √x transformation was performed to values of RSFC. Significant mean values were compared with LSD test.

RESULTS AND DISCUSSION

Rates of shoot-forming callus

In general, hard calli with shoots were induced in all combinations. Analysis of variance indicated that RSFC was significantly influenced by the safflower genotypes, doses of NAA and doses of TDZ and their interactions (Table 2). The response of safflower genotypes to callus induction varied and the highest and lowest RSFC among safflower genotypes were in Line 25 and Line 55, respectively. These results confirmed the findings of Rajendra Prasad et al. (1991) and Mandal and Gupta (2001) on callus induction and regeneration of safflower.

While the effects of increasing NAA doses on RSFC were positive, the lowest dose of TDZ resulted in the highest RSFC (Table 2). No callus induction was obtained on the medium including 0.5 mg L^{-1} of TDZ in Line 55 (Table 3). On the other hand, Line 25 produced the highest RSFC as averaged value over all NAA and TDZ combinations. Although NAA was not required for shoot forming callus induction in this study (Table 3), other research indicated that the media enriched with combinations of auxin and cytokinin is widely used in tissue cultures to increase

callus induction (Baskaran et al., 2006; Soheilikhah et al., 2013; Ghasempour et al., 2014; Ali and Afrasiab, 2014).

Shoots were produced from calli after 4-6 weeks of the beginning of culture (Fig. 1c and Fig. 1 d). The NSPC varied with the safflower genotypes, and also a high RSFC did not produce high shoot regeneration (Table 2 and Table 4). Radhika et al. (2006) and Nikhil et al. (2014) reported that there were significant differences in shoot induction from calli in different safflower genotypes. In many studies, it has been determined that safflower genotypes with Indian and Australian origins (Mandal

Table 2. The results of variance analysis and differences between mean values of RSFC and NSPC resulting from cultured cotyledonary node segments of safflower genotypes at various TDZ and NAA doses. (Mean ± standard error).

Factors	Ratios of shoot-forming callus [(%), (RSFC)]	Number of shoots per callus (NSPC)
Safflower genotypes		
Balcı	88.5±3.30^{b†}	2.11±0.35^{b}
Linas	87.7±3.70^{b}	2.42±0.51^{a}
Line 24	92.7±2.89^{ab}	1.13±0.05^{c}
Line 25	98.1±1.28^{a}	1.17±0.06^{c}
Line 55	72.2±6.85^{c}	1.17±0.15^{c}
NAA doses (mg L^{-1})		
0	81.7±4.16^{b}	2.44±0.36^{a}
0.2	89.1±3.24^{a}	1.26±0.05^{b}
0.5	92.7±2.19^{a}	1.12±0.07^{b}
TDZ doses (mg L^{-1})		
0.1	94.5±1.90^{a}	1.21±0.07^{b}
0.5	81.2±4.23^{c}	1.31±0.10^{b}
1	87.8±3.23^{b}	2.29±0.36^{a}
Analysis of variance		
Genotypes (A)	**	**
NAA doses (B)	*	**
TDZ doses (C)	*	**
A × B	*	**
A × C	**	**
B × C	*	**
A × B × C	**	**

*, **: Significant level of 5% and 1%, respectively †: Different letters indicate different groups at the 5% level.

Table 3. The effect of different NAA and TDZ doses on shoot-forming callus ratio (%) in different safflower genotypes. (Mean ± standard error).

		TDZ doses (mg L^{-1})		
Safflower genotypes	**NAA doses (mg L^{-1})**	**0.1**	**0.5**	**1**
Balcı	0	83±8.34^{abc*}	100±0^{a}	75±14.45^{bcd}
	0.2	100±0^{a}	87±6.78^{abc}	93±6.78^{ab}
	0.5	83±16.97^{abc}	83±16.97^{abc}	92±8.48^{abc}
Linas	0	100±0^{a}	50±0de	100±0^{a}
	0.2	100±0^{a}	73±6.78^{cd}	100±0^{a}
	0.5	83±16.97^{abc}	92±8.48^{abc}	92±8.48^{abc}
Line 24	0	85±7.77^{abc}	75±14.75^{bcd}	100±0^{a}
	0.2	100±0^{a}	92±8.48^{abc}	83±16.97^{abc}
	0.5	100±0^{a}	100±0^{a}	100±0^{a}
Line 25	0	92±8.48^{abc}	100±0^{a}	100±0^{a}
	0.2	100±0^{a}	100±0^{a}	100±0^{a}
	0.5	100±0^{a}	100±0^{a}	92±8.48^{abc}
Line 55	0	92±8.48^{abc}	0±0^{f}	75±0^{bbd}
	0.2	100±0^{a}	83±16.97^{abc}	25±0^{e}
	0.5	100±0^{a}	83±8.48^{abc}	92±8.48^{abc}

* Different letters indicate different groups at the 5% level for genotype × NAA × TDZ interactions.

and Gupta, 2001; Vijaya Kumar et al., 2008; Sri Shilpa et al., 2010; Belide et al., 2011) showed higher regeneration than those with Turkish, Chinese and Iranian origins (Başalma et al., 2008; Yang et al., 2009; Motamedi et al., 2011).

In general, increasing NAA doses with combinations of 0.5 and 1 mg/l TDZ negatively affected the NSPC except for Line 55 as indicated in Table 4. The highest NSPC was found in Linas at 1 mg L^{-1} of TDZ without NAA and this genotype was followed by the Balcı at the same dose (Table 4). A wide range of TDZ + NAA combinations was used for high shoot regeneration of safflower. Nikhil et al. (2014) supported the finding that the high shoot regeneration of safflower in a medium comprising of low NAA and high TDZ combination.

Rooting

Elongated shoots without vitrification were transferred to the rooting medium (Fig. 1e) and it was clear in the first week whether there would be rooting or not of these shoots. (Fig. 1f). When rooting was delayed, the shoots completely died off, and regeneration of the green shoot did not occur over again even if roots were induced from the callus. All safflower genotypes were rooted at different NAA doses, even at low frequency (Table 5). Better rooting in Balcı was achieved at 2 mg L^{-1} of NAA, 1 mg L^{-1} in Linas, and 0.1 mg L^{-1} in Line 25 and Line 55 and also poor rooting was obtained in Line 24 at only 2 mg L^{-1} of NAA dose.

Rooting and subsequent acclimatization are among the most challenging issues for safflower regeneration (Sujatha, 2007). Rooting studies on safflower genotypes have reported successful rooting at different doses of NAA such as 0.1 mg L^{-1} (Mandal and Gupta, 2003; Walia et al., 2007), 0.5 mg L^{-1} (Radhika et al., 2006), 1 mg L^{-1} (Mandal and Gupta, 2001). Also, Yang et al. (2009) determined that a combination of 2 mg L^{-1} of NAA and 0.5 mg L^{-1} of IAA (Indole-3-acetic acid) promoted the rooting of safflower.

Table 4. The effect of various NAA and TDZ doses on the number of shoots per callus in different safflower genotypes. (Mean ± standard error).

		TDZ doses (mg L^{-1})		
Safflower genotypes	**NAA doses (mg L^{-1})**	**0.1**	**0.5**	**1**
Balcı	0	$1.0\pm0^{i*}$	2.2 ± 0.27^{de}	6.7 ± 0.4^{b}
	0.2	1.2 ± 0.11^{hı}	1.2 ± 0.11^{hı}	2.1 ± 0.48^{ef}
	0.5	2.0 ± 0.98^{efg}	1.0 ± 0^{i}	1.4 ± 0.44^{ghı}
Linas	0	$1.8\pm0.14^{e-h}$	3.0 ± 0^{c}	9.6 ± 0.08^{a}
	0.2	$1.5\pm0.05^{f-ı}$	1.4 ± 0.18^{hı}	$1.7\pm0.24^{e-ı}$
	0.5	1.0 ± 0^{i}	1.0 ± 0^{i}	1.0 ± 0^{i}
Line 24	0	1.0 ± 0^{i}	$1.8\pm0.14^{e-h}$	1.0 ± 0^{i}
	0.2	1.0 ± 0^{i}	1.4 ± 0.22^{ghı}	1.0 ± 0.06^{i}
	0.5	1.0±0	1.0 ± 0^{i}	1.0 ± 0^{i}
Line 25	0	1.1 ± 0.06^{hı}	1.3 ± 0.22^{hı}	$1.8\pm0.44^{e-h}$
	0.2	1.0 ± 0^{i}	1.0 ± 0^{i}	1.0 ± 0.06^{i}
	0.5	1.0 ± 0^{i}	1.2 ± 0.16^{hı}	1.1 ± 0.06^{hı}
Line 55	0	1.4 ± 0.44^{ghı}	0.0 ± 0^{j}	2.9 ± 0.6^{cd}
	0.2	1.0 ± 0.06^{i}	1.2 ± 0.14^{hı}	1.0 ± 0^{i}
	0.5	1.0 ± 0^{i}	1.0 ± 0^{i}	1.0 ± 0^{i}

* Different letters indicate different groups at the 5% level for genotype × NAA × TDZ interactions

Table 5. The observation of NAA doses on rooting in different safflower genotypes.

Safflower genotypes	NAA doses (mg L^{-1})			
	0.1	**0.5**	**1**	**2**
Balcı	+	-	-	++
Linas	+	-	++	-
Line 24	-	-	-	+
Line 25	++	-	-	+
Line 55	++	-	-	-

+low, +++++high

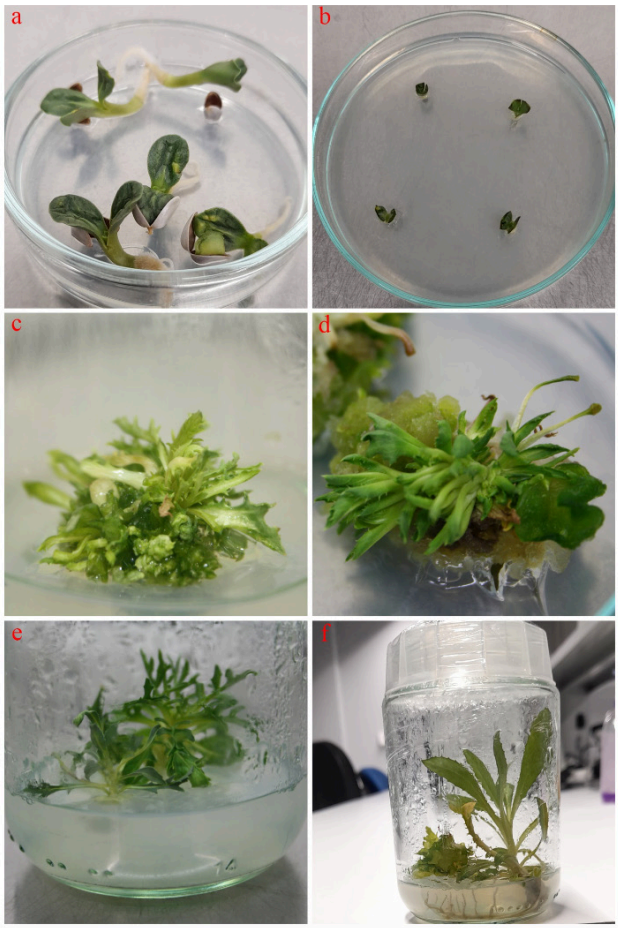


Figure 1. Callus-based shoot bud regeneration and rooting in different safflower genotypes. 1a: Stage of cotyledon node explants after germination from safflower seeds, 1b: Cotyledon node explants with half-cut cotyledon leaves, 1c and 1d: Shoots developing on callus in Linas and Balcı cultivars, 1e: Transferring of the elongated shoots into the rooting medium, and 1f: rooted safflower shoots.

CONCLUSION

In this study, callus-mediated organogenic shoot regeneration was obtained in different TDZ and NAA combinations by using cotyledon nodes in 2 cultivars and 3 inbred lines of safflowers. The NSPC was found to be low in genotypes with a high RSFC. Linas and Balcı cultivars produced high NSPC than inbred lines of safflower. Increasing doses of NAA and TDZ affected positively on RSFC and NSPC, respectively. It is possible to conclude that NAA has generally not any significant effect on the callus induction and shoot regeneration from cotyledonary segments of safflower. Rooting differed according to genotypes and occurred at a low frequency. In sum, Linas and Balcı indicated high number of shoots per callus in media enriched with 1 mg L^{-1} of TDZ without NAA.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

All authors declare that they have no conflicts of interest

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

This study was supported by Eskişehir Osmangazi University Scientific Research Projects Commission within the scope of the project coded 202123D04.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

Authors are thankful to Eskişehir Osmangazi University Scientific Research Projects Commission for their financial supports (Project No: 202123D04)

REFERENCES

Abd El-Lattief, E.A. (2012). Evaluation of 25 safflower genotypes for seed and oil yields under arid environment in upper Egypt. Asian Journal of Crop Science, 4(2): 72-79. Retrieved from https://scialert.net/abstract/?doi=ajcs.2012.72.79

Ali, N., Afrasiab, H. (2014). Effect of TIBA and other plant growth regulators on callogenic response from different explants of safflower (*Carthamus tinctorius*). International Journal of Agriculture And Biology, 16: 1112-1116. Retrieved from http://www.fspublishers.org/published_papers/44391_..pdf

Babaoglu, M., Guzel, M. (2015). Safflower (*Carthamus tinctorius* L.) breeding activities at Trakya Agricultural Research Institute. Ekin Journal of Crop Breeding and Genetics, 1(1): 20-25. Retrieved from https://dergipark.org.tr/tr/download/article-file/211565

Baskaran, P., Rajeswari, B.R., Jayabalan, N. (2006). Development of an in vitro regeneration system in sorghum [*Sorghum bicolor* (L.) Moench] using root transverse thin cell layers (tTCLs). Turkish Journal of Botany, 30: 1-9. Retrieved from https://journals.tubitak.gov.tr/botany/vol30/iss1/1/

Başalma, D., Uranbey, S., Mirici, S., Kolsarici, Ö. (2008). TDZ x IBA induced shoot regeneration from cotyledonary leaves and in vitro multiplication in safflower (*Carthamus tinctorius* L.). African Journal of Biotechnology, 8: 960-966. Retrieved from https://www.ajol.info/index.php/ajb/article/view/58585

Belide, S., Hac, L., Singh, S.P., Green, A.G., Wood, C.C. (2011). Agrobacterium-mediated transformation of safflower and the efficient recovery of transgenic plants via grafting. Plant Methods, 7(1): 12. https://doi.org/10.1186/1746-4811-7-12

Bérvillé, A., Gressel, J., McPherson, M., Breton, C., Good, A., Médail, F., Pinatel, C. (2005). Issues of ferality or potential for ferality in oats, olives, the Vigna Group, Ryegrass Species, Safflower, and Sugarcane. In: Crop Ferality and Volunteerism, CRC Press, 231–255. Retrieved from https://www.taylorfrancis.com/chapters/mono/10.1201/9781420037999-18/issues-ferality-potential-ferality-oats-olives-vigna-group-ryegrass-species-safflower-sugarcane-jonathan-gressel

Fan, L., Guo, M. (2013). Progress of safflower (*Carthamus tinctorius* L.) regeneration through tissue culture. Journal of Medical Colleges of PLA, 28(5), 289–301. https://doi.org/10.1016/S1000-1948(13)60045-3

Ghasempour, H., Soheilikhah, Z., Zebarjadi, A.R., Ghasempour, S., Karimi, N. (2014). In vitro micro propagation, callus induction and shoot regeneration in safflower L. cv. Lesaf'. Iranian Journal of Plant Physiology, 4 (2): 999-1004. Retrieved from https://journals.iau.ir/article_540310_fc3656ba47a7f941abfc499773945b81.pdf

Katkade, M.B., Syed, H.M., Andhale, R.R., Sontakke, M.D. (2018). Fatty acid profile and quality assessment of safflower (*Carthamus tinctorius*) oil. Journal of Pharmacognosy and Phytochemistry, 7(2): 3581-3585. Retrieved from https://www.phytojournal.com/archives/2018/vol7issue2/PartAX/7-2-364-260.pdf

Knowles, P.F. (1969). Centers of plant diversity and conservation of crop germplasm: Safflower. Economic Botany, 23: 324-349. Retrieved from https://link.springer.com/article/10.1007/BF02860678

La Bella, S., Tuttolomondo, T., Lazzeri, L., Matteo, R., Leto, C., Licata, M. (2019). An Agronomic Evaluation of New Safflower (*Carthamus tinctorius* L.) Germplasm for Seed and Oil Yields under Mediterranean Climate Conditions. Agronomy, 9(8): 468. https://doi.org/10.3390/agronomy9080468

Liu, L., Guan, L.L., Yang, Y.X. (2016). A review of fatty acids and genetic characterization of safflower (*Carthamus tinctorius* L.) seed oil. World Journal of Traditional Chinese Medicine, 2(2): 48-52. https://doi.org/10.15806/j.issn.2311-8571.2016.0006

Mandal, A.K.A., Gupta, S.D. (2001). Direct shoot organogenesis and plant regeneration in safflower. In Vitro Cellular & Developmental Biology, Plant, 37(1): 50-54. Retrieved from http://www.jstor.org/stable/4293417

Mandal, A.K.A., Dutta Gupta, S. (2003). Somatic embryogenesis of safflower: influence of auxin and ontogeny of somatic embryos. Plant Cell, Tissue and Organ Culture, 72(1): 27-31. https://doi.org/10.1023/a:1021264403398

Motamedi, J., Zebarjadi, A., Kahrizi, D., Salmanian, A.H. (2011). In vitro propagation and Agrobacterium-mediated transformation of safflower (*Carthamus tinctorius* L.) using a bacterial mutated aroA gene. Australian Journal of Crop Science, 5: 479-486. Retrieved from http://www.cropj.com/zebarjadi_5_4_2011_479_486.pdf

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nikhil, M., Dudhare, M.S., Jadhav, P.V., Moharil, M.P., Deshmukh, A.S. (2014). In vitro shoot regeneration and plantlet development in safflower (*Carthamus tinctorius* L.). The Bioscan 9(2): 551-555.

Patial, V., Krishna, R., Arya, G., Singh, V. K., Agarwal, M., Goel, S., Jagannath, A., Kumar, A. (2016). Development of an efficient, genotype independent plant regeneration and transformation protocol using cotyledonary nodes in safflower (*Carthamus tinctorius* L.). Journal of Plant Biochemistry and Biotechnology, 25(4): 421–432. https://doi.org/10.1007/s13562-016-0354-x

Radhika, K., Sujatha, M., Nageshwar Rao, T. (2006). Thidiazuron stimulates adventitious shoot regeneration in different safflower explants. Biologia Plantarum, 50: 174-179. https://doi.org/10.1007/s10535-006-0003-7

Rajendra Prasad, B., Khadeer, M.A., Seeta, P., Anwar, S.Y. (1991). In vitro induction of androgenic haploids in Safflower (*Carthamus tinctorius* L.). Plant Cell Reports, 10: 48–51. https://doi.org/10.1007/BF00233032

Soheilikhah, Z., Karimi, N., Ghasmpour, H.R., Zebarjad, A.R. (2013). Effects of saline and mannitol induced stress on some biochemical and physiological parameters of *Carthamus tinctorius* L. varieties callus cultures. Australian Journal of Crop Science, 7(12): 1866-1874. Retrieved from http://www.cropj.com/karimi_7_12_2013_1866_1874.pdf

Sri Shilpa, K., Dinesh Kumar, V., Sujatha, M. (2010). Agrobacterium-mediated genetic transformation of safflower (*Carthamus tinctorius* L.). Plant Cell, Tissue and Organ Culture, 103 (3): 387–401. https://doi.org/10.1007/s11240-010-9792-7

Sujatha M. (2007). Advances in safflower biotechnology. In: Functional Plant Science and Biotechnology. 1, Global Science Books, 160-170. Retrieved from http://www.globalsciencebooks.info/Online/GSBOnline/images/0706/FPSB_1(1)/FPSB_1(1)160-170o.pdf

Walia, N., Kaur, A. Babbar, S.B. (2005). In vitro regeneration of a high oil-yielding variety of safflower (*Carthamus tinctorius* var HUS-305). Journal of Plant Biochemistry and Biotechnology 14: 65–68. https://doi.org/10.1007/BF03263229

Walia, N., Kaur, A., Babbar, S.B. (2007). Proliferation and differentiation from endosperms of *Carthamus tinctorius*. Biologia Plantarum, 51(4): 749–753. https://doi.org/10.1007/s10535-007-0153-2

Vijaya Kumar, J., Ranjitha Kumari, B.D., Castaño, E. (2008). Cyclic somatic embryogenesis and efficient plant regeneration from callus of safflower. Biologia Plantarum, 52(3): 429-436. https://doi.org/10.1007/s10535-008-0087-3

Yang, J., Xiong, L.D., Li, T.H., Li, H.Y., Qu, Q., Fu, H.Q., Liu, X.M., Li, X.K. (2009). The effect of phytohormones on safflower regeneration plant. Zhong yao cai = Zhongyaocai = Journal of Chinese Medicinal Materials, 32(9): 1335-1338. PMID: 20034206. Retrieved from https://pubmed.ncbi.nlm.nih.gov/20034206/

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 107-116
DOI: https://doi.org/10.31015/jaefs.2023.1.12

Analysis of the foundations of market gardening activity in the Commune of Athiémé (Benin)

Marcelle-Agathe Monde[1] • **Claude Ahouangninou**[2] • **Djafarou Abdoulaye**[1]
Landry Denis Yawo Kiki[3] • **Samuel Massede**[4] • **Brice Tente**[1]

[1] Laboratoire de Biogéographie et Expertise Environnementale/UAC/ Bénin
[2] Laboratoire des Sciences Végétales, Horticoles et Forestières (LaSVHF)/École d'Horticulture et d'Aménagement des Espaces Verts ; Université Nationale d'Agriculture de Porto-Novo, Bénin
[3] Laboratoire Pierre PAGNEY : Climat, Eau, Ecosystèmes et Développement/UAC
[4] Département de Géographie et Aménagement du Territoire, Université d'Abomey-Calavi, Bénin ; B.P. 526, Cotonou, Bénin

Citation: Monde, M.A., Ahouangninou, C., Abdoulaye, D., Kiki, L.D.Y., Massede, S., Tente, B. (2023). Analysis of the foundations of market gardening activity in the Commune of Athiémé (Benin). International Journal of Agriculture, Environment and Food Sciences, 7 (1), 107-116

Received: 21 December 2022
Revised: 01 February 2023
Accepted: 05 February 2023
Published Online: 02 Martch 2023

Correspondence: Marcelle-Agathe Monde
E-mail: marcelleagathemondefifatin@gmail.com

Abstract

Vegetable production has been a booming activity in Benin for several years, as in most countries of the West African region. The Beninese state and many other organizations are working together to develop this activity, create jobs and strengthen food security in the country. In rural areas, this agricultural sub-sector is considered one of the important measures to guarantee food security and promote income diversification. However, questions arise about the viability of this form of activity. This study aims to analyze market gardening operations in the Commune of Athiémé in Benin. To achieve this objective, different data collection techniques are used, namely documentary research, observation, interviews and questionnaire surveys with market gardeners and an interview guide administered to local authorities. A sample of 206 market gardeners is questioned. The socio-demographic data of market gardeners and agricultural statistics (areas, production and yields, etc.) are collected. The results obtained showed that market garden production is a highly developed activity in the Commune of Athiémé and is practiced at all times of the year. The most developed methods of accessing land are by inheritance (31.9%) and by purchase (31.9%). Family labor was used most often on market gardening farms (76.21%). For water supply to farms, the dominant systems were those relating to irrigation, the use of rainwater and floodwater. The basic water distribution or irrigation equipment was motor pumps, which was used by 93.57% of market gardeners. For the fertilization, the majority of market gardeners prefer the use of Urea (44.44%) and NPK (35.28%). Only 10.83% use cow purse and poultry dung. For the control of crop pests and diseases, producers use a variety of chemical pesticides. Improved production systems, including training for producers, improved irrigation systems, soil fertilization and plant protection measures will promote the sustainability of vegetable farms.

Keywords: Analysis, vegetable farm, production system, vegetable crops, Athiémé

INTRODUCTION

Agriculture, the main rural activity in developing countries due to the number of people it occupies and the volume of its production, is one of the alternatives that allow people to ensure their survival (Atidegla *et al*., 2017). Agriculture is of paramount importance for strengthening the Beninese economy. Benin's agricultural sector contributes an average of 32.7 percent to Gross Domestic Product (GDP), 75 percent to export earnings, 15 percent to government

revenues, and provides about 70 percent of employment (Sossou, 2015). In Benin, vegetable production is characterized by a diversification of cultivated species (Ahouangninou *et al*., 2013). The income generated by this market gardening activity allows several hundred families to meet their daily needs (Adjatin *et al*., 2019). Market gardening is an important activity through which unemployment and the food crisis are mitigated, and also a means of obtaining income to support family needs (Ngakiama *et al*., 2019). According to Dossou-Yovo (2019), water availability for market gardening activities distinguishes between irrigated production systems in Benin, lowland production systems (that depend on seasonal rainfall and are reinforced by an irrigation system), and rainfed production systems in wetlands. The availability of water is a determining factor for market garden production. Market gardening is an important activity in the sense that in order to accelerate the growth of the agricultural sector, Benin adopted a Strategic Plan for the Recovery of the Agricultural Sector (PSRSA) in 2011, which is based on the development of 13 priority sectors, including the market gardening sector in view of the enormous potential it holds in terms of contribution to food and nutritional security, economic growth and rural job creation. Similarly, the Strategic Plan for the Development of the Agricultural Sector 2017-2025 places market gardening among the priority sectors to be developed in Benin (MESRS, 2017). The five-year action plan (2016-2021) and the government's action program (2017-2021) place market gardening among the nine priority sectors that should benefit from massive investments (PADMAR, 2019). At the national level, a total of five (5) major vegetable production areas have been identified based on criteria such as geographic location, types of vegetables produced, and production systems. At the national level, based on criteria such as geographical location, types of vegetables produced and production systems, five (5) major vegetable production areas are identified (Alinsato *et al*., 2018). The Commune of Athiémé is located in this part with high potential for vegetable production. This study aims to analyze the foundations of market gardening in the Commune of Athiémé.

MATERIAL AND METHODS

Study area

The Commune of Athiémé is located in the southwest of the Republic of Benin, between parallels 6°28′ and 6°40′ North latitude and meridians 1°35› and 1°47› East longitude (Figure 1). It covers an area of 238 km2 and shares a natural border with Togo, which is the Mono River. The Commune is located in the agro-ecological zone of the Mono department, which is made up of low valleys and alluvial formations. The climate is sub-equatorial, characterized by four seasons, including two dry seasons and two rainy seasons. It is favorable to market gardening activities which become intense during the months of November, December, January and February. This long period, corresponding to the great dry season, is a period of water withdrawal that leaves room for market gardening. Thermal conditions play an important role in vegetative growth. Temperatures are relatively high and vary between 26.15°C in August and 29.55°C in March.

The relief, marked by numerous depressions and banks (cordons) of sand and sandstone is monotonous and generally flat, eroded in places. The existing depressions constitute watersheds or valleys sheltered by ponds, swamps and plains, very suitable for off-season crops and market gardening. The soils on the whole are clayey, black hydromorphic clay, sandy clay or sandy clay soils very suitable for multi-cropping. The research environment is marked by the presence of the Mono River, which is the main river with a wide valley and watersheds and which irrigates almost all the villages of the Commune (INSAE, 2004).

At the last General Census of Population and Housing (RGPH4, 2013), this Commune had 56,483 inhabitants, 51.2% of whom were women compared to 48.8% men with a natural growth rate of 3.2%. This population constitutes a potential agricultural workforce in the Commune.

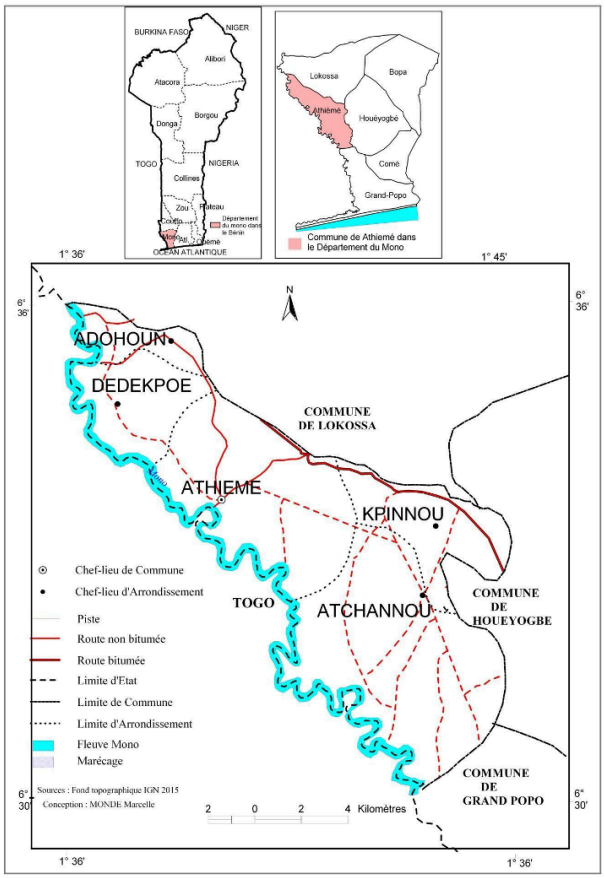


Figure 1. Geographical situation of study area

Data collection

This part concerns the data as well as the collection tools and techniques. These data include the socio-demographic characteristics of market gardeners, the main crops grown, the production systems, in particular data on the mode of access to land, the crops adopted, the type of labor, tools and materials, size of farm, water supply system, water supply sources, water distribution equipment, cropping operations i.e. types and means of fertilization, types of fertilizers used and forms of phytosanitary treatment. These data made it possible to analyze the foundations of market gardening in Athiémé. The different techniques used were: documentary research, observation, interviews, and surveys by questionnaire.

The sampling approach chosen is the non-probability approach, but the sample size n is obtained from the Schwartz (1995) formula:

$$n=Z\alpha^2\times pq/i^2$$

In this formula,

n is the sample size ;

Zα= Deviation set at 1.96 at a 95% confidence level;

p is the total proportion of market gardeners in the five arrondissements (Adohoun- Atchannou- Dédékpoe-Kpinnou and Athiémé) to the total agricultural populations of the Commune of Athiémé obtained as follows: (858/5300 = 0.1619) ;

q=1-p: (1-0.1619= 0.8381);

i: precision or risk of error fixed at 0.0503.

n = $(1.96^2\times0.1619\times0.8381) / (0.0503)^2$ = 206.02 market gardeners.

The distribution of locations and number of farms surveyed is presented in Table 1.

A total of 206 people were interviewed for the data and information collection. This number was selected according to a reasoned choice that took into consideration the number of years of practice, the size and the geographical location of the farms. In addition to this sample, three specialists were surveyed (in vegetable production, in plant production, in ecotoxicology). It is the same as the Head of the Local Development and Planning Department and the Head of the State Affairs and Environment Department of the Athiémé Town Hall. The five Arrondissement chiefs of the Commune contributed to the control of the occupation of the exploitation units.

Data processing and analysis

The data collected was processed in two phases. The first phase consisted of cleaning up the database. This step consisted mainly in harmonizing the response modalities served by each respondent. It was done in the Excel 2013 spreadsheet. The second phase, which is the analysis of the data, consisted mainly of the use of descriptive statistics (average, absolute and relative frequency) and proportion comparison tests to describe the data. These statistics were generated with the R 4.0.3 software. The analysis also focused on the qualitative aspects of the production system in order to assess the living and working conditions of the market gardeners. The production of graphs involved the use of the R software package ggplot2.

Table 1. Distribution of villages/towns and people surveyed.

Arrondissements	Total number of rural households	Total number of market gardeners surveyed by district (n)	Proportion of market gardeners surveyed (%)
Adohoun	1785	22	10,68
Atchannou	894	47	22,82
Kpinnou	630	50	24,27
Dedekpoe	531	32	15,53
Athiémé	1460	55	26,7
Total	**5300**	**206**	**100**

Source: Fieldwork results, January 2020

RESULTS

During this study, 206 market gardeners were registered. Analysis of Table II shows that 73.79% of the respondents were male. The study of the educational level of these actors shows that the vast majority of them have primary education, i.e. 33.01% of respondents. 93.03% were already married and the Kotafon were the ethnic group most involved in market gardening in the study area (Table 2).

Main crops grown

Market gardening is a highly developed activity in the Commune of Athiémé. It is practiced at all times of the year. However, in the different districts, some crops are produced more than others, depending on market demand. An analysis of Figure 2 below shows that the three market garden crops most often cited in Adohoun district and mainly produced by market gardeners are chili, tomato and vernonia. In the arrondissement of

Table 2. Socio-demographic characteristics of producers.

Variables	Modalities	Number	Percentage (%)	X^2 (p-value)
Sexe	Woman	54	26.21	<0.001
	Man	152	73.79	
Level of education	Any	25	12.14	0.022
	Primary school	68	33.01	
	Secondary school	56	27.18	
	University	57	27.67	
Marital status	Single	11	5.47	<0.001
	Maried	187	93.03	
	Widow/widower	3	1.49	
Ethnic	Adja	25	12.14	<0.001
	Adja Tala	37	17.96	
	Nago	1	0.49	
	Fon	4	1.94	
	Goun	1	0.49	
	Kotafon	85	41.26	
	Mina	5	2.43	
	No specified	18	8.74	
	Sahouè	5	2.43	
	Toguido	1	0.49	
	Watchi	23	11.17	
	Yorouba	1	0.49	

Table 3. Main crops.

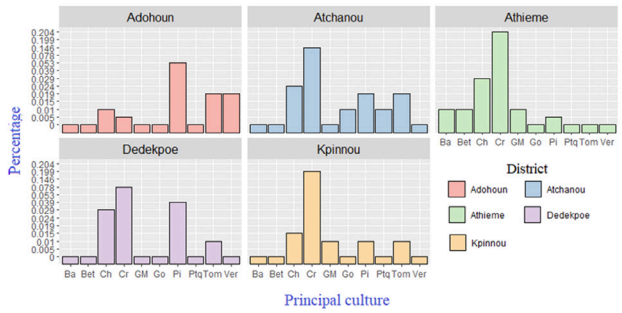
N°	Listed vegetable products (french name)	Scientific names	Local name KOTAFON	Local name MINA	Percentage(%)
1	Crincrin	*Corchorus tridens*	Adèmin	Adémin	63.1
2	Chou	*Brassica oleracea*	Chou	Chou	10.7
3	Piment	*Capsicum frutescens*	Vavo	Yebessé	12.6
4	Amarante	*Lycopersicum esculentum*	Têtè	Têtè	0.00
5	Grande-morelle	*Solanum macrocarpon*	Gboma	Gboma	1.90
6	Tomate	*Solanum lycopersicum*	Agbovi	Timanti	5.80
7	Gombo	*Abelmoschus esculentus*	Ninhou	Fétri	1.00
8	Concombre	*Cucumis sativus*	Concombre	Concombre	0.00
9	Pastèque	*Citrullus lanatus*	Watra	Watra	1.00
10	Tchiayo	*Ocimum gratissimum L*	Tchanmandido	Eslou/yandodo	0.00
11	Vernonia	*Vernonia galamensis*	Amanvivè	Aloman	1.90
12	Betterave	*Beta vulgaris L*			1.00

Source: Fieldwork results, April 2021

Atchanou, producers are more attracted to the production of crincrin, cabbage and, in equal proportions, chili pepper and tomato. In the arrondissement of Athiémé and Kpinnou, producers prefer to grow crincrin, cabbage and nightshade or gboma (*Solanum macrocarpon*). In the Dedekpoe zone, the production of crincrin, cabbage and chili peppers is more common (Figure 2).

Table 3 presents an inventory of the market garden products identified in the Commune. The results of the fieldwork allowed us to identify twelve (12) vegetables crops in the different farms surveyed. These crops are produced according to the preferences of each market gardener and the soil conditions of the plots. Thus, it appears that the cultivation of crincrin is the first preference of the producers, the production of chili pepper is the second and finally that of gboma is the third. The respondents gave the same reasons for their choice of crops that are high profitability, ease of marketing and food needs. In addition, the cultivation of crincrin, as the first crop, is linked to tradition in this area. It is therefore a traditional crop specific to the area. In addition, the relatively short harvest cycle of this crop (21 days after sowing) and the rapid availability of income help to solve financial problems.

Legend: Ba="Basil", Bet="Beetroot", Ch="Cabbage",



Cr'="Crin-crin", Go="Okra", GM="Grandmother", Ptq="Watermelon", Pi="Pepper", To="Tomato", Ver="Vernonia"

Figure 2. Main crops produced by district

Systems of Production

Most of the results of previous research consulted, in order to characterize production systems, refer to the mode of acquisition or mode of access to land, the type of labor, the work tools, the size of the farm, the water supply system, the sources of water supply, the cultivation operations, i.e., the types and means of fertilization, and the forms of phytosanitary treatment.

Cultural operations for soil preparation

The field surveys reveal a number of cultivation operations in the context of soil preparation. These include chemical clearing, which is a new form introduced, manual clearing, and manual or mechanical plowing. As for the cleaning of the soil, the producers proceed by incineration, by collection and/or burial and finish by transplanting (Figure 3).

Figure 3. Planting of greater nightshade in Ahoho (Shot: M. Mondé, 2020)

This transplanting session is done manually and is a sensitive step. When it is well done, the seedlings grow well. Otherwise, they can be asphyxiated or burned by the heat. The protection of these plants, especially for off-season crops, is done by several means available to each producer. For example, protection is provided by means of hedges made of palm branch stakes. Producers also sow maize to shade the crops one month after transplanting. Transplanting can also be done in banana plantations, but the latter practice increases the exposure of the crop to fungal diseases.

Mode of land acquisition and use of labor

Market gardeners get the land in several ways. This access depends on each district and the origin of the farmer. Figure 4 presents the different modes of acquisition of the farm units visited.

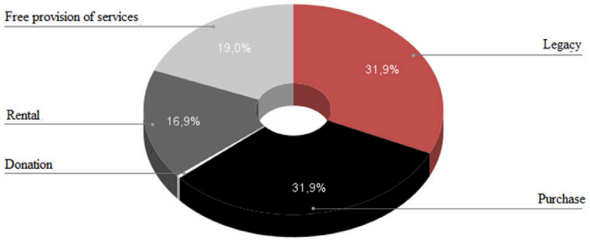


Figure 4. Methods of acquisition of farm units by market gardeners.
Source: Fieldwork results, December 2020

The analysis in Figure 2 shows that there are five modes of acquisition of market gardening farms in the Commune of Athiémé. The most developed methods are those of inheritance and purchase, which represent 31.9% of all methods. However, the donation of plots to be farmed is less developed and represents 3%. The practice of making land available free of charge is also well developed in the Commune (19.0%), thus allowing producers who do not have the means to purchase land to produce their products over a defined period. Within this system, there is also renting (16.9%), which mainly concerns foreigners from other Communes in the Departments of Mono and Couffo and neighboring Republic of Togo.

Fieldwork revealed that market gardeners use three types of labor (Figure 5): family labor, occasional hired labor and permanent hired labor.

Looking at Figure 4, it can be seen that family labor is highly used in vegetable farms (73.21%). This shows that market gardeners use family members more. However, they use permanent paid labor, which are people from the Commune. Also, the occasional salaried labor force (mostly made up of foreigners), is used by market gardeners. It should be noted that the use of salaried labor concerns market gardeners who have a relatively large financial power and size of operation.

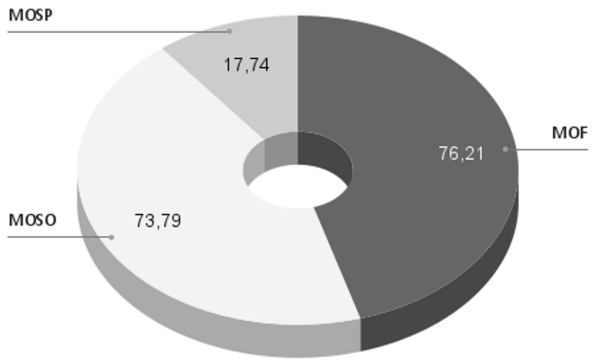


Figure 5. Proportions of labor use on vegetable farms (MOF=family labor; MOSO=casual hired labor; MOSP=permanent hired labor)
Source: Fieldwork results, December 2020

Areas farmed and equipment used on market garden farms

Land is a very important means of production in the farming system. In the Commune of Athiémé, depending on their financial means and social status, market gardeners have different portions of space to exploit. Figure 6 presents the size of the farm units allocated to market gardening in terms of the proportion of market gardeners.

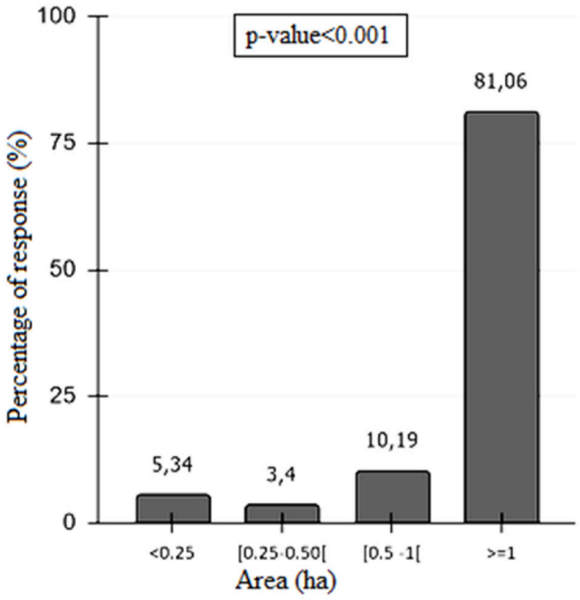


Figure 6. Areas farmed by market gardeners

Source: Fieldwork results, December 2021

Figure 5 shows a clear difference in terms of the amount of land farmed. Indeed, 81.06% of market gardeners have at least one hectare of land for market garden production. These issues indicate that the Commune of Athiémé is home to large market gardeners.

Irrigation systems

Field surveys revealed that in Athiémé, the dominant systems are those related to irrigation, rainfed and flood recession crops. In all five districts, rainfed crops are observed. In addition, the depressions are home to ponds, shallows and swamps and concern the Mono River valley, the Sazué and Saïdo rivers, and the Toho, Djèto and Whayè lakes. These rivers and bodies of water cross the villages of the various districts. From these river and lake networks, market gardeners organize various water supply systems for their crops. Others resort to drilling systems (Table 4).

Table 4. Water Distribution Equipment.

Equipment	Number (%)
Motor pumps	194 (33.66%)
Submersible pumps	15 (2.31%)
Perforated belts	45 (7.26%)
Turnstile	9 (1.65%)
Flexible hoses with sprinkler heads	60 (11.22%)
Displaceable hoses	162 (28.38%)
Watering can	72 (13.53%)
Drip tape	3 (0.66%)
Others	4 (1.32%)

Source: Field surveys, 2020

Examination of Table IV shows that the basic water distribution or irrigation equipment are motor pumps used at 33.66% by growers, submersible pumps used at 2.31%, perforated belts used at 7.26%, turnstiles used at 1.65% by market gardeners, flexible hoses with sprinkler heads used at 11.22%, movable piping at 28.38%, and drip waterers at 0.66% of surveyed market gardeners (Figure 7).

The water supply and distribution equipment and energy sources used by market gardeners are diverse. Figure 8 presents some of the energy sources identified in the research environment.

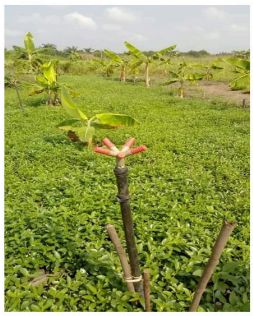
Figure 8 shows that efforts to modernize the pumping system are being made in the area. It is important to maximize this momentum and engage in the sustainable management of this equipment. According to field surveys, 93.57% of market gardeners use gasoline and 5.50% use diesel, not to mention photovoltaic energy.

In addition, other means are used on market garden farms. These are the tools, materials and machines used in the entire research sector. These means include the hoe and the cutter for all producers; rakes, tractors and accessories. Finally, work tools such as wheelbarrows, rope, rope for staking, decameters, hatchets and baskets are also noted.

Watering by apple and flexible pipes Djonougoui hamlet in Adjovè (Athiémé)

Irrigation system with 50 mm PVC movable pipes in Adjovè

Feeding by perforated bands in Adjovè



Feeding at the turnstile (Djonougoui in Adjovè)

Figure 7. Some water distribution equipment in Athiémé

Shot by M. Mondé, January 2020

Solar water pumping and treatment system for the Mono River (Aguidahoué to Adohoun)

Generator for water pumping

Figure 8. Energy sources for irrigation; Photo credit: M. Mondé, 2020

Fertilizer use and plant protection measures

Fertilizers

Several fertilizers (organic and chemical) are used to fertilize market gardens (Figure 9).

Soil fertilization is done in various ways and depends on the means available to market gardeners, particularly financial and human means. However, regardless of the type of operation, plants are amended either by chemical fertilizers or by organic matter or by both.

The analysis in Figure 6 shows that 80% of market gardeners use chemical fertilizers (NPK and urea). Only 11% use cow dung and poultry droppings. Others use liquid fertilizer, which is manufactured locally or imported.

Figure 10 shows the amendment of cabbage with urea (3.1) and the biological fertilization operation (3.2) of the plots.

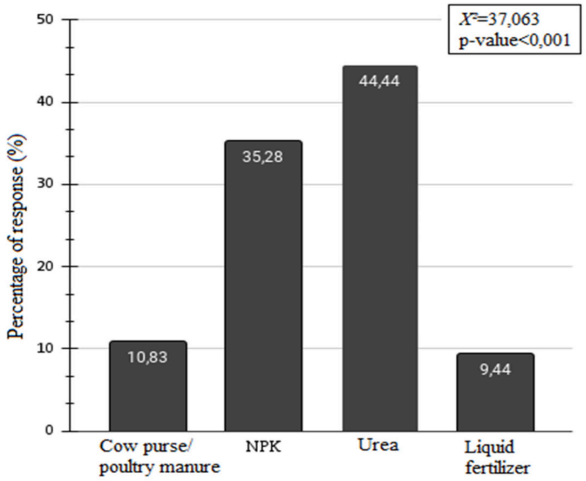


Figure 9. Proportion of farmers by type of fertilizer used

Source: Fieldwork results, December 2020

Urea amendment in Ahoho

Spreading organic fertilizer in Adjovè

Figure 10. Fertilizer products for Athiémé soil

Protection of the plants

In the Commune, the treatment of crops and the fight against pests are done in several ways. For the fight against pests, the producers resort to pesticides. The preparations that were the most recorded were the formulations of Isopropylamine, lambda-cyhalothrin, Emamectin benzoate, Abamectin, Haloxyfop-P-Methyl (Table 5).

The producers interviewed used pesticides, including herbicides, insecticides, fungicides and acaricides. The range of products identified includes 10 herbicides, 6 insecticides, 3 fungicides, 1 acaricide and 1 bactericide.

Protection measures taken by market gardeners during phytosanitary treatments

Phytosanitary treatment is a common practice in market gardening in the Commune of Athiémé. It is done by market gardeners without a minimum of personal protective measures. According to the surveys, 41.0% of market gardeners do not take any protective measures, although they are aware of the importance of wearing PPE.

Table 5. Chemical inputs used in the Commune.

Type of pesticide	Trade name	Active ingredients	Concentration	Chemical family
Herbicides	Finish 68 SG	Abamectine	18 g/L	Organophosphorous
	Malik 108 EC	Haloxyfop-R méthyl	104 g/l	Organophosphorous
	Force up	Glyphosate	360 g/L	Organophosphorous
	Terminator G4	Glyphosate Terbuthylazine Amitrole Oxyfluorfène	100 g/l 350 g/l 60 g/l 15 g/l	Organophosphorous
	Sunphosate 360 SL	Glyphosate	360 g/l	Organophosphorous
	Adwuma wura	Glyphosate sel d'isopropylamine	360 g/l	Organophosphorous
	Gallup super 360	Glyphosate	360 g/l	Organophosphorous
	Glyphader 360	Glyphosate	360 g/l	Organophosphorous
	Capizad	Haloxyfop-P-Méthyl acide éq. Haloxyfop-R-Ester méthylique EC	104 g/L 108 g/L	Propionate
	Sharp	Acétamipride	20 g/L	Néonicotinoïdes
Insecticides	Acarius 018 EC	Abamectine	18 g/L	Avermectines
	Emacot 050 WG	Emamectine benzoate	50 g/kg	Avermectines
	Pacha 25 EC	Acetamipride Lambda Cyhalothrine	10 g/l 15 g/l	Néonicotinoïdes Pyréthrinoïdes
	CYPERCAL 50 EC	Cyperme'thrine	50 g/L	Pyréthrinoïdes
	K-Optimal GHS 50.00	Lambda Cyhalothrine Acetamipride	15 g/L 20 g/L	Pyréthrinoïdes Néonicotinoïdes
	Sunpyrifos 48 EC	Chlorpyriphos-e'thyl	480 g/L	Organophosphorous
Fongicides	Acarius 018 EC	Abamectine	18 g/kg	Avermectines
	Idefix	Hydroxide of copper	65,6 g /100 g	Hydroxide of copper
	Coga 80 WP	Mancozeb	800 g/kg	Dithiocarbamates
Bactericides	Idefix	Hydroxide of copper	65,6 g /100 g	Hydroxide of copper
Acaricides	Acarius 018 EC	Abamectine	18 g/L	Avermectines

Source: Survey results

Figure 11 shows a producer spraying without protection.

Figure 11. Absence of PPE during spraying in Ahoho

The sensitivity of the chemical products requires, however, a protection by the use of the EPI for the sanitary safety of the producers.

DISCUSSION

Analysis of the foundations of the market gardening farms of the Commune of Athiémé has made it possible to appreciate certain specificities that should be underlined. Indeed, the market gardeners surveyed have an average level of education, the market gardeners are on average. 33.01% have primary school education. This finding is similar to the results obtained by Ouattara (2016) in Burkina Faso, where those with primary education are the most numerous (31.3%). The same observation is made by Ahouangninou (2013) who notes that in southern Benin, market gardeners for the most part do not have a high level of education.

The present study reveals that the most developed modes of access to land are those of inheritance and purchase, which represent an equal proportion of 31.9% of all modes. The opposite results exposed by Kasanda, (2016) in Lubumbashi in the Democratic Republic of Congo highlight that, access to land by inheritance, is

the least frequent mode in the study environment and represents 0.99%, or 1% of the study population. Those who have acquired the right to use the land by purchase represent 17.33% of the total population.

Renting is the most common mode of access in all the sites studied, and concerns 58.42% of farmers. In terms of land availability, the results of this research in Athiémé showed that 81% of market gardeners have more than one hectare of land under cultivation. These ending are in line with those of the study conducted by Dossou-Yovo (2019) in Sèmè-Podji, where 75% of producers farmed areas of more than 0.5 hectares. On the other hand, they contrast with those of Tori-Bossito, because the farmers in this Commune mostly farm small areas for their production activity. Only a few have areas larger than one hectare (Ahouangninou *et al.*, 2011).

Family labor is the most used 76.21% The present results allowed the identification of twelve (12) market gardening species in the different farms surveyed compared to nine (09) market gardening species identified in Grand-Popo in southern Benin by Adjatin *et al*., (2019).

The analysis of the results showed a high use of chemical fertilizers in the market garden farms (80% of the market gardeners use chemical fertilizer), organic fertilizers in a lesser proportion (11%). Going in the same direction, Mondé (2019) demonstrates that in the Commune of Tori-Bossito, to increase agricultural yields, producers use chemical fertilizers such as NPK and Urea.

In Athiémé, pest control is done with pesticides and a significant use of these products is noticed. According to the deductions of the survey, 21 commercial preparations were identified, including 10 herbicides, 6 insecticides, 3 fungicides, 1 acaricide and 1 bactericide. For weed control, 33.5% and 42.3% of producers use selective herbicides and total herbicides respectively. Only 24.2% of producers reported not using any herbicide and resorted to hand weeding to reduce crop vulnerability to weeds. These conclusions differ from those of Ahouangninou *et al*. (2011), who did not identify any herbicides, because people are more concerned with the control of insect pests and prefer manual weeding. The chemical preparations most commonly found in the study area were formulations of Methyl Isopropylamine, lambda-cyhalothrin, Emamectin benzoate, Abamectin, Haloxyfop-R methyl.

The same results regarding the use of phytosanitary products were obtained by Mondedji *et al*. (2015) in southern Togo, who showed that in developing countries, there is excessive and inappropriate use of pesticides but also failure to comply with the pre-harvest residual time of phytosanitary products. In terms of protection against the negative effects of the use of phytosanitary products, the results showed that market gardeners do not have PPE. The work of Ngakiama *et al*. (2019) in the town of Kinshasa showed similar results where no market gardeners have complete protective equipment such as gloves, boots, masks, dust covers, overalls, aprons, goggles. Only 36.9% of market gardeners use one or two pieces of protective equipment.

CONCLUSION

The research on the analysis of the foundations of market gardening in the Commune of Athiémé showed that market gardening is an activity carried out by a significant number of producers at all times of the year and in the five districts. A dozen market gardening activities were identified on the various farms visited. The dominant systems are those related to irrigation, rainfed and flooded crops. The distribution equipment and energy sources for the supply and distribution of water used by operators are diverse and efforts to modernize the pumping system are being made in the area. Plants are amended either by chemical fertilizers or by organic matter or by both. For pest control, producers use pesticides. The most common formulations were Isopropylamine, lambda-cyhalothrin, Emamectin benzoate, Abamectin, Haloxyfop-P-Methyl. In these farms, very few market gardeners use protective equipment during phytosanitary treatments. In view of the observations made from the analysis of the foundations of market gardening in Athiémé, it is urgent to deepen knowledge of current production methods in order to make this activity sustainable.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Adjatin, A. A., Bonou-Gbo Z., Boco A., Yédomonhan H., Dansi A. (2019). Diversité biologique et caractérisation de l'activité de maraîchage sur le site de Grand-Popo au Sud Bénin. International Journal of Biologie and Chemical Sciences 13(6): 2750-2764. https://dx.doi.org/10.4314/ijbcs.v13i6.26

Ahouangninou C. (2013). Durabilité de la production maraîchère au Sud-Bénin : un essai de l'approche écosystémique, Thèse de doctorat, Université d'Abomey-Calavi, Ecole doctorale pluridisciplinaire, Bénin. 344 pages.

Ahouangninou C, T Martin, S Bio-Bangana, J Huat, L Parrot, F Vidogbéna, D Medali, C Houssou, P Edorh, M Boko et B Fayomi, 2013. Characterization and diversity of the market-gardening production systems and theirs interactions with urban and peri urban environment in southern-Benin, West Africa. Continental Journal of Sustainable Development, 4(1): 24-38. https://doi.org/10.5707/cjsd.2013.4.1.24.38

Ahouangninou C., Fayomi B., Martin T. (2011). Évaluation des risques sanitaires et environnementaux des pratiques phytosanitaires des producteurs maraîchers dans la commune rurale de Tori-Bossito (Sud-Bénin). *Cah Agric* 20: 216-22. https://doi.org/10.1684/AGR.2011.0485

Alinsato A., Yagbedo U. (2018). « Analyse d'offre des produits maraîchers au Bénin », Université d'Abomey-Calavi, Bénin. 29 pages.

Atidegla C. S., Degbo H. E., Azonhè T., Agbossou Kossi E. (2017) Impacts socioéconomiques et sanitaires de l'exploitation agricole de la plaine inondable Todé Gbamè au Bénin. European Scientific, 13(18): 185-203 http://dx.doi.org/10.19044/esj.2017.v13n18p185

Dossou-Yovo S. (2019). Risques environnementaux et sanitaires liés à la pratique du maraîchage dans la commune de Sèmè-Podji sur le littoral du Bénin : évaluation de la contamination et propositions de stratégies de contrôles, Thèse de doctorat, Université d'Abomey-Calavi, 297 pages.

Kasanda Mukendi N., Mushagalusa Balasha A., Kitsali J. H., Nkulu Mwine Fyama J., Bogaert J. (2016). Maraichage périurbain à Lubumbashi : modes d'accès à la terre et gestion des superficies agricoles. International Journal of Innovation and Applied Studies ISSN 2028-9324 14 (1). 27-36.

Kpadenou C. C., Tama C., Dado Tossou B., Yabi A. J., (2019). Déterminants socio-économiques de l'adoption des pratiques agro-écologiques en production maraîchère dans la vallée du Niger au Bénin. International Journal of Biologie and Chemical Sciences 13(7): 3103-3118. https://dx.doi.org/10.4314/ijbcs.v13i7.11

Mondé M.-A. (2019). Evaluation des risques environnementaux de l'utilisation des intrants chimiques dans les cultures maraîchères de bas-fonds dans la Commune de Tori-Bossito. Mémoire de Master 2. Université d'Abomey-Calavi, Ecole doctorale pluridisciplinaire, Bénin, 72 pages.

Mondedji A., D., Nyamador W. S., Amevoin K., Adéoti R., Abbévi Abbey G., Koffivi Ketoh G., Glitho A. I. (2015). Analyse de quelques aspects du système de production légumière et perception des producteurs de l'utilisation d'extraits botaniques dans la gestion des insectes ravageurs des cultures maraîchères au Sud du Togo. International Journal of Biologie and Chemical Sciences 9(1): 98-107. https://doi.org/10.4314/ijbcs.v9i1.10

Ndiaye O., Diatta U., Abeudje A., Dramé M. (2021). Caractérisation des périmètres maraîchers institués par les groupements des femmes comme stratégie de résilience en zone post conflit (Casamance, Sénégal). European Scientific, 7431,17(13). https://doi.org/10.19044/esj.2021.v17n13p118

Ngakiama Ngweme G., Mbela Kiyombo G., Pole Sikulisimwa C., Kyela Mulaji C., Komanda Aloni J. (2019). Analyse des connaissances, attitudes et pratiques des maraîchers de la Ville de Kinshasa en rapport avec l'utilisation des pesticides et l'impact sur la santé humaine et sur l'environnement. Afrique International Journal of Innovation and Applied Studies ISSN 2028-9324, Vol. 26 No.2 345-356.

Ouattara Z. A. (2016). Caractérisation des systèmes de production maraichers et analyse des déterminants de la fertilité des sols sous cultures maraichères dans la province du Houet (Burkina Faso), Mémoire de fin de cycle, Université Polytechnique de Bobo-Dioulasso, 61 pages.

Projet d'Appui au Développement du Maraîchage. (2019). Manuel d'exécution revu du PADMAR, 151 pages.

Sossou C. H. (2015). Le financement de l'agriculture au Bénin : stratégies de gestion et d'adaptation des exploitations agricoles. Communauté française de Belgique, Université de Liège – Gembloux agro-Bio Tech, 199 pages.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 117-121
DOI: https://doi.org/10.31015/jaefs.2023.1.13

Production of recombinant expansin and detection by SDS page analysis in *Escherichia coli*

Serap Gunes[1] • **Demet Ektiren**[1] • **Mehmet Karaaslan**[2] • **Hasan Vardin**[2]

[1] Dicle University, Diyarbakir Vocational School of Agriculture, Department of Food Processing, Diyarbakir, Türkiye
[2] Harran University, Faculty of Engineering, Department of Food Engineering, Şanlıurfa, Türkiye

Abstract
The study aims to produce Expansin protein isolated from a young tomato plant by using *Escherichia coli* which is used in recombinant protein production. Continuous culture is the most common method used to grow cells for recombinant protein production. In the study, the K12 strain of *E. coli* was used as a culture for the production of Expansin protein. The used LeExp1 gene was isolated from a young tomato plant. Since the related gene is found in very small amounts in plants, it has been reproduced using the PCR method and has been made workable with this method. T17 vector (T7 RNA polymerase system), which is frequently used in the production of recombinant protein, was used as the bacterial expression vector. The T7 RNA polymerase system is a commonly used vector in *E. coli*. With the transfer, the *E. coli* bacterium was given the ability to produce recombinant protein. Whether the obtained recombinant protein expressed the appropriate protein was determined by SDS Page analysis.
Keywords: Recombinant protein, Expansin protein, LeExp1 gene, Escherichia *coli*, PCR

Citation: Gunes, S., Ektiren, D., Karaaslan, M., Vardin, H. (2023). Production of recombinant expansin and detection by SDS page analysis in *Escherichia coli*. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 117-121

Received: 17 October 2022
Revised: 11 December 2022
Accepted: 14 December 2022
Published Online: 10 March 2023

Correspondence: Serap Gunes
E-mail: serap.gunes@dicle.edu.tr

INTRODUCTION

Proteins are a group of macromolecules with various biochemical functions that are widely used for research and applications in fields such as the health and food industry, mainly thanks to biotechnological advances (Andersson et al., 2018; Rabert et al., 2013; Walsh et al., 2014). While obtaining protein from natural sources is very laborious and expensive, the efficiency of obtaining protein in these ways is low, but advances in DNA technology have made it possible to use expression systems more efficiently for recombinant protein production (Garcia-Fruitos, 2014; Gräslund et al., 2008; Sanchez-Garcia et al., 2016).

Today, the first publication on the creation of recombinant DNA and the replication of recombinant DNA in the cell, which comes to mind in the field of modern biotechnology, was published in 1972 and 1973 (Jackson et al., 1972; Katartaş, 2011). The first genetically modified DNA molecule was developed using recombinant DNA technology; It was obtained by Paul Berg in 1972 (Hughes, 2001). As a result of these developments, with the understanding of the importance of Recombinant DNA technology, companies using this technology (Genentech, Eli Lilly and Company) were established. The result of the studies was first carried out on the *E. coli* bacteria in 1978. It has been described that a new strain capable of producing insulin has been synthesized by performing a genetic manipulation on the bacterium (Ladisch et al., 1992).

Expansin proteins are a family of proteins in the plant cell wall structure. Expansin proteins are divided into four subgroups: Expansin A, Expansin B, Expansin A-like

and Expansin B-like. Expansin proteins were first isolated from the cucumber plant by Daniel Cosgrove in 1992. The two Expansin proteins revealed in the cucumber plant correspond to an average of 270 amino acids and their molecular weights are 29 kDa (kiloDalton) and 30 kDa. In general, A and B Expansins and Expansin-like proteins consist of approximately 300 amino acids and have molecular weights of around 25-28 kDa (Cosgrove, 2000). Expansin proteins have an important role in developmental processes in plant cell development, fruit softening, root hair growth, stigma and pollen tube development, and cell wall fragmentation (Hartley, 2006). Enzymes that break down the cell wall are used in many industries, such as the biofuel production, cellulose industry and the food industry.

Expansin proteins are found in very small amounts in plants and there is no microbial production of these proteins. In addition to the fact that the expansine proteins cannot be produced in pure culture, it is very costly to isolate these proteins from plants. Many of these problems can be eliminated by producing expansine proteins in the form of recombinant proteins. With the production of the recombinant Expansin protein, an alternative protein capable of modifying the cell wall structure can be obtained in addition to the enzymes that degrade the cell wall. To facilitate gene studies, the gene can be isolated and amplified. The method of isolating and amplifying a gene is to clone it by inserting the relevant gene into another DNA molecule that acts as a vector or tool that can be replicated in a living cell. It is the formation of a new DNA molecule as a result of combining these two DNAs of different origins. Although genetic events such as crossing over (change of parts in chromosomes) technically provide the formation of recombinant DNA, DNA molecules formed by the participation of segments obtained from different biological sources are generally used for this work. The recombinant DNA molecule is introduced into a prokaryotic or eukaryotic host cell. The host cell then makes copies of the existing DNA molecule and replicates the vector with its foreign DNA fragment. Foreign DNA is amplified with these developing processes and these amplified DNAs are purified for further analysis (Mullis, 1990).

In the study, the LeExp1 gene isolated from the tomato plant was amplified by PCR method and transferred to the T17 expression vector (T7 RNA polymerase system) by a suitable kit. Vector *E. coli* was transformed into bacteria in liquid culture and *E. coli* bacteria were given the ability to produce recombinant protein.

MATERIALS AND METHODS

Materials

Young tomato (Solanum lycopersicum) seedlings were used as the relevant gene source in the study. Tomato seedlings were grown in special capped plastic boxes in the medium without air. They were left to grow for 15 days. Gene DNA and RNA obtained from the grown seedlings were used as a source.

E. coli strain K12 and vector T17 were commercially available from Invitrogen. DNA restriction and DNA ligase enzymes required for the cloning process were obtained from New England Biolabs. The IPTG inducer and the necessary medium required for the research were obtained from Sigma company. The gene of interest in the study was selected based on the gene sequences stored in the NCBI (National Center of Biotechnology Information) database.

PCR Primer Design

Designing Forward and Reverse PCR primers used to amplify the gene of interest is a critical step for expression. Depending on the use of vectors, when designing PCR primers, the sequence should be known to facilitate cloning, and the N-terminal and C-terminal peptide tags should be known for cloning the PCR product (Anon., 2010).

Forward and Reverse Primers Used

The forward and reverse primers used in the study are shown in the Table 1.

Table 1. Forward and Reverse Primers

Primer name	Lenght	Melting temperature (°C)	GC%	Sequence (5'-3')
TOF1	24	62.2	33.3	CACCATGGGTATCATAATTTTCAT
LeExpR1	20	63.3	50	GCAGCCACTTCAACCTTTCT

DNA Extraction from Tomato Plant

DNA isolation method was performed as stated by Doyle and Doyle (1990). The method used in the research is an extraction process based on CTAB (Cetyl trimethylammonium bromide).

Blunt End PCR Product Production

The production of the blunt PCR product used in the study was carried out by taking into account the basic molecular biology rules (T. Maniatis, J. Sambrook, E.F. Fritsch). The extracted LeExp1 gene was amplified using a Biorad branded thermocycler. According to this method, the components were mixed in the PCR tube. PCR conditions for the prepared PCR mixture were determined as 3 minutes at 94 °C, 30 seconds at 94 °C, 60 °C, and 72 °C for 35 cycles, and finally 10 minutes at 72 °C. The duplication process was carried out taking into account the conditions. After this process, the PCR mixture was run on a 0.8% agarose gel and visualized.

Purification of LeExp1 Gene from PCR Reaction

After the PCR reaction, the DNA was purified. Purification was performed using the GE Healthcare GFX PCR DNA and Gel Band Purification Kit. The liquid phase obtained as a result of purification was stored at -20 ºC.

Cloning of LeExp1 Gene into TOPO Vector and Transformation into Chemical Competent Cells

After obtaining the desired PCR product, it was cloned into the pET TOPO vector. The recombinant vector TOP10 obtained by TOPO cloning was transformed into *E. coli* One Shot. When performing directional TOPO cloning, the molar ratio between the PCR product and the TOPO vector is critical for the reaction to be successful. To obtain high efficiency in the TOPO cloning reaction, the PCR product: TOPO vector ratio should be between 0.5:1 and 2:1. The ratio of PCR product and TOPO vector used in the study is 1:1. The cloned LeExp1 gene was transferred to the pET100/D TOPO expression vector by the Champion kit used in the study. The recombinant vector TOP10 obtained as a result of the TOPO cloning reaction was transformed into *E. coli* One Shot cells.

Isolation of Plasmid DNA from *E. coli* Cell and Transformation of Plasmids into Expression Cells

Isolation of plasmid DNA from *E. coli* cells and transformation of plasmids into expression cells were performed following the analytical steps specified in Champion™ pET100 Directional TOPO® Expression Kit.

Recombinant Protein Expression

E. coli cells must reach a certain concentration to produce recombinant protein. Cells were incubated for 4 hours in a 37 ºC shaker until the OD reached 0.5-0.8. When the bacterial density reached the desired level, the culture was divided into two 5 ml tubes. IPTG was added to one of the tubes containing the cultures at a final concentration of 0.5-1 mM (0.0005-0.001 M). The tube with the other culture was left for control purposes without adding anything. Incubation was continued in a 37 ºC shaker for the expression process. Marking was done for each culture at a time interval of 4-6 hours. The samples were centrifuged in a centrifuge to remove the liquid phase and the pellets were stored at -20 ºC.

RESULTS AND DISCUSSION

Extraction and purification of nucleic acids is the first step in most molecular biological studies and all recombinant DNA techniques. The purpose of nucleic acid extraction is to purify nucleic acids from different sources for specific analysis using PCR analysis.

Control of DNA Extraction by PCR

In the study, the nucleic acids used to isolate the LeExp1 gene encoding Recombinant Expansin proteins were isolated from young tomato plants. For this purpose, DNA coding for the LeExp1 gene was extracted from the genome of the tomato plant. Analysis by gel electrophoresis was performed to check whether the isolated nucleic acids could be used in molecular studies. 0.8% agarose gel image of DNA of LeExp1 gene is given in Figure 1. PCR samples used in the study were prepared using appropriate primers. The region multiplied by the primers used in the study is 991 bp. However, according to the gel image results, the DNA size was approximately 1.3 kb. It is predicted that the reason for this may be the coexistence of meaningful and meaningless regions.

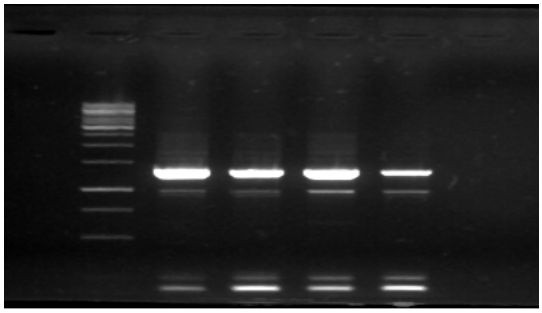


Figure 1. DNA marker (1st Well), 0.8% agarose gel image of DNA of LeExp1 (2nd - 5th well) gene

Control of Purification of the LeExp1 Gene

Purification was performed following the procedure using the GFX PCR DNA and Gel Band Purificiation Kit. To control the efficiency of the purification process, the obtained DNA was visualized by running on 0.8% agarose gel. The obtained electrophoresis gel image is given in Figure 2. The band size seen in Figure 2 is approximately 991 bp and it has been determined that the purification process has been done successfully according to the gel image.

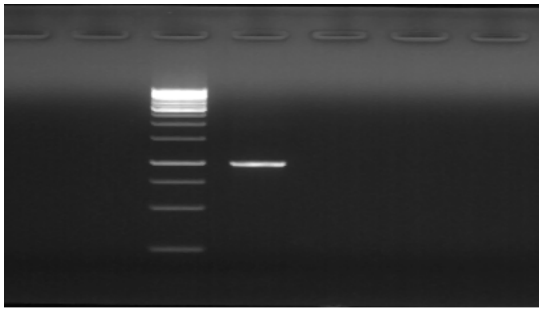


Figure 2. DNA marker (1st well), 0.8% agarose electrophoresis gel image of DNA belonging to LeExp1 (2nd – 5th well) gene to control the efficiency of the purification process

Control of Plasmid DNA Isolation

PCR analysis was performed to check whether the plasmid DNA isolation was performed effectively and whether the LeExp1 gene was inserted into the TOPO vector. The image of 0.8% electrophoresis gel obtained as a result of the analysis is given in Figure 3. In the electrophoresis gel image, there is a DNA marker in the first well, an A100 TOPO vector in the second well, and an

A200 TOPO vector in the third well. It is known that the size of the vectors is approximately 1000 bp according to the marker used and that special primers are used to amplify the DNA in the vector. Since the size of the DNA used is approximately 1000 bp, the result is successful in both wells, that is, the DNA has been successfully inserted into the vector. Since the band of the A100 TOPO vector is displayed more sharply in the electrophoresis gel image, subsequent analyzes were continued with this vector.

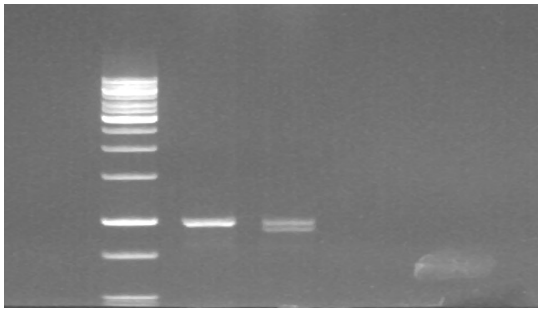


Figure 3. Plasmid DNA isolation 0.8% electrophoresis gel image, DNA marker (well 1), LeExp1 gene (well 2)

SDS Page (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) Analysis

Samples in tubes left for expression were collected every 5 hours, which continued for approximately two days until expression was complete. SDS Page analysis was performed to detect the presence of protein in the samples stored at -20 °C during the expression process. The gel image obtained as a result of the analysis is given in Figure 4. The gel image seen in Figure 4 is an image of the plasmid in protein synthesis without the addition of an inductor. The samples in the wells of the gel belong to the 0, 10, 20, 30, 40, and 45 hours, respectively. The size of the LeExp 1 protein is known to be approximately 30 kDa. The size of the bands observed as marked in corresponds to approximately, also 30 kDa.

Protein synthesis was continued by adding an IPTG inductor to observe whether the IPTG inducer positively affected protein synthesis. In the gel image shown in Figure 5, protein synthesized plasmids were visualized by adding an IPTG inductor. As a result of the use of IPTG at a concentration of 0.1 M in protein synthesis, it was observed that its synthesis improved positively.

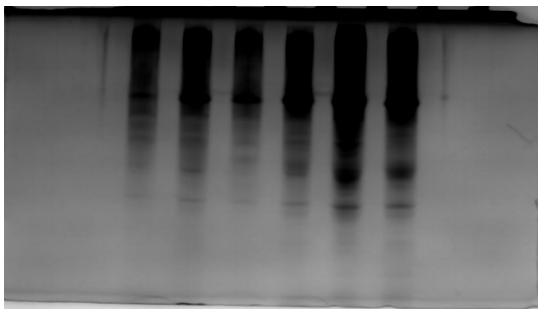


Figure 4. SDS Page gel image of Vector A

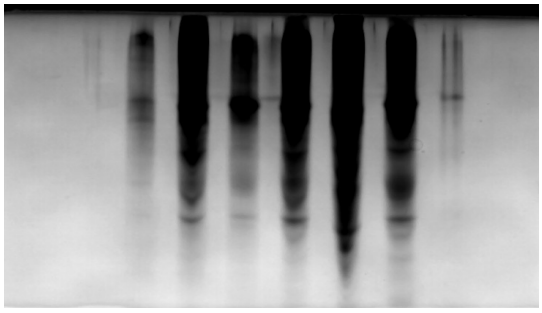


Figure 5. SDS Page gel image of vector A with added inductor

CONCLUSION

Today, due to the decreasing natural resources, microorganisms and bacteria are seen as potential in many production areas and intensive studies are carried out on this subject. As a result of the studies, many kinds of enzymes can be produced and these enzymes contribute greatly to the country's economy. 60% of these enzymes, which are an indicator of industrial development, are recombinant products. Expansin proteins are found in small amounts in plants and these proteins are not produced microbially. Expansin proteins cannot be produced in pure culture, and isolating them from plants is costly. The production of expansin proteins in recombinant protein formats would overcome these problems. In this study, a potential alternative protein to the enzymes that break down the cell wall was obtained by producing the recombinant Expanzine protein. Since the expansin protein is very costly to isolate and produce, the relevant gene (LeExp 1) was produced as a recombinant protein. Since this protein obtained in the study was produced recombinantly, a new biological agent was obtained that breaks down the cell wall. Whether this protein provides a synergistic effect when used with other enzymes and economic analysis of the production for industrial applications of recombinant expansion can be evaluated in further study.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

This work was supported by TUBITAK within the scope of its project numbered 113O392. Researchers would like to thank TÜBİTAK for its support.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgments

This study was a part of the Master's Thesis of Serap Güneş (Harran University, Department of Food Engineering, Turkey). The authors would like to thank all the staff members of the Food Engineering Department of Harran University.

REFERENCES

Andersson, P., Edenståhl, S., Eriksson, E., Nielsen, J., Pihlblad, A. (2018). The Future's Expression Systems for Complex Proteins: Evaluation of Twelve Expression Systems, Uppsala.

Anon. (2010). Champion™ pET Directional TOPO® Expression Kits. Five-minute, directional TOPO® Cloning of blunt-end PCR products into vectors for high-level, inducible expression in *E. coli*. Catalog no. K100-01, K101 01, K102-01, K151- 01, K200-01.

Cosgrove. D.J. (2000). Expansive growth of plant cell walls. Plant Physiology and Biochemistry 38:1-1.

García-Fruitós, E. (2014). Insoluble Proteins: Methods and Protocols, Insoluble Proteins Methods Protoc Vol. 1258, pp. 1–422, https://doi.org/10.1007/978-1-4939-2205-5

Gräslund, S., Nordlund, P., Weigelt, J., Hallberg, B.M., Bray, J., Gileadi, O., Knapp, S., Oppermann, U., Arrowsmith, C., Hui, R., Ming, J., Dhe-Paganon, S., Park, H., Savchenko, A., Yee, A., Edwards, A., Vincentelli, R., Cambillau, C., Kim, R., Kim, S.H., Rao, Z., Shi, Y., Terwilliger, T.C., Kim, C.Y., Hung, L.W., Waldo, G.S., Peleg, Y., Albeck, S., Unger, T., Dym, O., Prilusky, J., Sussman, J.L., Stevens, R.C., Lesley, S.A., Wilson, I.A., Joachimiak, A., Collart, F., Dementieva, I., Donnelly, M.I., Eschenfeldt, W.H., Kim, Y., Stols, L., R.Wu., Zhou, M., Burley, S.K., Emtage, J.S., Sauder, J.M., Thompson, D., Bain, K., Luz, J., Gheyi, T., Zhang, F., Atwell, S., Almo, S.C., Bonanno, J.B., Fiser, A., Swaminathan, S., Studier, F.W., Chance, M.R., Sali, A., Acton, T.B., Xiao, R., Zhao, L., Ma, L.C., Hunt, J.F., Tong, L., Cunningham, K., Inouye, M., Anderson, S., Janjua, H., Shastry, R., Ho, C.K., Wang, D., Wang, H., Jiang, M., Montelione, G.T., Stuart, D.I., Owens, R.J., Daenke, S., Schütz, A., Heinemann, U., Yokoyama, S., Büssow, K., Gunsalus, K.C. (2008). Protein production and purification, Nat. Methods, 5, s. 135–146. https://doi.org/10.1038/nmeth.f.202

Hartley, J.L. (2006). Cloning technologies for protein expression and purification, Current Opinion in Biotechnology, 17:359-366.

Hughes, S.S. (2001). Making dollars out of DNA. The First Major Patent in biotechnology and the commercialization of molecular biology, 1974-1980. Isis., 92(3): 541-75.

Jackson, D., Symons, R., Berg, P., (1972). Biochemical method for inserting new genetic information into DNA of simian virus 40: circular sv40 DNA molecules containing lambda phage genes and the galactose operon of Escherichia coli. Proc Natl Acad Sci, 69(10): 2904-2909.

Katartaş, Z. (2011). Drug production with recombinant DNA technology. Graduation Paper, Erciyes University, Faculty of Pharmacy, Pharmaceutical Biotechnology, Kayseri.

Ladisch, M.R., Kohlmann, K.L. (1992). Recombinant human insulin. Biotechnol. Prog., 8:469-478.

Mullis, K.B. (1990). Recombinant DNA technology and molecular cloning, Scientific American, 262(36): 181-231.

Rabert, C., Weinacker, D., Pessoa, A., Farías, J.G. (2013). Recombinants proteins for industrial uses: utilization of Pichia pastoris expression system, Braz. J. Microbiol. 44, 351–356, https://doi.org/10.1590/S1517-83822013005000041

Sanchez-Garcia, L., Martín, L., Mangues, R., Ferrer-Miralles, N., Vázquez, E., Villaverde, A. (2016). Recombinant pharmaceuticals from microbial cells: a 2015 update, Microb. Cell Fact., 15, pp. 1-. https://doi.org/10.1186/s12934-016-0437-3

Walsh, G. (2014). Biopharmaceutical benchmarks, Nat. Biotechnol. 32 (2014) 992–1000, https://doi.org/10.1038/nbt.3040

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 122-125
DOI: https://doi.org/10.31015/jaefs.2023.1.14

Quality parameters of *Paspalum paspalodes* in wetland of Kızılırmak delta

Sebahattin Albayrak[1]

[1] Ondokuz Mayıs University, Vocational School of Bafra, Samsun, Türkiye

Abstract

The Kızılırmak Delta, located in the north of Turkey, covers approximately 20,000 hectares and contains seven lakes of various sizes. There are Balık, Cernek, Gıcı, Liman, Uzun and Tatlı Lakes in the east and Karaboğaz Lake in the west and the width of these lakes is around 3,600 hectares. While the lakes have the largest area in the winter months, the water level decreases in the summer months and livestock are grazed in these areas. This study was carried out in the grassland areas formed by the withdrawal of water in the summer period in the south of Balık Lake. *Paspalum paspalodes* is the powerful plant species that appears with the withdrawal of water in the summer grazing period. In the present study, the CP, ADF, NDF and RFV values of the watergrass plant were found to be 8.92, 36.83, 60.57 and 92.45%, respectively. According to the results of the research; It was determined that *Paspalum paspalodes* was lovingly eaten by water buffaloes in the Kızılırmak Delta and the forage quality was medium.

Keywords: Kızılırmak Delta, Yield, Quality, Water grass, Buffalo

Citation: Albayrak, S. (2023). Quality parameters of *Paspalum paspalodes* in wetland of Kızılırmak delta International Journal of Agriculture, Environment and Food Sciences, 7 (1), 122-125

Received: 20 October 2022
Revised: 01 November 2022
Accepted: 04 November 2022
Published Online: 05 March 2023

Correspondence: Sebahattin Albayrak
E-mail: sebahattinalbayrak@omu.edu.tr

INTRODUCTION

Kızılırmak Delta is located in Ondokuz Mayis, Bafra and Alaçam districts of Samsun, Turkey. The size of the delta area is 23.686 hectares in total. There are seven lakes in the delta. Six of them are on the east side and one on the west side. There is Karaboğaz Lake in the west, Tatlı Lake, Balık Lake, Gıcı Lake, Cernek Lake, Uzun Lake and Liman Lake in the east. Wetlands (lakes) in the delta have an average (between 2015-2021) total area of 3595.53 ha. This size corresponds to approximately 15% of the delta. According to the 6-year average size of the wetlands, the maximum size is 6012.14 ha (approximately 25% of the area), and the minimum size is 1930.52 ha (approximately 8% of the area). Wetlands throughout the delta are at their widest in December-February; it also has the narrowest spread in August-September (Anonymous, 2021).

When the main habitat types and related habitats are examined in the Kızılırmak Delta, it has been determined that there are 12 different species groups (plant associations, plant communities). These groups of species have come together in different parts of the delta according to their relations and contacts with fresh and salt water, changes in soil structure and formed plant communities with different floristic compositions (Şahin et al. 2013). One of these plant communities is flooded meadows.

Flooded meadows develop in places where the altitude is very close to sea level

(0-1 m), where the ground water remains on the soil surface or in water for part of the year, close to lakes and in relatively shallow places. The surface area of the lakes expands with the increasing amount of water from autumn, and it is reduced to its normal size in the spring. Here, some of the parts that are under water with the expansion in this winter continue to remain wet in the summer, with the ground water not being fully withdrawn. These flooded meadows, which developed under the dominance of Paspalum paspalodes species in the delta, actually have the appearance of a swamp, as the clayey soils keep this water inside. However, the low depth of the soil prevents the swamp from being dangerous for living things. While Paspalum paspalodes shows a very good growth here, species diversity is very low due to the excessive amount of water and the tightly developed roots of the Paspalum do not allow other plants to cling. It offers a very good nutrition opportunity for both cattle and birds. These flooded meadows, which can be grazed all summer long, are sensitive to the operation of the delta system and changes in the amount of water, as they are formed close to the lakes with the arrival of alluvium spreading to the delta (Anomim, 2018).

In this study; the quality characteristics of Paspalum paspalodes (water meadow) plant, which emerged with the withdrawal of water in June in the pasture of Yörükler village, located in the south of Balık Lake in the Kızılırmak Delta, were investigated.

MATERIALS AND METHODS

This research was carried out in the pasture of Yörükler village, located in the south of Balık Lake in the Kızılırmak Delta, in the period of May-August 2022. The study area is at 41° 3201' N, 36° 0431' E, and its altitude is 1 m. This area is under water in winter and turns into grassland with the withdrawal of water in June and the area size is approximately 80 hectares. Paspalum paspalodes (water meadow) is the dominant plant species that emerges with the withdrawal of water in the summer period.

In the research; The inside of the 0.30 x 0.40 = 0.12 m^2 quadrats from 20 different points was made cut with a Bosh brand electric hand mower from 10 mm stubble height. Approximately 250 g of fresh watergrass samples taken randomly from the cut green grass cluster were dried in a drying cabinet at 70°C for 48 hours (Albayrak and Öten, 2020).

For crude protein, 1 g of each grinded sample was weighed and crude protein ratios were determined as % by applying the Kjeldahl method with the help of previously prepared solutions. ADF and NDF analyzes were made with the help of ANKOM 220 Fiber Analyzer device according to the principles reported by ANKOM technology (Albayrak and Öten, 2020). Samples were analyzed in 3 replications.

Total digestible nutrients (TDN), Dry matter intake (DMI), Digestible dry matter (DDM), Metabolic energy (ME) and Relative feed value (RFV) were calculated according to the equation specified by (Albayrak et al. 2012).

TDN = (-1.291 x ADF) + 101.35

DMI = 120% NDF % dry matter basis

DDM = 88.9-(0.779 x ADF % dry matter basis)

ME = 0.15 x ADF (MJ/kg KM)

RFV = DDM x DMI x 0.775

RESULTS AND DISCUSSION

Values of quality parameters of Paspalum paspalodes are given in Table 1. In general, in order to maintain its weight in various ruminant animals, the crude protein ratio of the forage consumed should be at least 6-8% CP (Esmaeli and Ebrahimi, 2003; El-Shatnawi and Mohawesh, 2000; Moinuddin et al. 2012). In our study, the CP ratio of Paspalum paspalodes was found to be 8.92%. The result obtained is within acceptable limits as part of the livestock maintenance diet.

ADF and NDF ratios of Paspalum paspalodes were found 36.83% and 60.57%, respectively. Total digestible nutritional value (TDN) was found to be 53.80%, while Dry matter intake (DMI) and Digestible dry matter (DDM) values were determined as 1.98% and 60.21%. ADF and NDF ratios of forage are directly related to DDM and DMI ratios. An increase in the ADF ratio decreases the DDM ratio, while an increase in the NDF ratio decreases the DMI ratio. Fibers contain the least digestible parts of the herb. NDF ratio is always higher than ADF ratio. ADF contains cellulose, lignin, silica, cutin and pectin, while NDF also contains hemicellulose (Cash and Bowman, 1993). In studies on the determination of ADF and NDF ratios of Paspalum paspalodes; Heydari et al. (2006) found the rate of ADF to be 38.52%, Filho and Rodriges (2001) 44.30%, Ertekin (2021) 36.73%. The change in NDF ratios was found as follows; Heydari et al (2006) 70.27%, Robinson et al. (2004) 53.8%, Suyama et al. (2007) 62.6%, Abideen et al. (2011) 54.6%, Filho and Rodrigues (2001)

Table 1. Average Crude protein ratio (CP), Fiber insoluble in acid solvents ADF), Insoluble fiber in neutral solvents (NDF), Total digestible nutrients (TDN), Dry matter intake (DMI), Digestible dry matter (DDM), Metabolic energy (ME) and Relative Feed Value (RFV)

CP (%)	ADF (%)	NDF (%)	TDN (%)	DMI (%)	DDM (%)	ME (MJ/kg)	RFV
8.92	36.83	60.57	53.80	1.98	60.21	5.52	92.45

73.7%, Ertekin (2021) 67.29%. In conclusion, Heydari et al. (2006) reported that wetland grasses have high NDF and ADF content, while their fiber is highly digestible.

Figure 1. Kızılırmak Delta and natural lake areas.

Figure 2. The area covered with water on March 27, 2022

Figure 3. Water withdrawal area on July 22, 2022.

The metabolic energies of Paspalum paspalodes was measured as 5.52 MJ/kg KM. Nutritional value parameters differed from species to species, and it has been reported in many studies that the nutritional values of various species may differ from each other. Indeed, it was found metabolic energy values of Paspalum paspalodes among 9.2 to 9.5(Heydari et al. 2006; Suyama et al. 2007; Ertekin, 2021).

The relative feed value is determined by evaluating the ADF and NDF ratios of the forage together. Therefore, ADF and NDF ratios should be low for the relative feed value of the forage to be high. It is reported that if the RFV value of dry forage is greater than 180, it is in the highest quality class, in the range of 150-180 superior, 125-150 good, 100-120 medium. If the RFV value of the herb is below 100, it has low quality values (Albayrak and Öten, 2020). In present study, the relative feed value of Paspalum paspaloides has low quality class.

CONCLUSION

It was concluded that the quality values of Paspalum paspalodes, which emerged with the withdrawal of water in the wetlands of the Kızılırmak Delta in the summer period, may be sufficient for the feeding of the water buffaloes grazing in the region. On the other hand, it is thought that it would be beneficial to examine the quality and yield values of Paspalum species for longer periods and at different points in the delta.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No support received

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Abideen, Z., R. Ansari., Ajmal Khan., M. (2011). Halophytes: Potential source of ligno- cellulosic biomass for ethanol production. Biomass and Bioenergy. 35: 1818-1822. https://doi.org/10.1016/j.biombioe.2011.01.023

Albayrak, S., Öten., M. (2020). Döl kontrolü parsellerindeki yonca (Medicago sativa L.) genotiplerinin verim ve kalite özellikleri ile genel kombinasyon yeteneklerinin belirlenmesi. Anadolu Tarım Bilim. Dergisi. 20(3): 353-360. https://doi.org/10.7161/omuanajas.743199 (in Turkish)

Albayrak, S., M. Türk., O. Yüksel., Yılmaz., M. (2012). Forage Yield and the Quality of Perennial Legume-Grass Mixtures under Rainfed Conditions. Not Bot Hort Agrobot Cluj. 39(1):114-118. https://doi.org/10.15835/nbha3915853

Anonymous. (2021). Kızılırmak Deltası Araştırma Projesi. T.C.

Çevre ve Şehircilik Bakanlığı. Tabiat Varlıklarını Koruma Genel Müdürlüğü. 604s. (in Turkish)

Anonymous. (2018). Kızılırmak deltası su ayak izinin belirlenmesi projesi. Çevre ve Şehircilik Bakanlığı. Ankara. 528s. (in Turkish)

Cash, D., Bowman., H.F. (1993). Alfalfa hay quality testing, Montguide, Agriculture, MT-9302.

El-Shatnawi, M.K., Mohawesh., Y.M. (2000). Seasonal chemical composition of saltbush in semiarid grasslands of Jordan. J. Range Manage., 53: 211-214. https://doi.org/10.2307/4003285

Ertekin., İ. (2021). Bazı çok yıllık taban merası yem bitkisi türlerinin yem değerleri yönünden karşılaştırılması. 3 Internatıonal Eurasıan Conference On Scıence, Engıneerıng And Technology. 15-17 December 2021 Ankara / Turkey. (in Turkish)

Esmaeli, N., Ebrahimi., A. (2003). Necessity of determining animal unit requirement based on the quality of forage. Iranian J. Nat. Resourc., 55: 579-596.

Filho, C.V.S., Rodrigues., A. (2001). Evaluatıon Of Ten Tropıcal Grasses In The Northwest Regıon Of The State Of São Paulo-Brazıl. The XIX International Grassland Congress took place in São Pedro, São Paulo, Brazil from February 11 through February 21, 2001.

Heydari, G., A.T. Yansari., Zali., H. (2006). Inspection on Three Plant Spices as an Animal Forage Source in Mazandran Wetland. Pakistan J. of Nutrition. 5(4): 382-386. https://doi.org/10.3923/pin.2006.382.386

Moınuddın, M., S. Gulzar., I. Aziz., A.R.A. Alatar., A K. Hegazy., M. Khan., A. (2012). Evaluatıon Of Forage Qualıty Among Coastal And Inland Grasses From Karachı. Pak. J. Bot., 44(2): 573-577.

Filho, C.V.S., L.R. de A. Rodrigues. 2001. Evaluatıon Of Ten Tropıcal Grasses In The Northwest Regıon Of The State Of São Paulo-Brazıl. The XIX International Grassland Congress took place in São Pedro, São Paulo, Brazil from February 11 through February 21, 2001.

Robinson, P.H., S.R. Grattan., G. Getachew., C.M. Grieve., J.A. Poss., D.L. Suarez., Benes., S.E. (2003). Biomass accumulation and potential nutritive value of some forages irrigated with saline-sodic drainage water. Animal Feed Science and Technology. 111. 175–189. https://doi.org/10.1016/S0377-8401(03)00213-X

Suyama, H., S.E. Benes, P.H. Robinson, S.R. Grattan, C.M. Grieve., Getachew., G. (2007). Forage yield and quality under irrigation with saline-sodic drainage water: Greenhouse evaluation. Agricultural Water Management, 88: 159-172. https://doi.org/10.1016/j.agwat.2006.10.011

Şahin, B., Aslan, S., Ayyıldız, G., Vural., M. (2013). Kızılırmak Deltasında görülen habitat tipleri. III. Ulusal Sulak alanlar Kongresi. 271-276. 23-25 Ekim 2013. Samsun. (in Turkish)

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 126-130
DOI: https://doi.org/10.31015/jaefs.2023.1.15

Evaluation of some Triticale (x *Triticosecale* Wittmack) cultivars and lines under Duzce ecological conditions

Huseyin Gungor[1] • **Mehmet Fatih Cakir**[2] • **Yagmur Turan**[3] • **Bahar Gedik**[1]
Berkay Hakki Kantar[1] • **Ziya Dumlupinar**[4]

[1] Department of Field Crops, Faculty of Agriculture, Duzce University, Duzce, Türkiye
[2] Environment and Health Coordination, Duzce University, Duzce, Türkiye
[3] Graduate Education Institute, Duzce University, Duzce, Türkiye
[4] Department of Agricultural Biotechnology, Faculty of Agriculture, Kahramanmaras Sutcu Imam University, Kahramanmaras, Türkiye

Citation: Gungor, H., Cakir, M.F., Turan, Y., Gedik, B., Kantar, B.H., Dulupinar, Z. (2023). Evaluation of some Triticale (x *Triticosecale* Wittmack) cultivars and lines under Duzce ecological conditions. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 126-130

Received: 14 November 2022
Revised: 03 December 2022
Accepted: 06 December 2022
Published Online: 06 March 2023

Correspondence: Huseyin Gungor
E-mail: hgungor78@hotmail.com

Abstract

This study was carried out with five triticale cultivars (Özer, Truva, Karma-2000, Alperbey and Tatlıcak-97) and 10 advanced triticale lines to determine the grain yield and yield components during 2020-2021 and 2021-2022 growing seasons under Duzce ecological conditions. In this study, the parameters of the genotypes such as spike length, number of spikelets per spike, number of grains per spike, grain weight per spike, thousand kernel weight, and grain yield were investigated. According to the two year results, whereas spike length, number of spikelets per spike, and number of grains per spike of genotypes changed 10.6-15.3 cm, 24.5-39.7, and 48.8-87.8, respectively, the grain related parameters such as grain weight per spike, thousand kernel weight, and grain yield were 2.518-4.261 g, 34.3-46.8 g, 720.2-1093.4 kg da^{-1}, respectively. The highest yielding genotypes were found to be T-5, T-8, T-9 and Özer.

Keywords: Triticale, Grain yield, Yield components

INTRODUCTION

Triticale (x *Triticosecale* Wittmack) is a self-pollinated allohexaploid species of cereals with AABBRR genome, which was derived from crossing *T. turgidum* and *Secale cereale* and mostly produced for its grain (Lukaszewski and Gustafson, 1987).

In Turkey, the triticale cultivation area was 93,990 ha, the production amount was 228,000 tons, and the average yield was 245 kg da^{-1} in 2021 (TUIK, 2022).

In terms of potential yield, triticale is similar to wheat, which is highly adaptable like rye. Triticale has high grain, green grass yield, rapid development, and growth characteristics. Additionally, its high lysine content is essential for both human and animal nutrition (Oral and Ulker, 2016).

Triticale (x *Triticosecale* Wittmack) is more suited for cultivation in marginal lands because it is more resistant to biotic and abiotic stress conditions than wheat (Villegas et al., 2010). The triticale has an essential place in eliminating the current roughage and concentrated feed deficit problem in Turkey (Mut and Erbas Kose, 2018).

It is crucial to expand triticale production and develop triticale varieties suitable for different regions to evaluate marginal areas and contribute to animal husbandry in Turkey (Senturk and Akgun, 2014).

This study was carried out to evaluate some triticale cultivars and advanced

lines in terms of yield and yield components in Duzce ecological conditions.

MATERIALS AND METHODS

The study was conducted in Duzce during the 2020–2021 and 2021–2022 growing seasons. 10 advanced triticale lines named as T-1, T-2, T-3, T-4, T-5, T-6, T-7, T-8, T-9, and T-10 as well as five registered triticale cultivars of Özer, Truva, Karma-2000, Alperbey, and Tatlcak-97 were both used in the study. The study was conducted in four replications using the randomized blocks experimental design. In both growing seasons, sowing was carried out manually in 5 m-long plots with 20 cm row spacing and six rows of 500 seeds per m^2 in the first week of November. At both planting and harvest, the experiment's parcel sizes were carried out to be 6 m^2. Herbicides were used for weed control in the trial plots, and no pesticides was applied for diseases and pests. After sowing, 50 kg ha^{-1} nitrogen and 50 kg ha^{-1} phosphorus as pure were applied, the top fertilizer was divided into two, and 90 kg ha^{-1} nitrogen was applied during the tillering period, and 60 kg ha^{-1} nitrogen was applied during the tillering period. Harvest was done in the first week of July in both growing seasons. In the study, parameters such as spike length, number of spikelets per spike, grain number per spike, grain weight per spike, thousand kernel weight, and grain yield were evaluated.

The data was analyzed using JMP 10 statistical analysis program (JMP, 2010), and the comparisons among the averages of the traits with differences were evaluated with the Duncan multiple comparison test.

RESULTS AND DISCUSSION

The mean values of the spike length and the number of spikelets per spike of the triticale genotypes in the experiment are given in Table 1. In terms of spike length and the number of spikelets per spike, the year and genotype × year interaction was statistically insignificant, but the difference among genotypes was significant (Table 1).

The average spike length varied between 10.6 and 15.3 cm in the study's first and second years, averaging 10.7 to 14.8 cm in the first year and 10.6 to 15.82 cm in the second year. According to the average of the two years, the average spike length was found to be 12.65 cm. The first and second years' average spike lengths were 12.6 cm and 12.7 cm, respectivly.

The shortest spike length was recorded in the T-3 line (10.7 cm, 10.6 cm, 10.6 cm), and the longest spike length was recorded in cultivar Truva, according to data from the first, second, and combined years (14.8 cm, 15.8 cm, 15.3 cm). (Table 1). The spike length varies between 9.10 to 16.08 cm, according to different studies (Tayyar and Kahriman, 2016; Dolgun and Cifci Aydogan, 2019; Sirat et al., 2020; Gungor et al., 2022).

The genotypes ranged from 24.7 to 40.3 in terms of spikelets per spike in the first year and from 24.3 to 39.0 spikelets per spike in the second year (Table 1). The cultivar Truva had the highest number of spikelets in both the first and second years of the experiment (40.3, 39.0 respectively) whereas the T-3 (24.7, 24.3) line had the lowest spikelets per spike (Table 1). The values for the combined years show that each spike had between 24.5 and 39.7 spikelets. The maximum number of spikelets per spike were found in the Truva (39.7), T-2 (33.7), and Özer (33.5) genotypes. The lowest number of spikelets per spike were determined in the T-3 (24.5), T-6 (25.5), and T-8 (25.7) lines (Table 1).; Senturk and Akgun (2014), Dolgun and Cifci Aydogan (2019), and Sirat et al. (2020), reported that the number of spikelets per spike varies between 21.1-24.8, 21.1-29.7, and 22.7-24.43, respectively.

Years were found to be insignificant in terms of the number of grains per spike, while the genotype × year interaction and genotypes were found to be statistically significant (Table 2). The number of grains per spike varied between 46.7-82.3 in the first year, 50.7-93.3 in the second year, and 48.8-87.8 in the average of years in triticale genotypes (Table 2). The highest number of grains per spike in the first year, the second year, and the average of two years were found in the T-2 line (82.3, 93.3, 87.8) whereas the lowest grain number per spike was found in the T-3 (46.7) line in the first year, T-1 (50.7) line in the second year T-3 (48.8) line in the average of the years (Table 2). According to the studies of Dolgun and Cifci Aydogan (2019), Oral and Ulker (2020), Sirat et al. (2020), and Gungor et al. (2022), the number of grains per spike varied between 34.3-54.3, 37.4-42.7, 42.28-59.32, and 53.2-79.9, respectively

In terms of grain weight per spike, the difference between years and genotype × year interaction was insignificant, but the difference among genotypes was statistically significant (Table 2)

In terms of grain weight per spike, it varied between 3.998-4.251 g in the first year, 2.532-4.525 g in the second year, and 2.518-4.261 g in the average of years. In the first year of the study, the highest grain weight per spike was determined in the T-9 (4.251 g) and T-2 (3.998 g) lines and the lowest grain weight per spike was determined in T-7 (2.347 g) and T-3 (2.438 g) lines. In the second year of the experiment, the highest grain weight per spike were determined in the T-2 (4.525 g), and T-10 (4.356 g) lines and the lowest grain weight per spike was determined in T-6 (2.532 g) and T-3 (2.598 g) lines. When the average of years was examined, the lowest grain weight per spike was found in the T-3 (2.518 g) line, and the highest grain weight per spike was determined in the T-2 (4.261 g) line (Table 2). In similar studies, the grain weight per spike was determined by Senturk and Akgun (2014), 1.25-1.51 g; Dolgun and Cifci Aydogan (2019), 1.5-2.8 g, Sirat et al. (2020), 1.98-2.66 g, Gungor et al. (2022), reported that it varies between 2.32-3.61 g. In the study, the findings obtained with grain weight per spike were similar to the

Table 1. Average of triticale genotypes of spike length (cm) and number of spikelets per spike

Genotypes	Spike length (cm)			Number of Spikelets/Spike (no)		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
Özer	14.1 ab	13.5 bcd	13.8 bc	33.0 b	34.0 bc	33.5 b
Truva	14.8 a	15.8 a	15.3 a	40.3 a	39.0 a	39.7 a
Karma-2000	11.2 gh	12.6 b-e	11.9 ef	29.0 cd	31.7 bcd	30.3 cd
Alperbey	12.8 cde	12.7 b-e	12.7 de	26.6 de	28.6 d-g	27.7 ef
Tatlıcak-97	12.3 def	12.1 d-g	12.2 def	33.0 b	31.7 cde	31.8 bc
T-1	11.6 fgh	11.5 efg	11.5 f	27.3 cde	27.0 f-ı	27.2 ef
T-2	14.0 ab	13.9 b	13.9 b	32.7 b	34.7 b	33.7 b
T-3	10.7 h	10.6 g	10.6 g	24.7 e	24.3 ı	24.5 g
T-4	14.1 ab	14.1 b	14.1 b	28.7 cd	28.3 d-h	28.5 de
T-5	11.3 gh	11.4 efg	11.3 fg	28.3 cd	27.3 e-ı	27.8 ef
T-6	11.9 efg	11.0 fg	11.4 fg	26.0 de	25.0 hı	25.5 fg
T-7	12.1 efg	12.2 def	12.1 def	26.6 de	28.3 d-h	27.5 ef
T-8	11.4 fgh	12.3 c-f	11.8 f	26.0 de	25.3 ghı	25.7 fg
T-9	13.8 bc	13.8 bc	13.7 bc	27.3 cde	26.6 ghı	27.0 ef
T-10	13.2 bcd	12.7 b-e	12.9 cd	30.3 bc	30.3 def	30.3 cd
Year mean	12.6	12.7	12.65	29.3	29.4	29.35
Genotype (G)	**	**	**	**	**	**
Year (Y)		ns			ns	
G x Y		ns			ns	

** Significant P < 0.01, * significant P < 0.05, and ns: not significant

Table 2. Average of triticale genotypes of number of grains per spike and grain weight per spike (g)

Genotypes	Grain number/spike (no)			Grain weight/spike (g)		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
Özer	80.0 abc	73.3 bc	76.7 ab	3.556 abc	3.661 a-d	3.608 abc
Truva	81.0 ab	70.0 bcd	75.5 bc	3.615 abc	3.025 cde	3.320 bcd
Karma-2000	59.3 def	79.3 ab	69.3 b-e	2.708 cd	3.563 a-e	3.136 b-e
Alperbey	64.0 cde	66.0 b-e	65.0 c-f	2.984 bcd	2.735 cde	2.860 de
Tatlıcak-97	64.7 b-e	63.3 b-e	64.0 def	3.062 bcd	3.251 cde	3.157 b-e
T-1	51.3 ef	50.7 e	51.0 gh	3.043 bcd	2.770 cde	2.906 cde
T-2	82.3 a	93.3 a	87.8 a	3.998 ab	4.525 a	4.261 a
T-3	46.7 f	51.0 e	48.8 h	2.438 d	2.598 de	2.518 e
T-4	73.6 a-d	74.3 bc	74.0 bcd	3.708 abc	3.363 b-e	3.535 a-d
T-5	57.7 def	58.7 cde	58.2 e-h	3.156 a-d	3.360 b-e	3.258 bcd
T-6	77.0 abc	58.3 cde	67.7 b-e	3.557 abc	2.532 e	3.045 cde
T-7	51.6 ef	72.3 bc	62.0 efg	2.347 d	3.759 abc	3.053 cde
T-8	57.0 def	55.0 de	56.0 fgh	3.071 bcd	3.444 b-e	3.258 bcd
T-9	81.0 ab	69.6 bcd	75.3 bc	4.251 a	3.360 b-e	3.805 ab
T-10	67.3 a-e	71.3 bc	69.3 b-e	3.235 a-d	4.356 ab	3.796 ab
Year mean	66.3	67.1	66.7	3.249	3.353	3.301
Genotype (G)	**	**	**	*	*	**
Year (Y)		ns			ns	
G x Y		*			ns	

** Significant P < 0.01, * significant P < 0.05, and ns: not significant

Table 3. Average of triticale genotypes of thousand kernel weight (g) and grain yield (kg da^{-1})

Genotypes	Thousand Kernel Weight (g)			Grain Yield (kg/da)		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
Özer	41.3 e-h	32.0 f	36.6 ef	994.6 ab	1043.8 ab	1019.2 ab
Truva	41.9 efg	36.0 d	39.0 c	939.7 bc	1034.5 ab	987.1 bc
Karma-2000	45.5 cd	28.9 h	37.2 de	833.2 cd	1013.7 abc	923.5 cd
Alperbey	39.2 ghı	31.6 fg	35.4 fg	830.6 cd	817.6 de	824.1 ef
Tatlıcak-97	38.1 hı	30.4 g	34.3 g	712.8 e	834.8 de	773.8 efg
T-1	37.9 ı	36.9 cd	37.4 cde	719.5 de	978.3 bc	848.9 de
T-2	42.6 def	34.5 e	38.6 cd	732.5 de	707.9 f	720.2 g
T-3	42.1 efg	34.6 e	38.4 cd	649.7 e	865.6 de	757.6 fg
T-4	44.5 de	38.4 b	41.5 b	862.4 c	985.3 bc	923.8 cd
T-5	55.2 a	38.3 b	46.8 a	1072.3 a	1114.6 a	1093.4 a
T-6	40.2 f-ı	38.0 bc	39.1 c	721.5 de	785.8 ef	753.7 fg
T-7	41.7 efg	35.6 de	38.7 cd	928.1 bc	1088.4 a	1008.2 b
T-8	44.3 de	41.7 a	43.0 b	997.7 ab	1106.1 a	1051.9 ab
T-9	48.7 bc	35.7 de	42.2 b	1058.6 a	1038.7 ab	1048.7 ab
T-10	49.6 b	36.7 d	43.2 b	924.5 bc	914.6 cd	919.6 cd
Year mean	43.5 a	35.3 b	39.4	865.2 b	955.3 a	910.3
Genotype (G)	**	**	**	**	**	**
Year (Y)		**			**	
G x Y		**			**	

** Significant P < 0.01, * significant P < 0.05, and ns: not significant

results of Senturk and Akgun (2014), Dolgun and Cifci Aydogan (2019) and Sirat et al. (2020).

In terms of thousand kernel weight and grain yield, the variations among genotype, year, and genotype year interaction were found to be significant (Table 3). The genotypes' thousand kernel weights ranged from 37.9 to 55.2 g in the study's first year, from 28.9 to 41.7 g in the following year, and from 34.3 to 46.8 g on average throughout the two years (Table 3). According to the average of the two years, the average thousand kernel weight was 43.5 g in the first year, 35.3 g in the second year, and 39.4 g in the average year. The highest thousand kernel weight was determined in the T-5 (55.2 g) line in the first year, the T-8 (41.7 g) line in the second year, and the T-5 (46.8 g) line in the average of years, whereas the lowest thousand kernel weight in the first year T-1 (37.9 g) line, Karma-2000 (28.9 g) line in the second year and Tatlıcak-97 (34.3 g) line in the average of two years (Table 3). They noted that the thousand kernel weight varied between 24.64-40.30 g in different ecological conditions (Lermi and Palta, 2018; Mut and Erbas Kose, 2018; Dolgun and Cifci Aydogan, 2019; Gungor et al., 2022).

In terms of grain yield, it varied between 712.8-1072.3 kg da^{-1} in the first year, 707.9-1114.6 kg da^{-1} in the second year, and 720.7-1093.4 kg da^{-1} according to the combined years, and the average grain yield was determined as 910.3 kg da^{-1} (Table 3). T-5 (1093.4 kg da^{-1}), T-8 (1051.9 kg da^{-1}), T-9 (1048.7 kg da^{-1}) lines, and Özer (1019.2 kg da^{-1}) cultivar had the highest grain yield, according to the average of the years. Low grain yield was obtained in T-2 (720.2 kg da^{-1}), T-3 (757.6 kg da^{-1}) lines, and Tatlıcak-97 (773.8 kg da^{-1}) variety (Table 3). In the studies of Senturk and Akgun (2014), Lermi and Palta (2018), Dolgun and Cifci Aydogan (2019), Mut and Erbas Kose, (2019), and Gungor et al. (2022), the grain yield was found to be varying between 475.0-564.0 kg da^{-1}, 325.8-805.8 kg da^{-1}, 189.2-314.2 kg da^{-1}, 230.4-366.1 kg da^{-1}, and 553.9-680.0 kg da^{-1}, respectively. In the study, the findings with the grain yield were higher than the results of previous studies suggesting that grain yield differs according to genetic structure, environmental factors and cultivation techniques (Senturk and Akgun, 2014; Mut and Erbas Kose, 2018; Gungor et al., 2022).

CONCLUSION

This study was carried out to evaluate five cultivars and 10 advanced triticale lines under Duzce ecological conditions. It was concluded that it would be appropriate to conduct experiments with the T-5, T-8 and T-9 lines standing out in grain yield in other regions where triticale cultivation is carried out.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Dolgun, C. and Cifci Aydogan, E., 2019. Determination of yield and quality characteristics of some triticale genotypes in Bursa ecological conditions. KSU J. Agric Nat 22(5), 664-671. https://doi.org/10.18016/ksutarimdoga.vi.518718

Gungor, H., Cakir, M.F. and Dumlupinar, Z. (2022). Evaluation of Triticale: Genotype by Environment Interaction and GGE Bıplot Analysis. Journal of Animal and Plant Sciences, 32(6), 1637-1647. https://doi.org/10.36899/JAPS.2022.6.0573

JMP, (2010). JMP User Guide, Release 10 Copyright © 2010, SAS Institute Inc., Cary, NC, USA, ISBN 978-1-59994-408-1. Retrieved from: http://www.jmp.com/getstarted

Lermi, A.G. and Palta, S. (2018). Research on seed yield of different triticale (x *Triticosecale* Wittmack) cultivars in West Black Sea eology. Journal of Bartin Faculty of Forestry, 20(2), 366-372. https://doi.org/10.24011/barofd.442283

Lukaszewski, A.J. and Gustafson, J.P.D. (1987). Cytogenetics of triticale. Plant Breed. Rev, 5, 41-93. https://doi.org/10.1002/9781118061022.ch3

Mut, Z. and Erbas Kose, O.D. (2018). Grain yield and some quality properties of triticale genotypes. Anadolu J. Agr Sci, 33, 47-57. https://doi.org/10.7161/omuanajas.336108

Oral, E. and Ulker, M. (2016). Path analysis and relations between features in triticale (x *Triticosecale* Witmack) varieties. Iğdır Univ. J. Inst. Sci. & Tech. 6(3), 153-160. Retrieved from: https://dergipark.org.tr/en/pub/jist/issue/34622/387915

Senturk, S. and Akgun, I. (2014). Determination of yield and yield components of some triticale genotypes grown in Western Transition Zone. Journal of the Faculty of Agriculture, 9(1), 16-26. Retrieved from: https://dergipark.org.tr/tr/download/article-file/308664

Sirat, A., Bahar, B. and Bahar, N. (2020). A research on grain yield and yield components of triticale (x *Triticosecale* Wittmack) cultivars in continental climate and arid agricultural conditions of Eastern Black Sea Region. Journal of Bahri Dagdas Crop Research, 9 (2), 134-146. Retrieved from: https://dergipark.org.tr/tr/pub/bdbad/issue/58586/846529

Tayyar, S. and Kahriman, F. (2016). Determination of yield and some quality characteristics of triticale genotypes grown under Biga conditions. Journal of Adnan Menderes University Agricultural Faculty, 13(2), 23 – 31. https://doi.org/10.25308/aduziraat.293416

TUIK, 2022. Turkish Statistical Institute. Retrieved from: https://www.tuik.gov.tr (Accessed date: October 20, 2022).

Villegas, D., Casadesus, J., Atienza, S., Martos, V., Maalouf, F., Karam, F., Aranjuelo, I., Nogues, S. (2010). Tritordeum, wheat and triticale yield components under multi-local Mediterranean drought conditions. Field Crops Res., 116, 68-74. https://doi.org/10.1016/j.fcr.2009.11.012

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 131-135
DOI: https://doi.org/10.31015/jaefs.2023.1.16

Pesticide residues in sauce manufactured from agricultural products

Tarik Balkan[1] • **Kenan Kara**[1]

[1] Department of Plant Protection, Faculty of Agriculture, Tokat Gaziosmanpaşa University, Tokat, Türkiye

Abstract

In this study, pesticide residues in sauce manufactured from agricultural products were examined. A simultaneous multiresidue analysis for 260 pesticides in sauce samples was performed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). The QuEChERS AOAC 2007.01 (quick, easy, cheap, effective, rugged, and safe) was selected as the most suitable protocol for routine determination of pesticide residues. In line with the SANTE/11312/2021 guideline two parameters were used as identification criteria: retention time (RT) with a tolerance of ± 0.1 min in relation to the RT of the analyte in matrix-matched calibration standard and ion ratio tolerance below 30%. The RT deviation in positive samples was always < 0.1 min, and the ion ratio tolerance was < 30%. A total 4 different pesticides were detected in 20 sauce samples. No active ingredient was found in 16 samples. The residue levels were between 10 and 94 µg kg. The residues of acetamiprid, ametoctradin, imazalil sulfate, and metalaxyl-M were below the European Union Maximum Residue Limits (EU-MRLs).

Keywords: Pesticide residue, Processing factor, Sauce, Tomato, LC-MS/MS, QuEChERS

Citation: Balkan, T., Kara, K. (2023). Pesticide residues in sauce manufactured from agricultural products. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 131-135

Received: 14 November 2022
Revised: 23 December 2022
Accepted: 25 December 2022
Published Online: 06 March 2023

Correspondence: Tarik Balkan
E-mail: tarik.balkan@gop.edu.tr

INTRODUCTION

Sauce is a French word (sauce) derived from the Latin word salsus, meaning "salty". Sauces are most commonly defined as a food complement and flavor enhancer in gastronomy. Sauce is considered as important as food is in world cuisines. They are mixtures that add additional flavor to the dish, complement the existing flavor and make it easier to swallow. People want to experience the cuisines of different countries thanks to the development of technology, the expansion of the transportation network, international gastronomy tourism and franchise models. Food sauces belonging to French, Indian, Chinese and Italian cuisines enter the lives of people from different countries. In addition, with the spread of fast-food culture, sauces are consumed intensively. The global tomato paste-sauce market has reached approximately 47 million tons by the end of 2020. This figure is expected to increase to 55 million tons in 2025. In Turkey, tomato paste-sauce market is 383 thousand tons in size according to 2020 data (Karaboğa, 2021).

Similar to the other crops, fruits and vegetables used in sauce making have also pest, disease and weed problems. Therefore, pesticides are used to eliminate the negative effects of these pests during growing season and also post-harvest period. Pesticides are any substance or mixture of substances used to protect crops from harmful organisms in order to secure agricultural food production

(EPA, 2022). On the other hand, excessive use of chemicals made without paying attention to the prospectus leave residue on the product. Pesticide residues affect food safety and threaten human health. Residue studies on vegetables such as tomatoes and peppers, which are frequently used in sauce production, reveal the presence of many active substances (Ersoy et al., 2011; Bakırcı et al., 2014; Zengin and Karaca, 2017; Gölge and Kabak, 2015; Polat and Tiryaki, 2018; Balkan and Kara, 2019; Soydan et al., 2021; Balkan and Kara, 2022; Sancar et al., 2022).

Food processing refer to the methods and techniques used to convert raw materials to food for consumption by humans or animals at home or by the food processing industry (Kaushik et al., 2009). These processes are baking, bread making, dairy product manufacture, drying, thermal processing, fermentation, freezing, infusion, juicing, malting, milling, parboiling, peeling juicing and wine making. As a result of the processes which are, pesticide amounts may tend to increase or decrease depending on the physico-chemical properties of the active substance, the nature of the product and the processes they are exposed (Burchat et al. 1998; Cengiz et al., 2007; Kong et al., 2012; Scholz et al., 2017).

The European Food Safety Authority (EFSA) has found that pesticides are higher in unprocessed products, but residues are also found in processed products. However, there are no fixed MRL values for processed vegetables and fruits. For this reason, pesticide residue studies should be carried out in these products. In this study, pesticide residues were investigated in sauces produced from various agricultural products such as tomatoes, peppers and carrots etc.

MATERIALS AND METHODS

Reagents and chemicals

Pesticide reference standards were supplied by Dr. Ehrenstorfer Laboratories GmbH (Augsburg, Germany). Acetonitrile (ACN >99% purity) was supplied by Honeywell (North Carolina, USA). Methanol (MeOH >99% purity), magnesium sulfate anhydrous ($MgSO_4$ >99.5% purity), ammonium formate (NH_4CO_2H >97.5% purity) and acetic acid (AcOH >99% purity) were procured from Merck (Darmstadt, Germany). Water was purified by MP Minipure Dest Up system from Mes (Ankara, Türkiye). The QuEChERS products was purchased from Restek (Bellefonte, USA).

Chromatographic analysis

This study was carried out on LC–MS 8050 model from the Shimadzu®. LC–MS/MS system equipped with UPLC: LC-30AD pump × 2, SIL-20A autosampler, DGU-20A3R degasser, CTO-20ACV column oven and triple quadrupole MS/MS detector. The operating conditions of the device and the gradient program are given in Table 1. All parameters of the instrument were controlled using LabSolution® Insight LCMS software) (Balkan and Yılmaz, 2022).

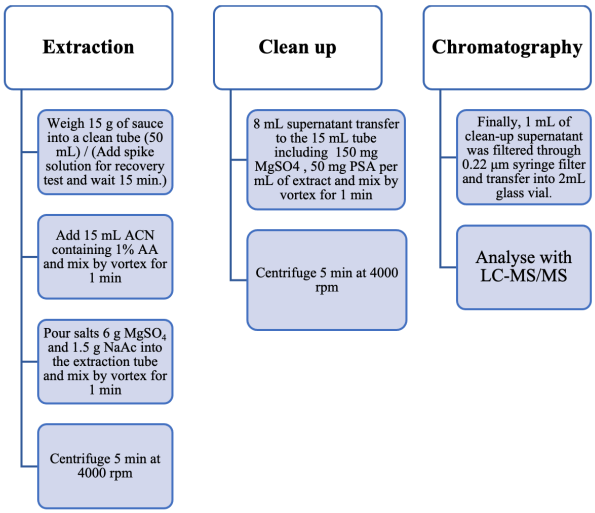


Figure 1. Analytical steps of the QuEChERS-AOAC Official Method 2007.01

Sample extraction and clean up

The official QuEChERS AOAC Method 2007.01 was used for the extraction and clean up procedures (Lehotay, 2007). The followed QuEChERS steps are illustrated in Figure 1. Each of the samples were analyzed in triplicates with LC-MS/MS.

For recovery studies, 15 g of tomato sauce sample were weighed into a 50 ml Falcon tube Then, 150 µl of pesticide mixture was added to 15 g of tube and vortexed for 60 seconds (Polat and Tiryaki, 2019; Dülger and Tiryaki, 2022). It was waited for 15 minutes for the pesticides to interact with the matrix and the steps in Figure 1 were followed.

RESULTS AND DISCUSSION

Two hundred sixty pesticides belonging to different groups (insecticide, acaricide, nematicide fungicide, herbicide, plant grow regulator and some metabolite) were discussed in the study. Analysis of 260 pesticides in sauce samples was performed using LC-MS/MS. In line with the SANTE guideline two parameters were used as identification criteria: retention time (RT) with a tolerance of ± 0.1 min in relation to the RT of the analyte in matrix-matched calibration standard and ion ratio tolerance below 30% (SANTE, 2021; Balkan and Karaağaçlı, 2023). The RT deviation in positive samples was always < 0.1 min, and the ion ratio tolerance was < 30% (Table 1). A total 4 different pesticides were detected in 20 sauce samples. No active ingredient was found in 15 samples. The residue levels were between 10 and 94 µg kg. The residues of acetamiprid, ametoctradin, imazalil sulfate, and metalaxyl-M were found above LOQs (Table 1). The chromatograms and calibration curves of the detected pesticides are given in Figure 2.

The processing factor is defined as the ratio of the pesticide residue level in the processed food to the

Table 1. LOQ, Recovery, Pesticide residue levels and requirements for identification

Sample	Pesticides found	Pesticide residue (mg/kg)	EU-MRL*	LOQ (µg/kg)	Recovery %	Standart RT (min)	Sample RT (min)	Quantification ion / Confirmation ion (m/z)	Standart ion ratio (%)	Sample ion ratio (%)
Sauce1	Acetamiprid	0.010	0.5	3.02	100.2	4.896	4.899	222.9/ 126.0	100	100
								223.0/ 56.10	18.49	18.68
Sauce2	Ametoctradin	0.015	2	2.41	90.3	8.277	8.226	276.1/ 149.15	100	100
								276.1/ 177.10	67.83	62
Sauce3	Imazalil sulfate	0.094	4	4.67	88.8	7.747	7.753	296.8/ 159.0	100	100
								296.8/ 255.0	32.83	41.66
Sauce4	Metalaxyl-M	0.033	0.3	5.29	106.9	6.198	6.150	280.05/ 220.05	100	100
								280.05/ 192.05	60.48	58.82
Sauce5	Metalaxyl-M	0.043					6.137	280.05/ 220.05	100	100
								280.05/ 192.05	60.48	66.08

*EU pesticide database (EC, 2022)

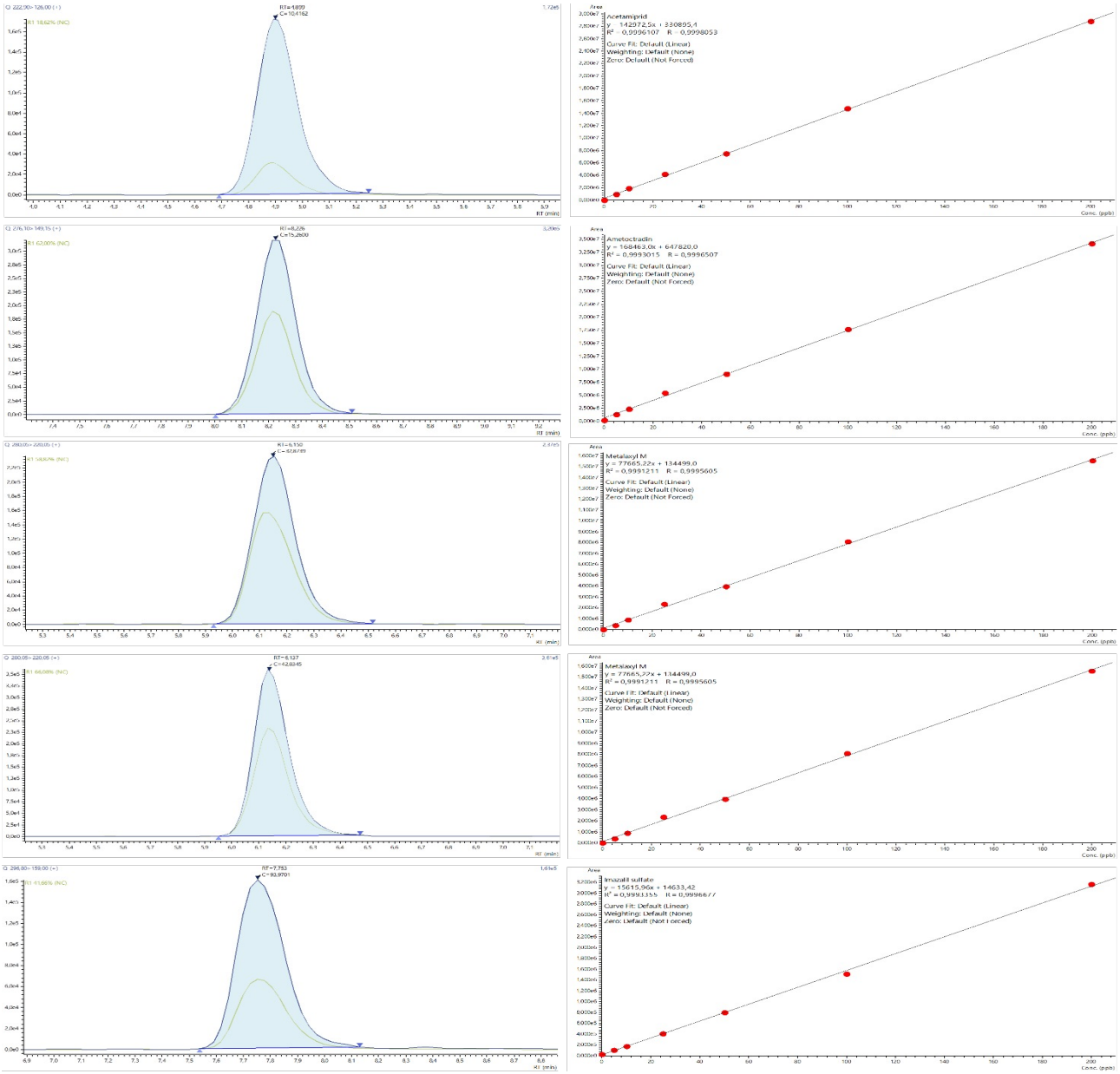


Figure 2. Acetamiprid(a), Ametoctradin(b), Imazalil sulfate(c) and Metalaxy-M (d and e) ion chromatograms for real samples (X axis: Retention time, Y axis: Signal intensity) and calibration curves

pesticide residue level in its raw form (Reddy et al., 2022). The processing factor is necessary to decide whether residues in products comply with legal standards and to assess the risks of residues to human, animal and environmental health in processed products (Scholz et al., 2017; Acoğlu et al., 2018). While determining the MRLs of the new processed products, processing factors are used according to international methods. The determined processing factors are published on the official website of the Ministry of Agriculture and Forestry. In the study, the raw results were evaluated because the residual changes of the product and active substances without processing factor could not be calculated (Görmez, 2019).

Frank et al. (1991) analyzed pesticides on tomato products, including hot pepper, ketchup, juice, tomato paste, and sauce, and found no detectable residue. Fukui et al. (2013) analyzed seventy-four processed food samples from markets in Japan and found pesticide residues above the maximum residue limit in 2 samples. Song et al. (2019) found residues of difenoconazole, dimethomorph, and tebuconazole below established MRLs of China in 10 chili sauce samples collected from local markets in Beijing. Tarifa et al. (2020) found pesticide presence above LOQ in 53% of 103 processed fruit products. In this study, the detected pesticides were below the EU-MRL values (Table 1). The pesticide residues were recorded in 4 tomato samples. The active substances were acetamiprid which were used for tobacco whitefly (*Bemisia tabaci*) and Greenhouse whitefly (*Trialeurodes vaporariorum*) in tomato, ametoctradin against soil-borne pathogens (*Pythium* sp., *Rhizoctonia* sp., *Fusarium* spp., *Verticillium* spp.,) and Downy mildew (*Phytophthora infestans*), metalaxyl-M, against seedling root rot (Collapse) (*Rhizoctonia solani*, *Fusarium solani*, *Pythium* spp., *Oxysporum* spp.), root rot (*Fusarium oxysporum*) and Downy mildew (*Phytophthora infestans*). It is thought that the residue in the sauce, in which the active ingredient of imazalil sulfate was detected, was caused by the orange juice in its content. Imazalil sulfate is a licensed fungicide against storage rot (Green mold rot) (*Penicillium digitatum*) and blue mold rot (*Penicillium italicum*) on oranges.

CONCLUSION

In this study, 4 pesticides were detected in 20 sauce samples. No active ingredient was found in 15 samples. The residue levels were between 10 and 94 µg kg. The residues of acetamiprid, ametoctradin, imazalil sulfate, and metalaxyl-M were found above LOQs. No pesticide residues were detected in onion sauce, chickpea sauce, pea sauce, eggplant sauce, red bean sauce, green lentil sauce, acuka, red beetroot, peach jam, hot sauce, carrot hot sauce, hot sweet sauce, red beet hot sauce, purple cabbage sauce, tomato puree. The results obtained from this study show that pesticide residues should be monitoring in processed agricultural products.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Acoğlu, B., Yolcı Ömeroğlu, P., & Çopur, Ö.U. (2018). Gıda İşleme Süreçlerinin Pestisit Kalıntıları Üzerine Etkisi ve İşleme Faktörleri. Gıda ve Yem Bilimi Teknolojisi Dergisi, 19, 42-54. Retrieved from: https://dergipark.org.tr/tr/pub/bursagida/issue/40169/477821

Bakırcı G.T., Acay D.B.Y., Bakırcı F., & Ötleş S. (2014). Pesticide residues in fruits and vegetables from the Aegean region, Turkey. Food Chemistry, 160, 379-392. https://doi.org/10.1016/j.foodchem.2014.02.051

Balkan, T., Kara, K. (2019). Tokat İlinde Tüketime Sunulan Domateslerde Neonikotinoid Grubu İnsektisitlerin Kalıntı Düzeylerinin Belirlenmesi Üzerine Araştırmalar Gaziosmanpaşa Bilimsel Araştırma Dergisi (Gbad), 8 (3), 50-58. Retrieved from: https://dergipark.org.tr/tr/pub/gbad/issue/50458/643592

Balkan, T., Kara, K. (2022). Determination of Pesticide Residues and Risk Assessment in Some Vegetables Grown in Tokat Province, Plant Protection Bulletin, 62 (2), 26-35. https://doi.org/10.16955/bitkorb.1053952

Balkan, T., Karaağaçlı, H. (2023). Determination of 301 pesticide residues in tropical fruits imported to Turkey using LC–MS/MS and GC-MS. Food Control, 109576. https://doi.org/10.1016/j.foodcont.2022.109576

Balkan, T., Yılmaz, Ö. (2022). Method validation, residue and risk assessment of 260 pesticides in some leafy vegetables using liquid chromatography coupled to tandem mass spectrometry. Food Chemistry, 384:132516. https://doi.org/10.1016/j.foodchem.2022.132516

Burchat, C.S., Ripley, B.D., Leishman, P.D., Ritcey, G.M., Kakuda, Y., Stephenson, G.R. (1998). The distribution of nine pesticides between the juice and pulp of carrots and tomatoes after home processing. Food Addit Contam 15, 61–71. https://doi.org/10.1080/02652039809374599

Cengiz, M.F., Certel, M., Karakas, B., Gocmen, H. (2007). Residue contents of captan and procymidone applied on tomatoes grown in greenhouses and their reduction by duration of a

pre-harvest interval and post-harvest culinary applications. Food Chemistry, 100, 1611-1619. https://doi.org/10.1016/j.foodchem.2005.12.059

Dülger, H., Tiryaki, O. (2022). Verification of an effective method for some pesticide residues determination in nectarine and peach samples. COMU Journal of Agriculture Faculty, 10 (1), 69-75. https://doi.org/10.33202comuagri.1006612

EC, 2022. European Commission, European Union (EU) pesticides database. Pesticide Residues MRLs. Directorate General for Health & Consumers. Retrieved from: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/mrls Accessed date: 29.10.2022

EFSA, European Food Safety Authority. (2018). The 2016 European Union report on pesticides residues in food. EFSA Journal, 16 (7), 5348. Retrieved from: https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5348 Accessed date: 28.10.2022

EPA, U.S. Environmental Protection Agency. (2022). What is Pesticide? Retrieved from: https://www.epa.gov/minimum-risk-pesticides/what-pesticide Accessed date: 28.10.2022

Ersoy, N., Tatlı Ö., Özcan S., Evcil E., Coşkun L.Ş., Erdoğan E. (2011). Determination of pesticide residues in some vegetable species by LC-MS/MS and GC-MS. Selçuk Tarım ve Gıda Bilimleri Dergisi, 25 (3), 79-85.

Frank, R., Braun, H.E., Ripley, B.D., Pitblado R. (1991). Residues of Nine Insecticides and Two Fungicides in Raw and Processed Tomatoes. Journal of Food Protection, 54 (1), 41–46. https://doi.org/10.4315/0362-028X-54.1.41

Fukui, N., Takatori, S., Kitagawa, Y., Okihashi, M., Kajimura, K., Obana, H. (2013). Study of multi-residue method for determining pesticide residues in processed foods manufactured from agricultural products by LC-MS/MS. Journal of the Food Hygienic Society of Japan, 54 (6), 426–433. https://doi.org/10.3358/shokueishi.54.426

Golge O., Kabak B. (2015). Evaluation of QuEChERS sample preparation and liquid chromatography-triple-quadrupole mass spectrometry method for the determination of 109 pesticide residues in tomatoes. Food Chemistry, 176, 319-332. https://doi.org/10.1016/j.foodchem.2014.12.083

Görmez, E. (2019). Ekonomik öneme sahip endüstriyel gıdalardan üzüm, domates ve biberde kullanılan pestisitlerin gıda prosesleri sonrasi kalıntı miktarlarının karşılaştırılması, Muğla Sıtkı Koçman Üniversitesi, Aydın. 187.

Karaboğa, Ö. (2021). Şefin imzası, lezzetin tamamlayıcısı: SOSLAR. Gastronomi Dergisi. (in Turkish) Retrieved from: https://www.gastronomidergisi.com/gida/sefin-imzasi-lezzetin-tamamlayicisi-soslar

Kaushik, G., Satya, S., Naik, S.N. (2009). Food processing a tool to pesticide residue dissipation – A review. Food Research International, 42 (1), 26-40, https://doi.org/10.1016/j.foodres.2008.09.009

Kong, Z., Dong, F., Xu, J., Liu, X., Zhang, C., Li, J., Li, Y., Chen, X., Shan, W., Zheng, Y., (2012). Determination of difenoconazole residue in tomato during home canning by UPLC-MS/MS. Food Control 23, 542-546. https://doi.org/10.1016/j.foodcont.2011.08.028

Lehotay S.J. (2007). Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: Collaborative Study. Journal of AOAC International, 90 (2), 485–520.

Polat B., Tiryaki O. (2018). Investigation of pesticide residues in tomato growing open-fields of Çanakkale province by QuEChERS method. COMU Journal of Agriculture Faculty, 6 (1), 71–79.

Polat B., Tiryaki O. (2019). Determination of some pesticide residues in conventional-grown and IPM-grown tomato by using QuEChERS method. Journal of Environmental Science and Health, Part B, 54 (2), 112-117. https://doi.org/10.1080/03601234.2018.1531663

Reddy, B.K.K., Bhuvaneswari, K., Geetha, P., Thamilarasi, N., Suganthi, A., Paramasivam. M. (2022). Effect of decontamination and processing on insecticide residues in grape (Muscat Hamburg). Environmental Science and Pollution Research, 29, 75790–75804. https://doi.org/10.1007/s11356-022-21165-2

SANTE, (2021). SANTE/11312/2021 Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. 1–55. Retrieved from: https://www.accredia.it/app/uploads/2021/02/SANTE_11312_2021.pdf Accessed date: 29.09.2022

Scholz, R., Hermann, M., Michalski, B. (2017). Compilation of processing factors and evaluation of quality controlled data of food processing studies. Journal of Consumer Protection and Food Safety, 12, 3-14. https://doi.org/10.1007/s00003-016-1043-3

Song, L., Han, Y., Yang, J., Qin, Y., Zeng, W., Xu, S., Pan, C. (2019). Rapid single-step cleanup method for analyzing 47 pesticide residues in pepper, chili peppers and its sauce product by high performance liquid and gas chromatography-tandem mass spectrometry. Food chemistry, 279, 237–245. https://doi.org/10.1016/j.foodchem.2018.12.017

Soydan, K.D., Turgut, N., Yalçın, M., Turgut, C, Karakuş, P.B.K. (2021). Evaluation of pesticide residues in fruits and vegetables from the Aegean region of Turkey and assessment of risk to consumers. Environmental Science and Pollution Research, 28, 27511–27519. https://doi.org/10.1007/s11356-021-12580-y

Valera-Tarifa, N. M., Santiago-Valverde, R., Hernández-Torres, E., Martínez-Vidal, J. L., Garrido-Frenich, A. (2020). Development and full validation of a multiresidue method for the analysis of a wide range of pesticides in processed fruit by UHPLC-MS/MS. Food chemistry, 315, 126304. https://doi.org/10.1016/j.foodchem.2020.126304

Zengin E., Karaca İ., (2017). Determination of pesticide residues in tomatoes grown in greenhouse in Uşak province. Journal of Natural and Applied Sciences, 21 (2), 554-559. https://doi.org/10.19113/sdufbed.72243

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 136-147
DOI: https://doi.org/10.31015/jaefs.2023.1.17

Comparison of genotype × trait and genotype × yield-trait biplots in Sunflower cultivars

Seyed Habib Shojaei[1] • **Khodadad Mostafavi**[2] • **Isa Ansarifard**[1]
Mohammad Reza Bihamta[3] • **Hossein Zeinalzadeh-Tabrizi**[4] • **Ali Omrani**[5]
Merve Gore[6] • **Seyed Mohammad Nasir Mousavi**[7]

[1] Department of Biotechnology and Plant Breeding, Islamic Azad University, Tehran, Iran
[2] Department of Agronomy and Plant Breeding, Islamic Azad University, Karaj, Iran
[3] College of Agriculture and Natural Resources (UCAN), University of Tehran, Karaj, Iran
[4] Department of Horticulture and Agronomy, Faculty of Agriculture, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan
[5] Crop and Horticultural Science Research Department, Ardabil Agricultural and Natural Resources Research and Education Center, Agricultural Research Education and Extension Organization (AREEO), Moghan, Iran
[6] Department of Medicinal and Aromatic Plants, Ödemiş Vocational Training School, Ege University, Izmir, Türkiye
[7] Institute of Land Use, Engineering and Precision Farming Technology, Faculty of Agricultural and Food Sciences and Environmental Management, Debrecen, Hungary

Citation: Shojaei, S.H., Mostafavi, K., Ansarifard, I., Bihamta, M.R., Zeinalzadeh-Tabrizi, H., Omrani, A., Gore, M., Mousavi, S.M.N. (2023). Comparison of genotype × trait and genotype × yield-trait biplots in Sunflower cultivars. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 136-147

Received: 16 November 2022
Revised: 11 December 2022
Accepted: 14 December 2022
Published Online: 10 March 2023

Correspondence: Merve Gore
E-mail: merve.gore@ege.edu.tr

Abstract

The selection of genotypes based on various characteristics is a critical challenge in plant breeding. An experiment was carried out in a randomized complete block design (RCBD) in three replications over two crop years, 2018-2019, to compare the effects of genotype × trait (GT) and the genotype × yield-trait (GYT) methods as well as to investigate the relationships between grain yield and different agronomic traits. Plant materials included ten sunflowers (*Helianthus annuus* L.) genotypes. Based on the combined analysis of variance, there was a significant difference in all parameters except leaf length. The effect of year × genotype was significant in all traits except plant height, stem diameter, and leaf length ($P \leq 0.01$). Gabur, Azargol and Favorite genotypes were ranked as high-yielding genotypes in the years of the experiment. Based on the graphical analysis performed on the effect of genotype × trait (GT), Zaria genotype was selected as the best and stable genotype. The genotype × yield-trait (GYT) biplot ranked genotypes by yield and other desired trait levels and depicts their trait profiles, or strengths and weaknesses. The correlation biplot revealed positive correlations between most traits with grain yield. This method is pictorial, objective, effective, and simple compared to the genotype × trait (GT) method. The GYT biplot technique is based on the paradigm shift that genotypes should be assessed by their yield levels in combination with other variables rather than individually. The graphical analysis of the effect of genotype × yield-trait (GYT) revealed that Gabur and Armavirski genotypes were selected as the best genotypes for all traits investigated and could be recommended for cultivation in the Karaj location.

Keywords: Sunflower, GT, GYT, Graphical analysis, Year × genotype, Multi-traits

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the major oilseed plant with the largest cultivation area after soybean, rapeseed and peanuts (Hu et al. 2010). Selection based on morphological traits and yield components with high heritability can be a fast and accurate way to screen plant populations to increase and improve crop yield (Gholizadeh and Dehghani 2016). Genetic control of yield is directly affected by parameters correlated with yield. Recognizing the correlation of yield and its components and finding the type of relationships between them can increase yield (Torres et al. 2004). The correlation study is significant in plant breeding to see the relationships between traits. The correlation coefficient indicates the intensity or weakness and direction of changes in two variables of plant traits, each of which is influenced by genetic and environmental structure (Malik et

al. 2010). GGE biplot can easily facilitate the selection of stable genotypes by graphical plotting (Yan 2001). Yan and Rajcan (2002) were the first researchers who studied the genotype by trait interaction (GT biplot), which is one of the GGE biplot methods. Genotype × trait (GT) biplots have also been used to identify reliable traits for indirect selection of a target main parameters (Akinwale et al. 2014). The GGE biplot method also has been used for evaluating the correlation of the characteristics by the genotype-trait biplot graphics (Akcura and Kokten 2017). In recent years, investigating the effect of genotype × yield-trait (GYT) has become a new method for multi-traits selection and screening of crop plants (Yan and Frégeau-Reid 2018). GYT biplot ranks genotypes based on the combination of grain yield with other evaluated traits and shows the strengths and weaknesses of genotypes with regards to trait weights (Yue et al. 2022). GYT method can be used to identify the best genotypes in the correlation between yield and other traits. As a result, the problem of genotype selection based on different traits can be overcome by the GYT method (Purwati et al. 2022). One of the superior of the GYT biplot is reducing the cost of measuring traits by identifying redundant traits (Mohammadi 2019). Gholizadeh and Ghaffari (2022) on 24 sunflower hybrids used GYT graphical analysis and reported a significant and positive correlation between the number of seeds per head and 100-grain weight combined with grain yield. This method has also been used in the study of various crop plants, including durum wheat (Kendal 2019), bread wheat (Hamid et al., 2019), sesame (Boureima and Abdoua 2019), maize (Mousavi et al. 2021) and oilseed rape (Zeinalzadeh-Tabrizi and Amiri Oghan 2021).

The objectives of this research were: 1) to investigate the effect of genotype × trait (GT) and select the best genotype based on this method, 2) to investigate the effect of genotype × yield-trait (GYT), compare with (GT) method and select the most desirable genotype based on this method, 3) to determine grain yield relationships and related traits, 4) to classify of the genotypes in terms of all traits studied.

MATERIALS AND METHODS

Experimental specifications

In this research, ten sunflower genotypes were evaluated for the effect of genotype × trait (GT), genotype × yield-trait (GYT) and also to investigate the relationship between yield and yield components in a randomized complete block design (RCBD) in three replications during two cropping years 2018-2019 in the research field of Islamic Azad University, Karaj Branch, Iran. According to meteorological statistics and the ombrothermic curve, this location has a Mediterranean arid-warm climate with 150-180 dry days per year. For its warm, dry summers and cold, rainy winters, it also has a dry moisture regime. The average annual precipitation in this area is 243 mm, according to Karaj's 30-year climatic data. Rainfall is most common in late autumn and early spring. The average temperature over the last 30 years has been 13.5 °C, while the soil temperature is 14.5 °C; hence, this location is categorized as having a thermic regime. According to the graphic derived for the number of freezing days/Year in Karaj agricultural weather station, the temperature decrease begins in October-November and lasts for the majority of the days in December, January, February, and March. Table 1 shows the genotype name, code and origin of the sunflower genotypes evaluated in this research. The Geographical and meteorological specifications of the experimental location are shown in Table 2.

The studied traits were: plant height (PH), flower diameter (FD), stem diameter (SD), leaf length (LL), leaf width (LW), grain width (GW), grain length (GL), grain diameter (GD), 100-grain weight (WHG) and grain yield (YLD).

Table 1. Origin, names and code of sunflower varieties studied in the study

Genotype code	Genotype name	Origin	Genotype code	Genotype name	Origin
G1	Progress	Russia	G6	Master	Russia
G2	Gabur	Russia	G7	SHF81-90	Russia
G3	Zargol	Iran	G8	Zaria	Iran
G4	Armavirski	Russia	G9	Favorite	Russia
G5	Azargol	Iran	G10	Record	Romania

Table 2. Geographical and meteorological specifications of the experimental location

Location	Longitude	Latitude	Elevation (m)	Average rainfall (mm)
Karaj	50°54'E	35°55'N	1312	243

Table 3. Soil characteristics of the cultivated area in the experiment

Location	EC(ds/m)	Acidity	Lime (%)	Organic carbon (%)	Organic materials (%)	Clay (%)	Silt (%)	Sand (%)
Karaj	0.20	8.2	7	32	45	32	25	22

Experimental plots were designed in four rows; each row was 5 m long with a row distance of 75 cm. Irrigation was done regularly during the growing seasons. All maintenance operations and harvesting were carried out on time. To eliminate marginal effects, sampling was done from the two middle rows. The soil property of the experimental location showed in Table 3.

Statistical analyses

Before variance analysis, normality of data and homogeneity of error variances were checked using Grubbs's test and Bartlett's test, respectively. Combined analyses of variance were then performed to evaluate the genotype and genotype × years effects.

The mean values of all traits in the first, second and average two years of the experiment were subjected to the GT and GYT biplot analyses using polygon biplots, genotype rankings, genotype rankings based on ideal genotype, and correlation of traits. The following equation described by Yan and Rajcan (2002) was used to study the effect of genotype × trait:

$$\frac{\alpha_{ij}-\beta_j}{\sigma_j}=\sum_{n=1}^{2}\lambda_n\xi_{in}\eta_{jn}+\varepsilon_{ij}=\sum_{n=1}^{2}\xi^*_{in}\eta^*_{jn}+\varepsilon_{ij} \quad (1)$$

In this equation, αij: mean of genotype i in the trait j, βj: mean of the trait j on all genotypes, σj: standard deviation of the trait j between the mean of genotypes, λn: singular value for principal component (PCn), ξin: PCn value for genotype i, ηjn: PCn value for genotype j, εij: residual value of genotype i for trait j in the model. Z score transformation was also used to eliminate the units of different traits as the following formula:

$$Z=\frac{X-\mu}{\sigma} \quad (2)$$

In this equation, Z: standard score, X: initial trait data, μ: mean of the trait, σ: standard deviation of the trait.

GYT table was obtained based on multiplying/dividing each trait with YLD based on the desirableness/undesirableness of each trait according to the method described by Yan and Frégeau-Reid (2018). Therefore, by this method, in the GYT table, a larger value is always more desirable. SAS v9.2 software was used for combined ANOVA. Genstat v12 software was used to perform GT and GYT analyses. Excel software was used for the mean comparison graph.

RESULTS AND DISCUSSION

Based on the combined analysis of the evaluated traits, a significant difference was observed between the genotypes in all characteristics except LL at the probability level of 0.01.

Table 4. Combined analysis of variance in studied traits of 10 sunflower genotypes during two years of the experiment

The effect of year also demonstrated significant difference in SD, FD and YLD. The effect of genotype × year was also significant in parameters except PH, SD and LL. The highest coefficient of variation was related to the SD (30.3), and the lowest was related to the FD (6.64) (Table 4).

Due to the importance of the YLD and also the significance of the genotype × year effect for this trait (Table 4), the average of the YLD was compared over the experimental years (Figure 1). Gabur, Azargol and Favorite genotypes had the best rank in the first year of the experiment. Gabur in the second year also had the highest rank among the genotypes studied. Accordingly, based on the data of the first year of the experiment, the highest mean YLD was related to Gabur with 4712 kg/ha and then Azargol with 4585 kg/ha. The lowest mean YLD was related to Zargol genotype with 3589 kg/ha. In the second year of the experiment, the highest YLD was related to Gabur and Master Genotypes, with 3501 and 3102 kg/ha, respectively, while the lowest mean GY was related to Azargol genotype with 2191 kg/ha (Table 5).

The significant effect of the year × genotype interaction showed that grain yield cannot give a correct picture of cultivar stability across different environments. So, the GYT table is obtained based on the combination of each trait with YLD (Table 5). According to the GYT table, PH, FD, SD, LL, LW, GW, GD and WHG was multiplied by the YLD; therefore yield-trait data table for GYT analysis was obtained. Genotypes were evaluated based on genotype × grain yield-trait (GYT) interaction for two purposes: first, YLD is one of the most important traits, and other traits are important when combined with YLD. Second, the desirability of a genotype should be based on the levels of the combination of YLD with other traits (Yan and Frégeau-Reid 2018).

Evaluation of genotypes based on genotype × trait (GT) biplot

Polygon biplot

A polygon biplot was used to examine the genotypes and characteristics evaluated in the research's first, second, and average two crop years (Figure 2). This biplot is drawn by connecting the farthest genotypes from the plot's origin, and the other genotypes are located within this polygon. In each section, genotypes closer to certain traits are more desirable regarding that trait.

Accordingly, in the first year of the experiment, Record, Zargol, Master, Favorite, Gabur and Armavirski genotypes had the most significant distance from the plot's origin and were determined as desirable genotypes. In each

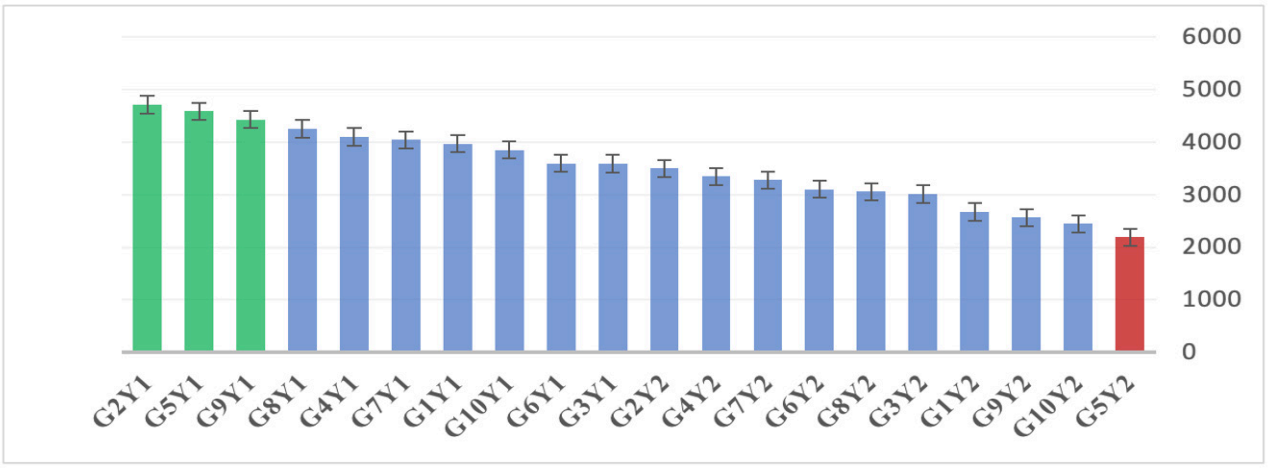


Figure 1. Comparison of the mean of genotype × year in grain yield on 10 sunflower genotypes in two cropping years

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7: SHF89-90, G8: Zaria, G9: Favorite, G10: Record).

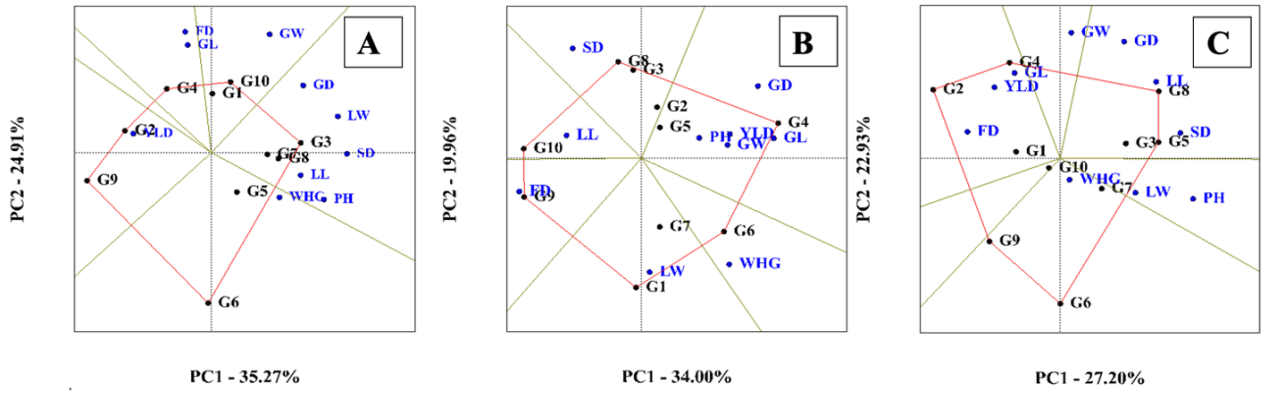


Figure 2. Polygon biplot of genotypes based on the genotype × trait (GT), A: first year of the experiment, B: second year of the experiment, C: average of the first and second year of experiment.

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7: SHF89-90, G8: Zaria, G9: Favorite, G10: Record). (FD: Flower Diameter, PH: Plant Height, SD: Stem Diameter, LL: Leaf Length, LW: Leaf Width, GL: Grain Length, GW: Grain Width, GD: Grain Diameter, WHG: Weight of 100 Grain, YLD: Grain Yield).

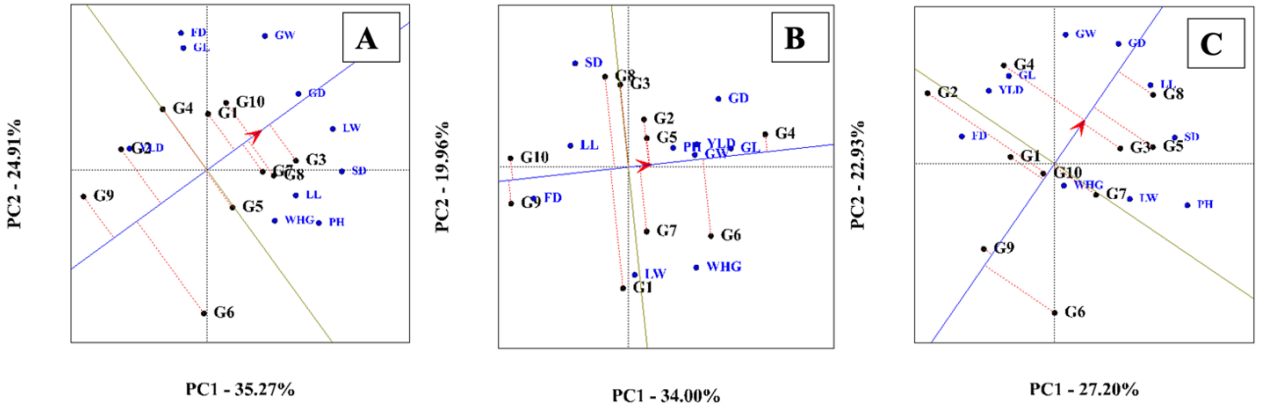


Figure 3. Ranking biplot of genotypes based on overall superiority and strengths and weaknesses in terms of genotype × trait (GT), A: first year of the experiment, B: second year of the experiment, C: average of the first and second year of experiment.

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7: SHF89-90, G8: Zaria, G9: Favorite, G10: Record. (FD: Flower Diameter, PH: Plant Height, SD: Stem Diameter, LL: Leaf Length, LW: Leaf Width, GL: Grain Length, GW: Grain Width, GD: Grain Diameter, WHG: Weight of 100 Grain, YLD: Grain Yield).

Table 4. Combined analysis of variance in studied traits of 10 sunflower genotypes during two years of the experiment

S.O.V	df	FD	PH	SD	LL	LW	GL	GW	GD	WHG	YLD
Year	1	13.87*	175.4^{ns}	0.342*	0.01^{ns}	5.79^{ns}	0.05*	0.28^{ns}	0.01^{ns}	887.04^{ns}	21420375**
Genotype	9	10.75**	1812.7**	0.14*	7.22^{ns}	10.7*	0.01**	3.5*	0.68**	7111.4**	465530.1*
Error1	4	657	649.17	0.02	6.11	5.59	0.0009	1.41	0.04	2244.1	397244.95
Year × Genotype	9	10.46**	348.01^{ns}	0.06^{ns}	12.78	16.6*	0.01*	38.3*	0.46**	4475.7*	531180.3*
Error2	36	1.7	453.05	0.11	9.93	5.87	0.01	1.45	0.08	1885.2	76462.5
CV %	-	6.64	12.24	30.3	18.9	15.32	12.62	30.1	23.1	14.95	24.85

*,** and ns: Significant at 5%, 1% and non-significant.
(FD: Flower Diameter, PH: Plant Height, SD: Stem Diameter, LL: Leaf Length, LW: Leaf Width, GL: Grain Length, GW: Grain Width, GD: Grain Diameter, WHG: 100-grain weight , YLD: Grain Yield)

Table 5. Average grain yield and other agronomic traits in the experimental years of the studied genotypes

	Genotype	FD	PH	SD	LL	LW	GL	GW	GD	WHG	YLD
Year 1	G1	24.3	171.4	1.1	16.8	15.1	0.9	5.2	1.3	310.9	3969.3
	G2	20.6	132.4	0.9	15.7	14.2	0.9	3.9	1.2	303.0	4712.3
	G3	21.1	176.3	1.4	19.1	16.5	0.8	4.1	2.2	308.8	3589.3
	G4	21.9	163.0	0.9	14.3	15.1	1.0	4.3	1.4	279.1	4101.0
	G5	18.1	193.5	1.1	21.3	16.5	0.8	3.6	0.9	259.5	4585.3
	G6	16.2	180.3	1.2	16.5	14.7	0.8	1.1	0.4	344.8	3595.7
	G7	19.1	183.7	1.4	14.2	16.9	0.9	3.8	1.3	383.1	4045.3
	G8	17.6	191.0	1.4	18.4	15.8	0.9	3.9	2.1	317.5	4252.0
	G9	20.1	159.3	0.9	13.6	13.2	0.9	1.3	0.6	218.3	4425.3
	G10	21.8	170.7	1.3	16.5	17.1	1.0	4.2	1.1	215.8	3849.3
Year 2	G1	19.5	150.1	0.8	16.4	18.1	0.8	3.9	1.1	345.0	2672.3
	G2	20.0	148.1	0.9	16.6	13.4	0.9	3.3	1.6	264.0	3501.0
	G3	18.3	175.6	1.3	15.7	14.7	0.8	3.1	1.3	251.6	3011.3
	G4	17.0	179.5	0.9	16.9	15.3	0.9	5.2	1.6	323.6	3347.0
	G5	18.9	196.1	1.2	16.0	16.8	0.8	4.6	1.6	287.0	2191.7
	G6	19.0	199.4	0.7	14.7	16.5	0.9	3.7	1.3	318.6	3102.7
	G7	18.3	184.8	0.9	17.2	20.3	0.8	2.82	1.2	286.3	3282.0
	G8	19.1	195.3	1.2	17.5	14.3	0.8	4.0	1.4	257.3	3056.3
	G9	21.4	177.9	1.0	17.6	16.1	0.7	3.8	0.9	281.3	2566.0
	G10	19.8	148.7	1.1	17.1	15.5	0.8	2.7	0.9	249.0	2444.6
Mean of two years	G1	21.9	160.8	0.9	16.5	16.6	0.8	4.6	1.2	327.9	3320.8
	G2	20.3	140.3	0.9	16.3	13.8	0.9	3.6	1.4	283.5	4106.7
	G3	19.7	175.9	1.3	16.8	15.5	0.8	3.6	1.6	280.2	3291.3
	G4	19.4	171.3	0.9	16.0	15.2	0.9	4.8	1.5	301.3	3724.0
	G5	18.5	194.8	1.1	17.8	16.6	0.8	4.1	1.4	273.2	3388.5
	G6	17.6	190.0	0.9	15.3	15.6	0.8	2.4	1.0	331.7	3361.2
	G7	18.7	184.2	1.1	16.1	18.6	0.9	3.3	1.3	334.7	3663.7
	G8	18.3	193.1	1.3	17.7	15.0	0.8	3.9	1.7	287.4	3654.3
	G9	20.7	168.6	0.9	16.2	14.6	0.8	2.6	0.8	249.8	3501.7
	G10	20.8	159.7	1.2	16.9	16.3	0.9	3.4	0.9	232.4	3153.0

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7:SHF89-90, G8: Zaria, G9: Favorite, G10: Record). (FD: Flower Diameter, PH: Plant Height, SD: Stem Diameter, LL: Leaf Length, LW: Leaf Width, GL: Grain Length, GW, Grain Width, GD: Grain Diameter, WHG: 100-grain weight , YLD: Grain Yield).

section, genotype Record in terms of GW, genotype Zargol with regards to LW and SD and Gabur genotype in terms of YLD were more desirable than other genotypes (Figure 2. A). Based on the polygon biplot obtained from the data of the second year of the experiment, the genotypes Armavirski, Master, Progress, Favorite, Record and Zaria were identified as the best genotypes, considering that they were the most distant from the origin of the biplot. Also, Zaria genotype in terms of SD, Armavirski genotype in terms of FD and GL, Master genotype in terms of WHG, Progress genotype with regards to LW and Favorite genotype in terms of flower

Table 6. Value of genotype × yield-trait in 10 sunflower genotypes in the first, second and average of two cropping years

	Genotype	Y*FD	Y*PH	Y*SD	Y*LL	Y*LW	Y*GL	Y*GW	Y*GD	Y*WHG	Y*FD
Year 1	G1	96547.4	680343.7	4366.3	66817.1	60069.2	3667.6	20762.2	5004.0	1234066	96547.4
	G2	97231.1	623912.9	4445.3	73826.6	66852.3	4291.3	18786.5	5500.8	1427837	97231.1
	G3	75871.3	632679.8	5120.8	68675.9	59104.4	3093.5	14685.1	7061.4	1108506	75871.3
	G4	89680.7	668463.0	3909.6	58781.0	61925.1	4199.4	17850.2	6717.4	1144452	89680.6
	G5	83174.9	887414.8	4982.7	97667.6	75658.0	4074.8	16650.8	4826.8	1189894	83174.8
	G6	58398.4	648418.6	4218.9	59208.6	52856.3	2886.1	4118.2	2327.5	1239786	58398.4
	G7	77478.9	742992.9	5717.4	57308.9	68500.9	3932.0	15550.3	4288.0	1549902	77478.9
	G8	74772.8	812132.0	5782.7	78095.1	67039.9	4067.7	16449.5	7934.2	1350152	74772.8
	G9	89005.2	705103.1	4041.8	60037.0	58414.4	4263.0	6003.7	4977.0	9661970	89005.2
	G10	84069.4	657209.5	5106.8	63385.7	65951.9	4016.1	16103.0	3823.6	8306860	84069.4
Year 2	G1	52110.5	401170.7	2271.5	43870.8	48378.1	2262.5	10573.5	2654.5	921955	52110.5
	G2	70113.3	518568.1	3092.5	58233.3	46971.7	3220.9	11459.9	4924.7	924264	70113.3
	G3	55137.5	528890.5	3844.5	47303.0	44326.8	2670.0	9294.9	4828.1	757852	55137.5
	G4	56954.7	600842.3	3280.1	56720.5	51209.1	3257.7	17404.4	4451.5	108331	56954.7
	G5	41429.8	429676.3	2564.2	35212.8	36929.6	1877.5	10001.3	3324.0	629008	41429.8
	G6	59105.8	619706.0	2306.3	45764.3	51328.4	2833.7	11459.2	5181.4	988716	59105.8
	G7	60038.7	606458.9	2910.1	56450.4	66777.7	2910.0	9277.1	3916.5	939746	60038.7
	G8	58335.2	597003.8	3647.2	53526.6	43776.9	2516.3	12225.3	4278.8	786496	58335.2
	G9	54809.7	456431.5	2694.3	45170.1	41432.3	1984.3	9746.5	2762.7	721901	54809.7
	G10	48575.5	363530.1	2819.5	41958.6	38096.1	2077.9	6555.7	2159.4	608722	48575.5
Mean of two years	G1	72764.9	533857.2	3237.8	54978.2	55186.7	2940.0	15254.8	3594.6	1089067	72764.9
	G2	83488.5	576001.1	3750.7	66984.3	56678.8	3758.9	14907.2	5449.0	1164240	83488.5
	G3	64918.2	579110.1	4448.7	55458.9	51322.8	2877.5	11812.6	5676.4	922396	64918.2
	G4	72403.2	637766.0	3599.8	59865.4	56604.8	3719.0	17787.0	5335.2	1122289	72403.2
	G5	62759.5	660051.6	3823.3	60352.9	56503.2	2957.0	13883.8	4615.1	925907	62759.5
	G6	59310.0	638733.7	3221.1	51500.5	52507.0	2883.8	8131.7	4467.3	1115011	59310.0
	G7	68594.8	674939.0	4213.2	59310.6	68290.7	3404.7	12219.5	4209.1	1226351	68594.8
	G8	67005.8	705895.4	4665.3	65039.0	54979.4	3252.3	14377.3	5683.7	1050377	67005.8
	G9	72611.7	590398.5	3437.4	56929.3	51381.1	3040.6	9025.5	3826.1	874833	72611.7
	G10	65755.8	503591.9	3909.7	53383.8	51577.8	2984.8	10822.6	2900.7	732757	65755.8

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7: SHF89-90, G8: Zaria, G9: Favorite, G10: Record)

diameter had better performance than other genotypes (Figure 2. B). The polygon biplot drawn based on the average data of the two crop years of the experiment showed that Zaria, Azargol, Master, Favorite, Gabur and Armavirski genotypes had the longest distance from the origin. Zaria genotype with regards to LL, Azargol genotype with regards to SD and Armavirski genotype were better and more desirable in terms of GL and yield than other genotypes (Figure 2. C). According to the polygon biplots drawn in the first, second and average two crop years, it can be concluded that in terms of all traits, Master, Favorite and Armavirski genotypes are selected and determined as the most desirable genotypes compared to the others. Rahmati and Ahmadi (2020) used this method in their analysis of wheat and obtained similar results.

Ranking of genotypes based on overall superiority in terms of all traits

In this biplot, which is connected to the mean of the traits from the origin of linear coordinates, the genotypes that are at the positive beginning of this axis have higher performance and desirability, thus the genotypes that are close to this axis have a higher value in terms of the trait studied. Based on the ranking biplot of genotypes in terms of all traits (Figure 3), in the first year, Zargol, Record, Zaria and SHF81-90 genotypes were determined as the best genotypes. Favourite and Master genotypes were also selected as undesirable genotypes. The order of genotypes from most desirable to most undesirable is as follows: (Figure 3. A)

Zargol> Record> Zaria> SHF81-90> Progress> Armavirski> Azargol > Gabur> Master> Favorite.

Based on the biplot obtained from the second year of the experiment, the Armavirski genotype was pointed out as the preferred genotype. Favourite and Record genotypes

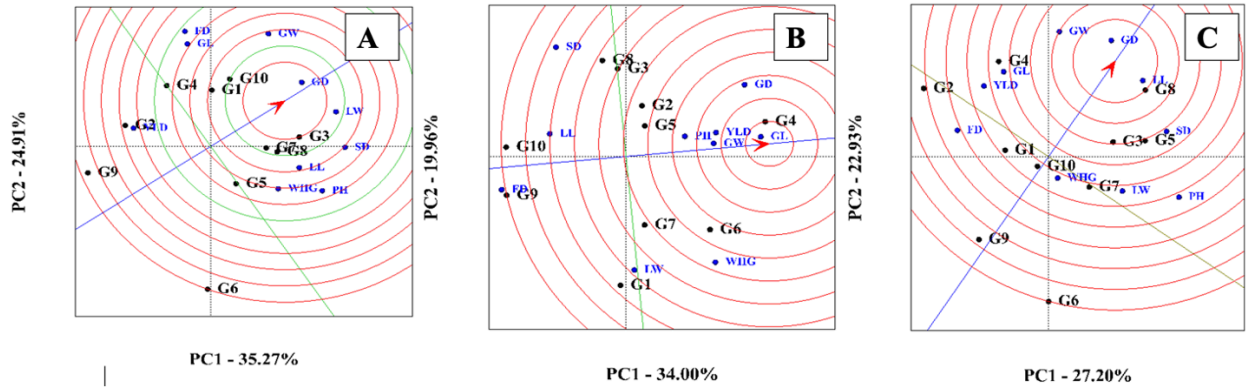


Figure 4. Genotype ranking biplot based on the ideal genotype in terms of genotype × trait (GT), A: first year of the experiment, B: second year of the experiment, C: average of the first and second year of experiment.

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7: SHF89-90, G8: Zaria, G9: Favorite, G10: Record). (FD: Flower Diameter, PH: Plant Height, SD: Stem Diameter, LL: Leaf Length, LW: Leaf Width, GL: Grain Length, GW: Grain Width, GD: Grain Diameter, WHG: Weight of 100 Grain, YLD: Grain Yield).

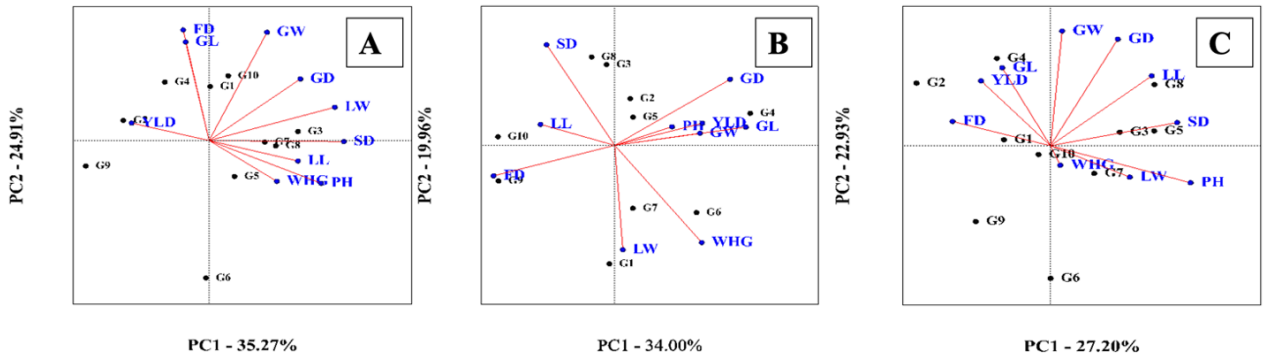


Figure 5. Correlation biplot between the traits evaluated in the experiment, A: the first year of the experiment, B: the second year of the experiment, C: the average of the first and second year of the experiment.

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7: SHF89-90, G8: Zaria, G9: Favorite, G10: Record). (FD: Flower Diameter, PH: Plant Height, SD: Stem Diameter, LL: Leaf Length, LW: Leaf Width, GL: Grain Length, GW: Grain Width, GD: Grain Diameter, WHG: Weight of 100 Grain, YLD: Grain Yield)

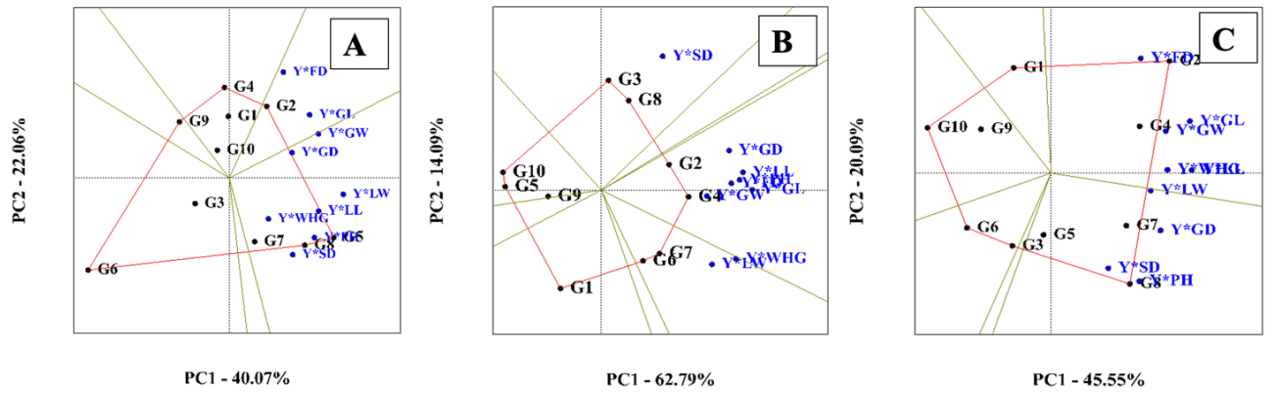


Figure 6. Polygon biplot based on genotype × yield-trait (GYT), A: first year of the experiment, B: second year of the experiment, C: average of the first and second year of experiment.

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7: SHF89-90, G8: Zaria, G9: Favorite, G10: Record). (FD: Flower Diameter, PH: Plant Height, SD: Stem Diameter, LL: Leaf Length, LW: Leaf Width, GL: Grain Length, GW: Grain Width, GD: Grain Diameter, WHG: Weight of 100 Grain, YLD: Grain Yield).

were selected as undesirable genotypes. The order of genotypes from most desirable to most undesirable is as follows: (Figure 3. B)

Armavirski> Master> Azargol> Gabur> SHF81-90> Zargol> Zaria> Progress> Record> Favorite.

Based on the data of the average of the first and second year of the experiment, Zaria and Azargol genotypes were determined as superior genotypes Master and Favorite genotypes were pointed out as undesirable genotypes. The order of genotypes from the best genotype to the most unfavorable genotype is as follows up: (Figure 3.C)

Zaria> Azargol> Armavirski> Zargol> SHF81-90> Record> Gabur> Progress> Favorite> Master.

Selection of the best genotype based on the ideal genotype biplot

The ranking biplot of genotypes was drawn based on the ideal genotype to select the best genotype (Figure 4). In this biplot, the best point is the center of the concentric circle, which is marked with an arrow, and other genotypes are ranked based on this point. The hypothetical ideal genotype is described based on the most stable and high-yielding genotype (Yan and Kang 2002). According to this biplot, in the first year of the experiment, Zargol, SHF81-90 and Zaria genotypes were selected as superior genotypes and Master and Favorite genotypes were selected as unfavorable genotypes. The ranking of genotypes from the best to the most unfavorable genotypes is as follows: (Figure 4.A)

Zargol> SHF81-90> Zaria> Record> Progress> Armavirski> Azargol> Gabur> Favorite> Master.

Based on the biplot of the second year of the experiment, the Armavirski genotype was determined as the most desirable genotype compared to other cultivars, and Favorite and Record genotypes were stated as undesirable genotypes. The order of genotypes in the second year of the experiment is as follows: (Figure 4.B)

Armavirski> Master> Azargol> Gabur> SHF81-90> Zargol> Zaria> Progress> Record> Favorite.

The biplot on the average of the data of the first and second years of the experiment indicated that the Zaria genotype was specified as the preferred genotype and Master and Favorite genotypes as the undesirable genotype. The order of genotypes from the most desirable to the most undesirable genotype is as follows: (Figure 4.C)

Zaria> Azargol> Zargol> Armavirski> SHF81-90> Record> Progress> Gabur> Favorite> Master.

Correlation between evaluated traits

According to this biplot, the cosine of the angle between the vectors of traits represents an estimate of the correlation coefficient between them. The acute angle between vectors represents a positive correlation, and the obtuse angle between vectors represents a negative correlation. If the angle between the vectors of the attributes is 90 degrees, it represents no correlation between them (Yan and Kang 2002). According to the correlation biplot drawn in the first crop year, GW, LW and SD, LL, PH, WHG and YLD, GL and FD were positively correlated. Considering the vector angle between the two traits of YLD and LL, which shows an angle of 180 degrees, a negative and significant correlation was evident between these two traits. The correlation between LL and GW traits was also estimated to be zero (Figure 5. A). The results of the correlation biplot in the second-year data also showed a positive correlation between GD, PH, YLD, GW and GL. Also, a positive correlation was observed between WHG and LW traits and between SD and LL traits. WHG was negatively correlated with SD, FD with PH. According to the angle between the two vectors, WHG traits with FD and SD traits with GD were estimated to be zero (Figure 5. B). The results of correlation between the evaluated traits in the mean of the first and second-year crop data are also a positive correlation between GW, GD, LL, SD, PH, LW and WHG, and GL, YLD and FD were observed together. Also, the degree of correlation between FD and PH traits was negative. The correlation between trait GD with the FD and PH traits was zero (Figure 5. C). Sincik and Goksoy (2014) reported a positive correlation between FD and YLD.

Evaluation of genotypes based on genotype × yield-trait (GYT) biplot

Polygon diagrams

Figure 6 shows the polygon biplot of genotypes in terms of genotype × yield-trait (GYT). As in Figure 2, the genotypes with the greatest distance from the origin of the biplot are specified as the most desirable trait. In each section, genotypes close to certain traits are more desirable than that trait (Figure 6). Based on the graph drawn in the first year of the experiment (Figure 6. A), the first two principal components explained 40.07% and 22.06%, respectively, and a total of more than 62% of the data variance. The high percentage of the first two principal components indicates the high validity of the GYT biplot in justifying the percentage of the data variance (Hamid et al. 2019). This biplot identified Armavirski, Gabur, Azargol, Zaria, Master and Favorite genotypes as the most desirable genotypes. In each section, Gabur genotype had the highest value of Y × FD, Y × GL, indicating that this was more desirable than other genotypes in combining YLD with a FD and GL. Azargol and Zaria genotypes also had the highest values in Y × LW, Y × LL, Y × PH and Y × SD and were superior in terms of YLD combination with PH, LW, LL and SD. In the second year of the research, the first and second major components covered 62.79 and 14.09, respectively, and 76.98% of the variance of the data. Zargol, Zaria, Gabur, Armavirski, SHF81-90, Master, Progress, Azargol and

Record genotypes were selected as the best genotypes according to the distance from the origin of the biplot. In each section, Gabur and Armavirski genotypes in terms of Y × GD, Y × LL, Y × PH, Y × FD, Y × GL and Y × GW show the combination of YLD with traits of SD, LL, PH, FD, GL and GW, the best genotypes were identified. Zargol and Zaria genotypes were also more desirable than other genotypes in terms of Y × SD, which shows the combination of YLD with SD (Figure 6. B). Gabur, Zaria, Zargol, Master, Record and Progress genotypes were determined as the best genotypes in studying the average data of two crop years. In each part, Gabur genotype in terms of Y × FD (combination of YLD in FD) and Zaria genotype in terms of Y × SD, Y × PH and Y × GD (combination of yield in SD, PH and SD were pointed out as the best genotypes (Figure 6. C). According to the graphs of the first, second and average years of the experiment, the Zaria genotype was identified as the upper genotype in all years and also this genotype was the best known compared to other genotypes in terms of Y × SD (combination of YLD with SD) in all years.

Ranking of genotypes based on genotype effect × yield-trait (GYT)

In this biplot, the genotypes at the positive end of the mean axis are identified as superior genotypes in terms of all traits (Figure 7). Based on the ranking biplot of genotypes in the first year of the experiment (Figure 7. A), Azargol, Zaria and Gabur genotypes were identified as the best genotypes in terms of a combination of traits, while Master Favorite genotypes were identified as undesirable genotypes. Based on this biplot, Azargol and Zaria genotypes were identified as desirable genotypes in combining YLD with LW, LL, WHG, PH and SD. The order of genotypes in the first year of the experiment from the most desirable genotype to the most undesirable genotype is as follows:

Azargol> Zaria> Gabur> SHF81-90> Progress> Armavirski> Record> Zargol> Favorite> Master.

Based on the second-year biplot, Armavirski and Gabur genotypes were identified as superior genotypes, whereas Azargol and Record genotypes were identified as undesirable genotypes. Armavirski genotype was ranked high compared to other genotypes in combining YLD with GW. The order of genotypes from the most desirable genotype to the most undesirable genotype is as follows: (Figure 7. B)

Armavirski> Gabur> SHF81-90> Master> Zaria> Zargol> Progress> Favorite> Azargol> Record.

According to the average data of the first and second years, Gabur, SHF81-90 and Armavirski genotypes were identified as the best genotypes while, Record, Master and Favorite genotypes were identified as unfavorable genotypes. Gabur and Armavirski genotypes were more desirable by combining YLD with FD, GL, GW, WHG and LL traits. Zaria genotype can also be selected as more desirable than other genotypes in combining YLD with PH and SD traits. The order of genotypes from the most desirable genotype to the most undesirable genotype is as follows: (Figure 7. C)

Gabur> Zaria> Armavirski> SHF81-90> Azargol> Zargol> Progress> Favorite> Master> Record.

According to the biplots of different years of the experiment, Gabur and Armavirski genotypes can be identified as desirable genotypes.

Selection of the best genotype based on the ideal genotype

The genotypes were ranked based on the ideal genotype ranking biplot, which is similar to Figure 4 (Figure 8). In the first year of the experiment, Azargol and Zaria genotypes were determined as the best genotypes while, Master genotype was ranked as undesirable. According to this biplot, the order of genotypes from the best genotype to the most unfavorable genotype is as follows: (Figure 8. A)

Azargol> Zaria> Gabur> SHF81-90> Progress> Record> Armavirski> Zargol> Favorite> Master.

Based on the biplot of the second year of the experiment, Armavirski and Gabur genotypes were identified as desirable genotypes while, Record was ranked as undesirable genotype. The ranking of genotypes from favorable genotype to unfavorable genotype is as follows: (Figure 8. B)

Armavirski> Gabur> SHF81-90> Master> Zaria> Zargol> Favorite> Progress> Azargol> Record.

Based on the biplot of the average of two years of the experiment, Armavirski, SHF81-90, Gabur and Zaria genotypes were identified as desirable genotypes while, Record genotype remained undesirable genotype, respectively. The ranking of genotypes based on the average biplot of the first and second year of the experiment from the best genotype to the most unfavorable genotype is as follows: (Figure 8. C)

Armavirski> SHF81-90> Gabur> Zaria> Azargol> Zargol> Progress> Favorite> Master> Record.

Correlation biplot of Yield × Trait data

In this biplot, the vector length indicates the discrimination of genotypes (Figure 9). In other words, traits with longer vector lengths have more discrimination power. This biplot, like Figure 5, can show the correlation between the traits based on the angles of the vectors. Since yield performance as a component is present in all yield×trait combinations, different yield×trait combinations tend to be positively correlated. According to the biplot in the first year of the experiment, there was a positive correlation between Y × FD, Y × GL, Y × GW and Y × GD, indicating the high utility of combining FD, GL, GW and GD with YLD. Also, a positive correlation was observed between Y × LW, Y × LL, Y × WHG, Y × PH and Y × SD, which

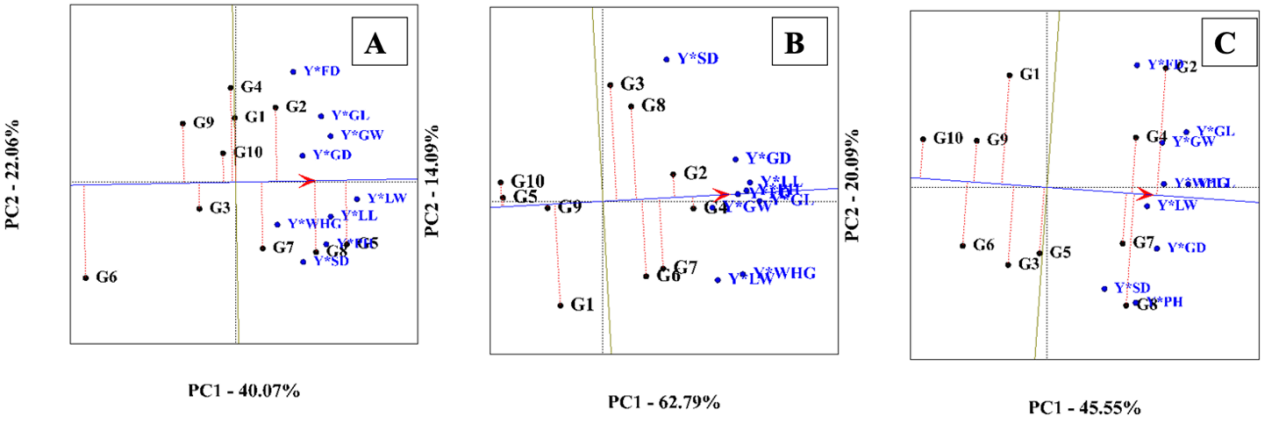


Figure 7. Genotype ranking biplot based on overall superiority and strengths and weaknesses in terms of genotype × yield-trait (GYT), A: first year of the experiment, B: second year of the experiment, C: average of the first and second year of experiment.

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7: SHF89-90, G8: Zaria, G9: Favorite, G10: Record). (FD: Flower Diameter, PH: Plant Height, SD: Stem Diameter, LL: Leaf Length, LW: Leaf Width, GL: Grain Length, GW: Grain Width, GD: Grain Diameter, WHG: Weight of 100 Grain, YLD: Grain Yield).

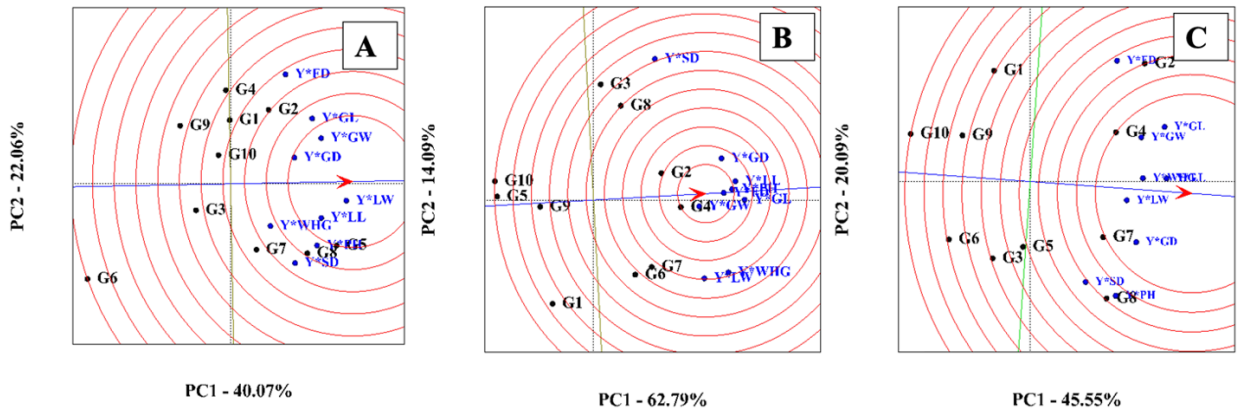


Figure 8. Genotype ranking biplot based on ideal genotype in terms of genotype × yield-trait (GYT), A: first year of the experiment, B: second year of the experiment, C: average of the first and second year of experiment.

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7: SHF89-90, G8: Zaria, G9: Favorite, G10: Record). (FD: Flower Diameter, PH: Plant Height, SD: Stem Diameter, LL: Leaf Length, LW: Leaf Width, GL: Grain Length, GW: Grain Width, GD: Grain Diameter, WHG: Weight of 100 Grain, YLD: Grain Yield).

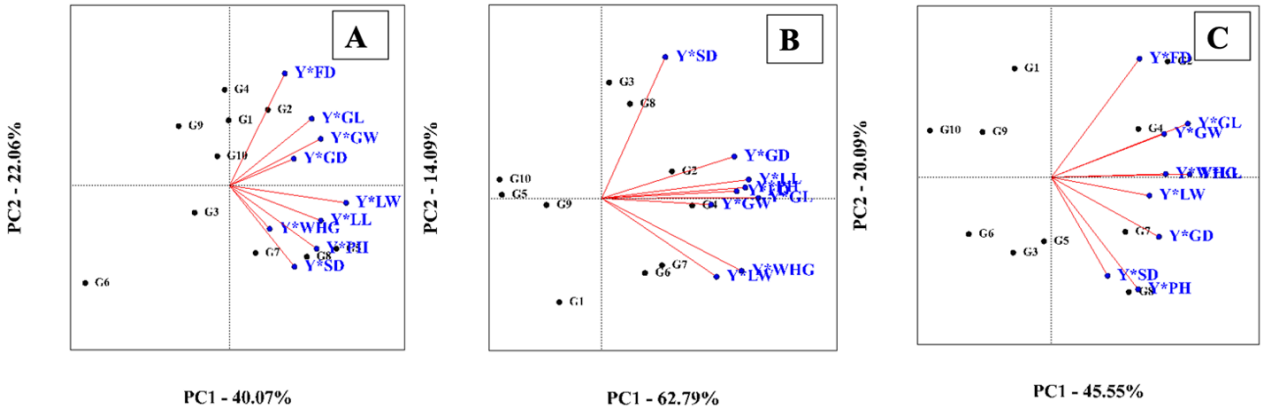


Figure 9. Correlation biplot between grain yield compounds in the traits evaluated in the experiment, A: first year of the experiment, B: second year of the experiment, C: average of the first and second year of the experiment.

(G1: Progress, G2: Gabur, G3: Zargol, G4: Armavirski, G5: Azargol, G6: Master, G7: SHF89-90, G8: Zaria, G9: Favorite, G10: Record). (FD: Flower Diameter, PH: Plant Height, SD: Stem Diameter, LL: Leaf Length, LW: Leaf Width, GL: Grain Length, GW: Grain Width, GD: Grain Diameter, WHG: Weight of 100 Grain, YLD: Grain Yield).

indicated the high usefulness of the combination of LW, LL, WHG, PH and SD with YLD. (Figure 9. A). In the biplot of the second year of the experiment, there is a positive correlation between Y × SD, Y × GD, Y × LL, Y × PH, Y × FD, Y × GL and Y × GW together. Positive correlations were observed between Y × WHG and Y × LW (Figure 9. B). The positive correlation between Y × FD, Y × GL, Y × GW, Y × WHG, Y × LL and between Y × LW, Y × GD, Y × SD and Y × PH were observed (Figure 9. C).

CONCLUSION

The selection of genotypes based on various characteristics is a critical challenge in plant breeding. Combined analysis of variance indicated a significant effect among genotypes in terms of all traits except LL. Comparison of the mean interaction effect of genotype × year in the YLD trait also identified Gabur, Azargol and Favorite genotypes as high-yielding genotypes in the years of the experiment. Based on the graphical analysis performed on the effect of genotype × trait (GT), Zaria genotype was selected as the best and stable genotype. The genotype × yield-trait (GYT) biplot ranked genotypes by yield and other desired trait levels and depicts their trait profiles, or strengths and weaknesses. This method is pictorial, objective, effective, and simple compared to the genotype × trait (GT) method. The GYT biplot technique is based on the paradigm shift that genotypes should be assessed by their yield levels in combination with other variables rather than individually. The graphical analysis of the effect of genotype × yield-trait (GYT) revealed that Gabur and Armavirski genotypes were selected as the best genotype for all traits investigated and could be recommended for cultivation in the Karaj location.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

All data associated with this research were indicated and used in the manuscript submitted.

Consent for publication

All authors consented to the publication of this manuscript.

REFERENCES

Akcura, M., Kokten, K. (2017). Variations in grain mineral concentrations of Turkish wheat landraces germplasm. Quality Assurance and Safety of Crops & Foods, 9(2): 153-159. https://doi.org/10.3920/QAS2016.0886

Akinwale, R., Fakorede, M., Badu-Apraku, B., Oluwaranti, A. (2014). Assessing the usefulness of GGE biplot as a statistical tool for plant breeders and agronomists. Cereal Research Communications, 42(3): 534-546. https://doi.org/10.1556/crc.42.2014.3.16

Boureima, S., Abdoua, Y. (2019). Genotype by yield* trait combination biplot approach to evaluate sesame genotypes on multiple traits basis. Turkish Journal of Field Crops, 24(2): 237-244. https://doi.org/10.17557/tjfc.655165

Gholizadeh, A., Dehghani, H. (2016). Graphic analysis of trait relations of Iranian bread wheat germplasm under non-saline and saline conditions using the biplot method. Genetika, 48(2): 473-486. https://doi.org/10.2298/GENSR1602473G

Gholizadeh, A., Ghaffari, M. (2022). Simultaneous selection for seed yield and agronomic traits in sunflower. Isfahan University of Technology-Journal of Crop Production and Processing, 11(4): 1-15. https://doi.org/10.47176/jcpp.11.4.36103

Hamid, A.E., Aglan, M., Hussein, E. (2019). Modified method for the analysis of genotype by trait (Gt) biplot as a selection criterion in wheat under water stress conditions. Egyptian Journal of Agronomy, 41(3): 293-312. https://doi.org/10.21608/AGRO.2019.16580.1177

Hu, J., Seiler, G., Kole, C. (2010). Genetics, genomics and breeding of sunflower. CRC Press, Routledge, USA.

Kendal, E. (2019). Comparing durum wheat cultivars by genotype× yield× trait and genotype× trait biplot method. Chilean Journal of Agricultural Research, 79(4): 512-522. http://dx.doi.org/10.4067/S0718-58392019000400512

Malik, S.R., Bakhsh, A., Asif, M.A., Iqbal, U., Iqbal, S. (2010). Assessment of genetic variability and interrelationship among some agronomic traits in chickpea. International Journal of Agriculture and Biology, 12(1): 81-85.

Mohammadi, R. (2019). Genotype by yield* trait biplot for genotype evaluation and trait profiles in durum wheat. Cereal Research Communications, 47(3): 541-551. https://doi.org/10.1556/0806.47.2019.32

Mousavi, S.M.N., Bojtor, C., Illés, Á., Nagy, J. (2021). Genotype by trait interaction (GT) in Maize hybrids on complete fertilizer. Plants, 10(11): 2388. https://doi.org/10.3390/plants10112388

Purwati, R., Anggraeni, T., Machfud, M. (2022). Genotype by yield* trait biplot analysis to evaluate Jatropha curcas genotypes based on multiple traits. IOP Conference Series: Earth and Environmental Science, 974:012013. https://doi.org/10.1088/1755-1315/974/1/012013

Rahmati, M., Ahmadi, A. (2020). Assessment of interrelationship between agronomic traits of wheat genotypes under rain-fed conditions using double and triple biplots of genotype, trait and yield. Iranian Dryland Agronomy Journal, 9(1): 1-20. https://doi.org/10.22092/IDAJ.2020.122220.284

Sincik, M., Goksoy, A.T. (2014). Investigation of correlation between traits and path analysis of confectionary sunflower genotypes. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 42(1): 227-231. https://doi.org/10.15835/nbha4219429

Torres, V., Davila, J., Mendoza, A., Godina, F., Maiti, R. (2004). Importance of agronomic characteristics in the grain yield of maize under irrigated and rainfed conditions. Crop Research, 27(2): 169-176.

Yan, W. (2001). GGEbiplot-a windows application for graphical analysis of multienvironment trial data and other types of two-way data. Agronomy Journal, 93(5): 1111-1118. https://doi.org/10.2134/agronj2001.9351111x

Yan, W., Frégeau-Reid, J. (2018). Genotype by Yield* Trait (GYT) Biplot: a Novel approach for genotype selection based on multiple traits. Scientific Reports, 8(8242): 1-10. https://doi.org/10.1038/s41598-018-26688-8

Yan, W., Kang, M.S. (2002). GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. CRC press. DOI: https://doi.org/10.1201/9781420040371

Yan, W., Rajcan, I. (2002). Biplot analysis of test sites and trait relations of soybean in Ontario. Crop Science, 42(1): 11-20. https://doi.org/10.2135/cropsci2002.1100

Yue, H., Wei, J., Xie, J., Chen, S., Peng, H., Cao, H., Bu, J., Jiang, X. (2022). A study on genotype-by-environment interaction analysis for agronomic traits of maize genotypes across Huang-Huai-Hai Region in China. Phyton,91(1): 57. DOI: https://doi.org/10.32604/phyton.2022.017308

Zeinalzadeh-Tabrizi, H., Amiri Oghan, H. (2021). Selection of superior spring rapeseed genotypes using new approach: Genotype by Yield×Trait biplot (GYT). Proceedings of the 7th Iranian Plant Physiology Conference. Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. September 1-2, 2021. Pp 170-173.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 148-155
DOI: https://doi.org/10.31015/jaefs.2023.1.18

Effects of various soil structure and irrigation regimes on rio-red grapefruit yield and morpo-physiology

Uğur Kekeç[1] • **Berken Çimen**[2]

[1] Vocational School of Yumurtalik, Çukurova University, Yumurtalik, Adana, Türkiye
[2] CukurovaUniversity, Faculty of Agriculture, Department of Horticulture, Adana, Türkiye

Abstract

The research was carried out in 2011 using Rio-Red grapefruit trees in Research Station of Cukurova University, Agricultural Faculty, Citrus experiment Station, Adana Latitude, 5°2 ' N Longitude, 6°50' E, altitude 27 m).In the study, it was aimed to determine the effects of the amount of irrigation water applied at different levels to the trees growing in soils of different soil texture on fruit development and yield, tree trunk development, tree canopy volume development, leaf area index(LAI) and photosynthesis rate. The research area soils contain three different groups in terms of resistivity values and the trees are grown in soils electrical conductivity (ECa) with T1: 86-109, T2: 23-37 and T3: 62-72 ECavalues.In the experiment, three different irrigation levels I100, I70 and I50 water was applied. The average amount of irrigation water applied to grapefruit trees ranged from 332,48 mm (I100) to 178,92 mm (I50). Actual plant water consumption was between 810.5 mm (I100) and 694.4 mm (I50) according to the water budget method. Yields related to irrigation on trees in the plot varied between 883 (I70) and 828 (I50) kg per tree on average. It has been determined as 1050 kg on average from the fully irrigated I100. Photosynthesis values were measured as 2.64 umol/m2/s for I50, 3.48 umol/m2/s for I70 and 4.77 umol/m2/s for I100. Consequently, the effects of irrigation treatmentson fruit yield are not statistically significant, water reduction can be recommended for the region in order to save water for the farmers in this study.

Keywords: Fruit Size, DeficitIrrigation, Citrus Paradisi, Photosynthesis

Citation: Kekec, U., Cimen, B. (2023). Effects of various soil structure and irrigation regimes on rio-red grapefruit yield and morpo-physiology. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 148-155

Received: 07 October 2022
Revised: 15 February 2023
Accepted: 17 February 2023
Published Online: 09 March 2023

Correspondence: Ugur Kekec
E-mail: ukekec@cu.edu.tr

INTRODUCTION

Studies conducted in the world and in our country; reveals that one of the plant groups with the highest amount of water consumption is citrus fruits.

Citrus fruits need precipitation or an adequate water source as irrigation water throughout the year, as they remain constantly green. The water requirement varies and the needed water is applied with various irrigation methods.The annual water requirement of citrus fruits varies between 900-1200 mm depending on the soil, climate and physiological condition of the trees (Doorenboss and Kassam, 1979). Considering the potential irrigated areas of Turkey and the water use of plants, only about 33% of the total irrigated areas could be irrigated with the potential of existing water resources. For this reason, it has become imperative to consider approaches such as limited irrigation management, which provides water increase in order to be able to irrigate more effectively and to irrigate more areas with our existing water resources allocated for agriculture (Ünlü et al, 2008). The potential benefits of the scarce irrigation technique have three major factors: reduction of production costs, higher water use efficiency and lower water costs. The effective use of deficit irrigation thinking depends on a good

understanding of the importance of these three factors (Stegman et al., 1990; Kanber et al., 2007). In the deficit irrigation approach, the plant is faced with a lack of water in all or some periods of the growing season; Increases in irrigation water are provided without significant reductions in yield. For this reason, the approach mentioned is called by different names. These are Partial Irrigation; Regulated Deficit Irrigation; ET Deficit Irrigation and Limited Irrigation approaches (English et al., 1990; Kanber et al., 2007). Deficit irrigation practices, which were first introduced in Australia and New Zealand in the early 1970s, can save a significant amount of water without causing any loss in yield in plants with high water requirements (Chalmers et al., 1981). This study was carried out in 2011 using Rio Red grapefruit trees in Çukurova University Faculty of Agriculture Research and Application Farm. Electrical conductivity (ECa) maps of the soils of the trial area were drawn in order to make a detailed structure analysis. In the study, it was aimed to determine the effects of the amount of irrigation water applied at different levels to the trees growing in soils of different soil texture on fruit development and yield, tree trunk development, tree canopy volume development, leaf area index(LAI) development and photosynthesis rate.

MATERIALS AND METHODS

In the region that the research is carried out, is dominant by Mediterranean climate; Summers are hot and dry, winters are mild and rainy. The long-year climate data of the area where the experiment was carried out are from the records of Adana Meteorology Regional Directorate, which is affiliated to the General Directorate of Meteorology; The data related to the research years were obtained from the automatic climate observation station located in the experimental area. The research was carried out in the grapefruit orchard in Çukurova University Faculty of Agriculture Research and Application Farm in 2011. The trial garden is located in the Eastern Mediterranean Region, at 36°59'N, 35°18'E north and east latitudes and longitudes and at an altitude of 20 m from the sea. Grapefruit orchard was established in 1993 and its area is 39.1 decares and there are a total of 612 trees (16 tree decares^{-1}). Trees were planted at 8×8 m intervals.

In 2011, when the study was conducted, the lowest temperature was -3°C in February; the highest temperature was determined as 41°C in September. However, in the long-term (1930-2007) the lowest and highest relative humidity values were recorded in October with 58.1% and in April and July with 65.9%, respectively. In the trial year, these values were 49.4% in October; 72.3% in July; In its second year, it was measured as 55.9% in March and 77.7% in December. The average monthly rainfall in the region between 1932 and 2007 is 644.9 mm. Most of the precipitation occurs during the winter months. The highest precipitation amount in the study year was measured in December with 169.4 mm. There was no precipitation in July and August. The irrigation water source of the trial area is an open channel irrigation system and the irrigation water quality is C2S1. Irrigation water samples taken from the irrigation canal were analyzed using the methods detailed in USSL (1954) and the results are given in Table 1.

The applications in the study were created on the west side of the trial garden. Irrigation levels are arranged as described below.

Controlirrigation: It is the maximum amount of water supplied from the existing irrigation system in the trial area applied to the parcels. (I100, Control).

Deficit irrigation I: where 70% of the irrigation water given to I100 is applied (I70).

Deficit irrigation II: where 50% of the irrigation water given to I100 is applied (I50).

Trial were arranged in a randomized block design in the field. Each treatment was repeated 3 times. The replicates are arranged side-by-side in strip-like blocks. Throughout the study, irrigation applications could be carried out with an average dripper flow rate of 2.2 L/h. I100 is the level where the highest amount of water is applied.In this case, the irrigation interval in the study was determined to be 10-15 days, according to the water supply process, which is necessarily connected to the water source.

Considering the calculated water amounts, the application time (day, hour) was the applied total irrigation time, and the deficit applications were calculated by reducing the total time by 70% and 50%.

Measurement of Soil Resistivity

Electrical conductivity (ECa) maps of the soils of the trial garden were drawn in order to make a detailed structure analysis. For this purpose, the Wenner Mechanism, which measures the resistivity of the soil (ECa; resistivitymeter), developed by the ATP (Agro-TechniqueBornim, Germany) Institute was used. The mentioned ECa map was obtained

Table 1. Analysis Results of Irrigation Water Used in Experiment

irrigation water class	ECw (dS/m)	pH	Cations(me/L)				Ations(me/L)				SAR
			Na^+	K^+	Ca^{++}	Mg^{++}	CO^{-3}	HCO^{-3}	Cl^-	SO^{-4}	
C2S1	0,35	7,0	0,45	0,07	1,54	1,54	-	1,6	0,94	1,06	0,36

by using the said device separately for each tree.

The specified Wenner Arrangement is a geophysical method tool used to reveal the geological structure of the region to be investigated and, accordingly, the detailed structure distribution of the trial area (Özdemir, 2008).

In the method described, electricity is sent into the earth with two current electrodes and the potential distribution created by this current with two potential electrodes is determined through measurements made from the surface.

In the method, the Wenner Electrode Arrangement approach developed by the researcher, called the mechanism, is used. Vertical discontinuities determined sensitively by using the offset measurement technique. Information could be obtained from a depth equal to approximately one-third of the distance between the current electrodes. Since the impact (penetration) depth is low, it can detect shallow soil structures with high resolution.

The obtained map is arranged according to soil resistivity (ECa) values. The structure value of the soil at the point where each tree is taken into consideration is revealed.

Constituent classes are classified according to the ECa values read. It is accepted that as the ECa values increase, there is a finer soil texture, and as the ECa values decrease, there is a coarser soil texture.

Trunk Diameter (circumference) Measurements and Fruit Development

In the trial, on 26.06.2011; Before the start of irrigation, the trunk circumference of the trees was measured 10 cm above the grafting point and before the start of irrigation on 11.06.2012.

In order to determine fruit development, 10 fruits showing equal development and homogeneously distributed around the crown were marked in the plots of the trial treatments (30 fruits in total for each treatment. The circumferences of the marked fruits were measured once a month on days 221, 262, 292, 314 and 345 of the year from mid-August to mid-December in the first year of the experiment.

Canopy Volume

Canopy volumes of trial trees were measured. For this purpose, trees from which trunk circumference was measured were used in plots related to each treatment. Measurements in the working year were on 05.03.2012, YGS: 64; made on the day. The approach given by Westwood (1978) was used to calculate the tree canopy volume. Measurements were made at noon, when the sun's rays were perpendicular. First of all(firstly), the shade diameter of the tree canopy was measured in the north-south and east-west directions. The average canopy diameter obtained from these measurements was compared with the canopy height of the tree. Crown heights were determined by mira readings. Tree canopy volume, if these two values are equal to each other, equation 1a: if not, it was estimated using equation 1b.

$$V = \frac{4}{3}\left(h \times r^3\right) r \leq h \qquad (1a)$$

$$V = \frac{4}{3} h \left(a \times b\right)^2 r > h \qquad (1b)$$

In the formulas, h is the tree crown height, m; r, crown mean radius, m; a and b are the short and long radius of the tree canopy, respectively, m.

The measurements made regarding the tree canopy were punctuated against time, and the temporal dimension change/development of cover development was obtained. In addition, after each irrigation, the width of the wetness occurring within the crown projection of the tree and around the lateral of the drop was measured. In the measurements, the wetness formed at a depth of 5 cm from the soil surface was taken into account.

Photosynthesis Measurements

Portable photosynthesis meter system CI-340 model was used to measure the amount of photosynthesis. In order to monitor the internal water status of the plants under different applications, photosynthesis measurements were carried out with a portable photosynthesis meter (CI-340 Handheld Photosynthesissystem) at noon (11:00 - 13:00) on three plants from each plot, which are completely sun-facing and newly developed leaves (Figure 1).

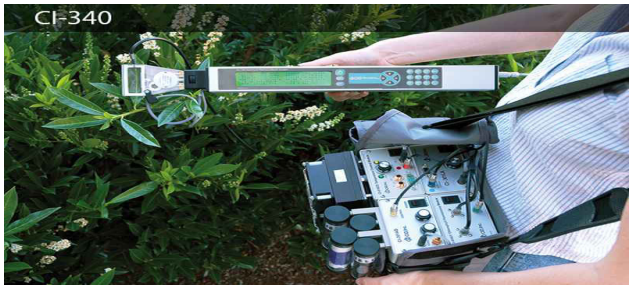


Figure 1.CI-340 HandheldPhotosynthesissystem

Statistical analysis

The experiment was organized as 3 x 3, nine row, three replicates, respectively, in a 'Randomized Block Design'. The means and calculated standard deviations were stated.

Least significant difference (LSD) test was used for mean comparison and the F test was significant at $P < 0.05$. The 'Correlation coefficients' were also calculated between all measured parameters. Data used in the ANOVA by the SAS v9 statisticalanalysessoftwareandSigmaPlot® v11 (Systat Software, San Jose, CA, USA).

RESULTS AND DISCUSSION

Irrigation and Water Consumption

Trial irrigation applications in the study were 11 times; for the first irrigations on 30.06.2011; started; and it was terminated on 31.10.2011. Thus, the length of the irrigation season for the grapefruit plant varied between 123 days depending on the climate, plant growth and soil conditions. In the study, 332.48 mm irrigation water was given to I100, 240.35 mm to I70, and 178.92 mm to I50. The ET values calculated with the water budget approach has changed between 810.5mm for I100 and 694.4mm for I50.

Resistivity Change in Trial Field Soils

The colors in the general resistivity change map of the field reflects different soil texture groups. For example, dark colors represent fine textured soils and light colors represent coarse textured or gravel areas. It is understood from the map that there are 10 different texture groups in the experimental garden(Figure 2).

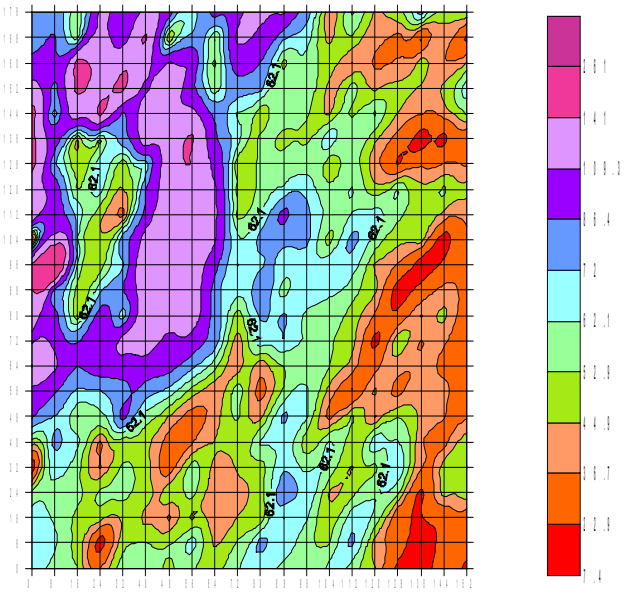


Figure 2. General ECa map of trial garden soils.

As seen from the map, the pink colors represent the heavy tecture class; dark red color represents coarse textured or gravel areas. In this case, it is seen that the trees grown on soils of different texture classes; It can be stated that the situation summarized above should be taken into account in estimating water and fertilizer requirements and monitoring their developments.

Figure 3.shows the soil resistivity values for each tree in the field. In particular, it has been determined that the trees, which are shown in light-colored or even white colors on the eastern sides of the garden, which was established on the lands of the river bed, are mostly on the conglomerate and gravel areas. In this section, the soil depth is quite shallow, around 1.0 m.

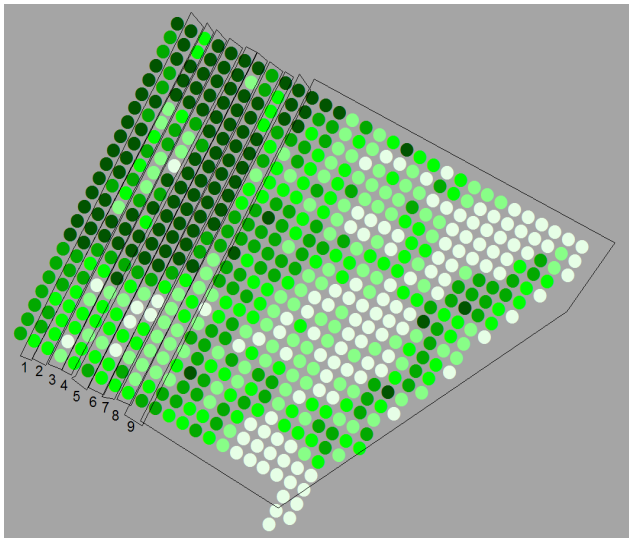


Figure 3. Resistivity values of trees in the experimental garden according to their location

Trunk Development in Trial Trees

Trunk circumferences were measured on 27.06.2011 at the beginning of the study in order to understand whether all trees in the trial plots were homogeneous in terms of development and the effect of the applied water restriction on the tree circumference and diameter. The homogeneity of the experimental trees in terms of development is a very important factor in understanding the effects of applied irrigation regimes on yield and other considerations and in comparing the regimes with each other. Therefore, the measurement results were evaluated statistically.

Kanber.etal.(1999) and Abdel-Messih and Nokrashy (1977) found that grapefruit trunk development changes at the beginning and end of the season. They determined that the tree trunk circumference increased with irrigation. Kırda et al (2007), determined that the circumference of the mandarin main stem increased between 1.53 and 3.33 cm according to the various irrigation regimes. Perez et al. (2014) in star ruby variety grapefruit trees, the water restriction applied at different stages of development, the tree trunk growth rate and they stated that it decreased in the cell division phase, but at the harvest it was the same as the control treatment. On the contrary, Gonzalez-Altozano and Castel (2000), conducted a study on mandarin and stated that water restriction applied in the same period caused an increase in trunk growth.

Many researchers have found a correlation between tree environment and irrigation, as in this study. According to these researchers; They determined that measuring trunk circumference can be used to compare the response of trees in the same garden to different irrigation practices (Wiegand and Swanson, 1982; Dasberg et al, 1981).

Fruit development

In the study, 10 fruits were selected from the trees in 3 different soils (soils with different resistivity values; ECa: 86.4-109.3 for T1; ECa:22.9-36.7 for T2 and ECa:62.1-72.0 for T3) for each irrigation regime circumferences were measured. Measurements were made at five different times between 08 August and 11 December 2011. The last reading values were considered as the harvest values.

The measurement results which are an indicator of fruit development, were evaluated statistically and graphically. Thus, at the end of the first year of the experiment, the effects of the applied irrigation programs on tree growth according to the soil characteristics of the area where the tree is located; The contribution of different irrigation treatmentsto fruit development in the same season has been tried to be examined. Fruits are smaller in other deficitirrigation than in fully watered regimes. For example, fruits in the I-70 were 5% smaller than the I-100, and the fruits in the I-50 were 15% smaller. From this, it could be said that regardless of soil conditions, fruit size is directly affected by irrigation programs and the amount of water given.The average fruit circumference values of the irrigation treatmentsfor the trial year are given in Figure 5. Compared to the fully watered regimes, the fruits are smaller in other deficit irrigation. For example, the goldenball fruits in the I50 were 6% smaller than the I100, and the fruits in the I70 remained 4% smaller than the I100. From this, it could be said that fruit size is directly affected by irrigation programs and the amount of water given. In the study, a tree was determined in each replication for each irrigation and the circumferences of 10 fruits on this tree were measured at different times. Measurements were made at five different times between 8 August and 11 December 2011.

The temporal variation of fruit development is given in Figure 4. Thus, the contribution of irrigation programs applied in the trial year to fruit development was tried to be examined.

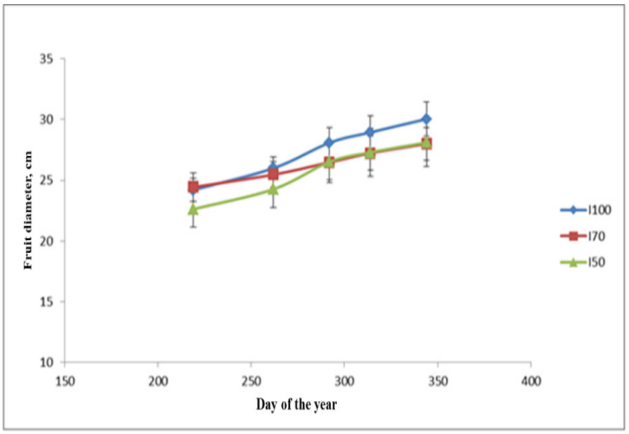


Figure4. The temporal variation of fruit development

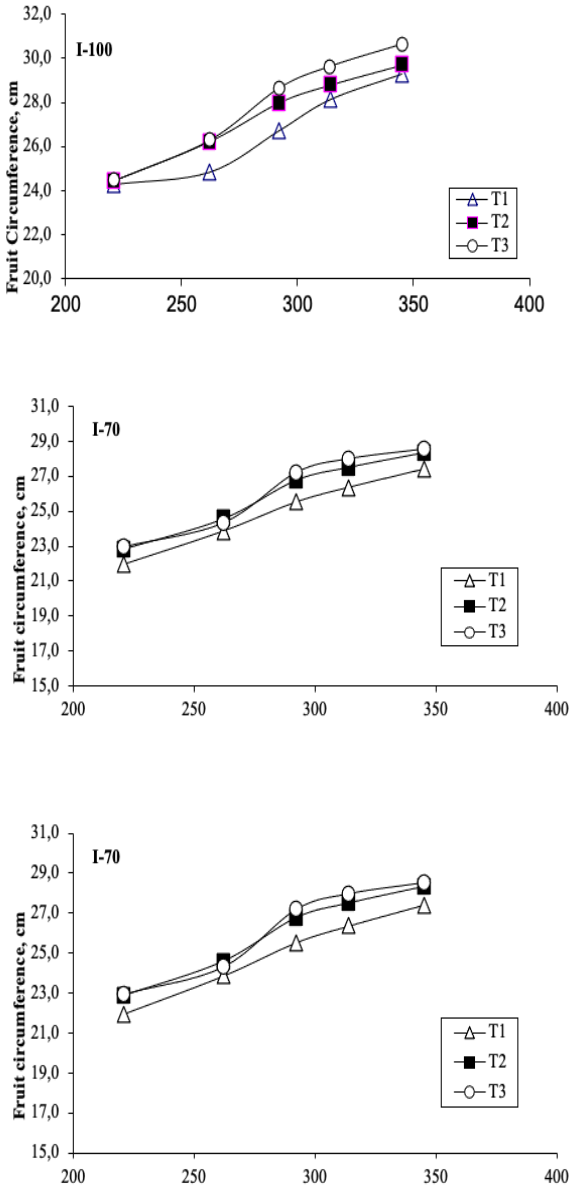


Figure 5. Temporal changes in the development of fruits in different soil and irrigation programs.

The temporal changes in the development of fruits on the trees considered in the study in different soil and irrigation programs are shown schematically in Figure 5.

As mentioned, T1 indicates heavy textured, T2 light textured and T3 medium textured soils. In Table 3, fruit development in irrigation treatmentsshowed differences according to soil textures.

For example, in the treatmentof I100, it was determined that the fruits developed better in medium and light textured soils than in heavy textured soils. A similar situation is observed for I70. As the soil texture gets heavier, fruit development worsens. On the other hand, on the I50 treatment, where the water shortage is more severe, a contrasting situation has emerged compared

to other treatment. n the I50 line, this time, the fruits on the trees grown on heavy textured soils showed a better development. In this regard, fruits developed more in soils with T1 symbol than other T2 and T3 soils. It can be thought that the described situation arises as a result of the deterioration of aeration in heavy textured soils in areas where excess water is applied and the negative effect of fruit development due to the decrease in water intake as a result.

As can be seen from Table 2, the photosynthesis change and the transpiration change are similar to each other in the grapefruit tree. The highest transpiration rate was obtained from the I100 treatmentwhere full irrigation was attempted. This was followed by the water-restricted I70 treatment. The least transpiration rate was measured on the I50 with the highest water restriction. Transpiration values measured according to irrigation treatmentsin the grapefruit tree ranged from 0.57 (I100) to 0.28 mmol/m2/s (I50). From this, it can be explained that transpiration rate in grapefruit trees, like photosynthesis rate, varies depending on the amount of irrigation water applied.As can be seen from Table 2, the statistically significant net photosynthesis values were measured as 2.64 umol/m^2/s for I50, 3.48 umol/m^2/s for I70 and 4.77 umol/m^2/s for I100. The highest net photosynthesis value was obtained in I100, which was tried to be fully irrigated. This was followed by the I70, and the smallest value was found on the I50, where the water was significantly reduced. From this, it can be said that the rate of photosynthesis is directly related to the water level in the plant root zone under the same climate, soil and cultural application conditions and varies significantly depending on irrigation applications. In particular, it could be said that the rate of photosynthesis changes depending on the amount of irrigation water used in the grapefruit trees. Panigrahi et. al., (2014) found that photosynthesis, stomatal conductivity and transpiration decrease with decreasing irrigation water amount, similar to the study conducted in Kinnow mandarin variety.In another way, it can be expressed that the mandarin plants could sustain their photosynthesis rate with 50% reduction of water supply, which is called as the photosynthetic acclimatisation nature of citrus (Tomar and Singh, 1986; Vu and Yelenosky, 1988; Zhihui et al., 1990). The gs and Tr values followed the same trend of Pn in different irrigation treatments.

Table 2. Photosynthetic active radiation ranged between 820 and 1296 at leaf surface during gas exchange measurements where leaf surface temperature in °C varied from 21.4 to 24.9.

		photosynthetic rate, µmol $m^{-2}s^{-1}$	transpiration rate, mmol$m^{-2}s^{-1}$	Stomatal conductance, mmol $m^{-2}s^{-1}$	WUE
Irrigation treatment					
I50		2.64 c	0.28 c	28.22 c	9.56 a
I70		3.48 b	0.43 b	33.56 b	8.11 b
I100		4.77 a	0.57 a	39.22 a	8.39 b
Soil structure					
ECa: 22.9-36.7		3.60 b	0.43 b	33.67 b	8.56
ECa: 62.1-72.0		3.25 c	0.38 c	31.78 c	8.84
ECa: 86-109.3		4.06 a	0.47 a	35.56 a	8.67
irragation	soil				
I50	ECa:22.9-36.7	2.68	0.28fg	28.00 f	9.71 ab
	ECa:62.1-72.0	2.38	0.24 g	25.67 g	9.90 a
	ECa: 86-109.3	2.87	0.32ef	31.00 e	9.07abc
I70	ECa:22.9-36.7	3.47	0.44 d	33.33 cd	7.81 e
	ECa:62.1-72.0	3.02	0.36 e	32.67 de	8.41cde
	ECa: 86-109.3	3.97	0.49 c	34.67 c	8.10 de
I100	ECa:22.9-36.7	4.65	0.57 ab	39.67 a	8.15cde
	ECa:62.1-72.0	4.34	0.53bc	37.00 b	8.19cde
	ECa: 86-109.3	5.33	0.60 a	41.00 a	8.83bcd
Prob> F					
irragation		<.0001*	<.0001*	<.0001*	<.0001*
soil		<.0001*	<.0001*	<.0001*	0.2207
irragation*soil		0.1675	0.0326*	0.0030*	0.0065*

Table 3. Statistical evaluation of the effect of irrigation water applied at different levels on trees grown in soils with different soil textures on fruit development, fruit yield, tree trunk development, canopy volume development and leaf area index (LAI).

		Fruitperimeter (cm)	Trunkdiameter (cm)	Canopyvolüme (m^3)	LAI	Yield (kg/tree)
Irrigation treatment						
I50		26.33	67.22	48.07 b	7.06	828b
I70		25.74	71.11	52.70 ab	6.35	883b
I100		27.44	73.39	63.21 a	7.01	1050a
Soil structure						
ECa: 22.9-36.7		26.69	67.66	52.41	7.00 a	877b
ECa: 62.1-72.0		26.56	72.38	55.06	7.52 a	1155a
ECa: 86-109.3		26.25	71.69	56.51	5.89 b	727b
irragation	soil					
I50	ECa:22.9-36.7	26.65	61.57	36.57	7.80	633c
	ECa:62.1-72.0	25.55	68.90	53.41	7.81	1000b
	ECa: 86-109.3	26.78	71.20	54.22	5.56	850bc
I70	ECa:22.9-36.7	26.00	72.70	61.55	6.19	1083b
	ECa:62.1-72.0	26.19	69.37	43.24	7.11	700c
	ECa: 86-109.3	25.02	71.27	53.32	5.74	866b
I100	ECa:22.9-36.7	27.43	68.70	59.10	7.03	1766a
	ECa:62.1-72.0	27.94	78.87	68.55	7.64	916b
	ECa: 86-109.3	26.94	72.60	61.98	6.36	466c
Prob> F						
irragation		0.0698	0.5076	0.0218*	0.2397	116*
soil		0.8060	0.6317	0.7143	0.0063*	217*
irragation*soil		0.6324	0.7913	0.0741	0.5461	369*

As seen in Table 3, there is no statistically significant difference between tree trunk circumference measurements at the beginning of the experiment. Trees are very homogeneous among themselves. At the end of the statistical analysis, no statistically significant difference was found between the irrigation levels.

In this case, it can be said that the differences between the regimes may have occurred by chance.

In the analyzes (canopy volume and main trunk circumference and height measurements), it was understood that the trees included in the experiment developed quite homogeneously and there was no statistical difference in terms of growth. It was determined that the irrigation levels were statistically significant in Canopy volume and yield, soil structure was statistically significant in LAI and yield, and interaction was statistically significant only in yield.

Experimental garden soils contain three different groups in terms of resistivity values (ECa); It has been determined that trees develop in soils with these soil structures. Accordingly, the tested trees were T1: 86-109; It is grown in soils with ECa values of T2: 23-37 and T3: 62-72. According to the ECa values mentioned, T1 soils are heavy; T2 soils represent light and T3 soils represent medium structures groups(Table 3).

In the study, the yields for irrigating the trees in the plot taken for the experiment varied between 883 (I70) and 828 (I50) kg per average tree. An average of 1050 kg was obtained from the fully irrigated I-100 treatment. The figures given are quite high. It can be said that this situation is due to the fact that the multi-year average has been overestimated during the year. Table 3.

CONCLUSION

This study was carried out in 2011 using Rio Red grapefruit trees in Çukurova University Faculty of Agriculture Research and Application Farm. Electrical conductivity (ECa) maps of the soils of the trial garden were formed in order to make a detailed structure analysis. In the study, it was aimed to determine the effects of the amount of irrigation water applied at different levels on the fruit growth and yield, tree trunk development, tree canopy volume development, leaf area index development and photosynthesis rate on trees growing in soils of different body classes. The highest net photosynthesis value was obtained in I100, which was tried to be fully irrigated. This was followed by I70, and the smallest value was found on

I50, where the water was significantly reduced. From this, it could be said that the rate of photosynthesis is directly related to the water level in the plant root zone under the same climate, soil and cultural application conditions and varies significantly depending on irrigation applications. In particular, it can be said that the rate of photosynthesis changes depending on the amount of irrigation water used in grapefruit trees. It was understood that the fruits of the other treatments with water restriction were smaller compared to the fully irrigated treatmentin the change of fruit size with the amount of irrigation water. From this, it can be said that regardless of soil conditions, fruit size is directly affected by irrigation programs and the amount of water given. Within the scope of this study, since the effects of irrigation treatmentson fruit yield are not statistically significant, water reduction can be recommended for the region in order to save water for the farmers.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the correspondingauthor to the study is 70%, that of the other author is 30%.
All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

All data associated with this research were indicated and used in the manuscript submitted.

Consent for publication

All aouthors consented to the publication of this manuscript.

REFERENCES

Abdel-Messih, M.N., El-Nokrashy, M. A. E. G. (1977) Effect of Different Soil Moisture Levels on Growth, Yield and Quality of Washington Navel Orange. Egypt Agric. Res. Rev. 55(3): 47-57.Agricultural Water Management96:565–573.

Chalmers, D.J., Mitchell P.D, Van Heek, L. (1981). Control of Peach Tree Growth and Productivity by Regulated Water Supply, Tree Density and Summer Pruning. J.Am. Soc. Hor. Sci.106:307–312.

Dasberg, S., Bielorai, H., Erner, Y. (1981).Partial Wetting of The Root Zone and Nitrogen Effect on Growht and Quality of Shamouti Orange. ActaHortic. 119: 103-105. https://doi.org/10.17660/ActaHortic.1981.119.9

Doorenbos, J., Kassam A.H. (1979). Yield Response to Water.FAO United Nations, Irr. and Drain. Paper, No. 33,Rome, 193 S.

Engısh, M.J., Musick, J.T., Murty, V.V.(1990). Deficit Irrigation. Management of Farm Irrigation Systems, Edit. G.J. Hoffman., T.A. Howell., K.H. Solomon."Chap. 17. An ASAE Monograph,St. Joseph, MI, 631-663 S.

González,A.P., and Castel, J.R. (2000). Regulated Deficit İrrigation in Clementina De Nules' Citrus Trees. II.Vegetative Growth. Journal of Horticultural Science & Biotechnology75:388–392. https://doi.org/10.1080/14620316.2000.11511256

Kanber, R., Köksal, H., Yazar, A., Özekici, B., Önder, S. (1999). Effects of Different Irrigation Programs on Fruit, Trunk Growth Rates, Quality and Yield of Grapefruit Trees. Tr. J. of Agriculture and Forestry, 23, 401-411.

Kanber,R.,Ünlü,M., Tekin, S., Koç, L., Kapur, B. (2007). Akdeniz İklim Koşullarında Kimi Tarla Bitkilerinin Su KullanımRandımanlarının İrdelenmesi Türkiye VII. Tarla Bitkileri Kongresi, 25-27 Haziran2007, Erzurum (Poster Bildiri).(in Turkish)

Kırda, C., Topaloğlu, F., Topçu, S., Kaman, H.(2007). Mandarin Yield Response to Partial Root Drying and Conventional Deficit Irrigation. Turkish Journal of Agriculture For. 31. 1-10 .

Özdemir, A. (2008). Çokelektrotlu Jeofizik Rezistivite Ölçümlerinin Yeraltısuyu ve Jeotermal Arama Alanlarındaki Uygulamalarının Değerlendirilmesi. (in Turkish)

Panigrahi, P., Sharma, R. K. Hasan, M. and Parihar, S. S. (2014). Deficit irrigation scheduling and yield prediction of Kinnow mandarin (*Citrus reticulate* Blanco) in a semiarid region. Agricultural Water Management, 140: 48-60. https://doi.org/10.1016/j.agwat.2014.03.018

Perez-Perez, J.G., Robles, J.M., Botia, P. (2014). Effects of Deficit İrragation İn Different Fruit Growft Stages on"StarRuby"Grapefruit Trees İn Semi-Aridconditions. Agricultural Water Management 133. 44-54. https://doi.org/10.1016/j.agwat.2013.11.002

Stegman, E.C., B.G. Schatz, J.C. Gardner.(1990). Yield Sensitivities of Short Season Soybeans to Irrigation Management. Irrigation Sci. 11(2):111-119.

Tomar, N.S., Singh, R., 1986. Effect of various moisture regimes on photosynthesis and photorespiration in plant lemon. Indian J. Hort. 43 (1 & 2), 46–50.

Ünlü, M., Kanber , R., Kapur , B., Koç, D.L., Tekin, S. (2008).Tarımsal Sulamada Su Artırımı: Kısıntılı Sulama Yaklaşımı 5. DünyaSu Forumu Bölgesel Hazırlık Süreci DSİ Yurtiçi Bölgesel Su Toplantıları Sulama – Drenaj Konferansı Bildiri Kitabı 81-95. (in Turkish)

USSL,(1954). Diagnosis and Improvement of Saline and Alkali Soils,Agriculture Handbook, No:60, 160s., USA.

Vu, J.V., Yelenosky, G., 1988. Water deficit and associated changes in some photosynthetic parameters in leaves of 'Valencia' orange (Citrus sinensis L. Osbeck). Plant Physiol. 88, 375–378. https://doi.org/10.1104/pp.88.2.375

Westwood, M.N.(1978). Fruit and Nut Species. In: Temperate-Zone Pomology, M.N. Westwood (Ed) Chapter.3, W.H. Freeman and Co., San Francisco CA, Pp. 41-76.

Wiegand, C. L., Swanson, W. A.(1982).Citrus Response to Irrigation. III Tree Trunk and Canopy Growth. J. Rio Grande WalleyHortic. Soc. 35: 97-107.

Zhihui, C., Liangcheng, Z., Guanglin, W.U., Shonglong, Z., 1990. Photosynthetic acclimation to water stress in citrus. In: Proc. Int. Citrus Symp., 5-8 November, 1990, Guangzhou, China, pp. 413–418.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 156-162
DOI: https://doi.org/10.31015/jaefs.2023.1.19

Techno-economic analysis of a solar-powered agricultural irrigation system using PV*Sol software: A case study in Konya

Mehmet Ali Biberci[1]

[1] Mechanical Engineering Department, Engineering Faculty, Cankiri, Türkiye

Citation: Biberci, M.A. (2023). Techno-economic analysis of a solar-powered agricultural irrigation system using PV*Sol software: A case study in Konya. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 156-162

Received: 29 January 2023
Revised: 24 February 2023
Accepted: 26 February 2023
Published Online: 12 March 2023

Correspondence: Mehmet Ali Biberci
E-mail: m.alibiberci@karatekin.edu.tr

Abstract

Agricultural irrigation is essential for crop growth and yield, but traditional irrigation systems are often associated with high costs, energy consumption, and negative environmental impacts. The development of alternative irrigation systems, such as solar-powered systems, has gained increasing attention in recent years. In this study, a techno-economic analysis of a grid-connected solar photovoltaic (PV) system was carried out for the electrical energy needed in irrigation of an agricultural area located in Konya, Turkiye. The electrical energy produced by the solar energy system was used to meet the energy needs of the electrical equipment and the water pumping system required for agricultural irrigation. According to the simulation results, the annual energy requirement for agricultural irrigation is 6,735 kWh and the peak load is 2.1 kW. In addition, in line with the simulation results, the PV system can feed the load with a self-consumption of 15.9%, a self-sufficiency level of 46.6% and a performance rate of 83% when the desired rate is set as 115% for a year reference period. When the financial analysis of the simulation is examined, it is estimated (or computed) that an asset return of 9.14% and a depreciation period of 8.7 years. The annual gain loss of the energy system due to shading is 1.5% and the annual CO_2 emission prevented by the system is 9.201 kg. Considering these results, it could be stated that the proposed energy system is technically and economically appropriate for agricultural irrigation systems. At the same time, these results might provide valuable insights for farmers and policymakers seeking to adopt sustainable and cost-effective irrigation systems for agricultural production.

Keywords: Agricultural irrigation, Solar-powered systems, PV*Sol, Techno-economic analysis

INTRODUCTION

Agriculture is a critical sector for the economies of many countries and water for irrigated agriculture is essential for crop growth and increasing yield (Mancosu et al., 2015; Özhüner, 2020a, 2020b, 2022). Agricultural irrigation is, thus, an essential part of agricultural production in arid and semi-arid regions where rainfall is limited, and it accounts for over 70% of global water use (Deng et al., 2006; Wallace, 2000). Traditional irrigation systems such as flood irrigation, furrow irrigation and sprinkler irrigation have been used for centuries to provide water to crops (Hassanli et al., 2010; Jensen, 2007). However, these methods have several drawbacks including high water loss due to evaporation and runoff, high energy consumption and dependence on unreliable power sources. In recent years, solar-powered irrigation systems have emerged as an alternative solution that can overcome some of these challenges. The photovoltaic design and simulation (PV*Sol) program is a tool that can be used to design and analyze the techno-

economic feasibility of solar-powered agricultural irrigation systems (Arnell, 1999; Nkuriyingoma et al., 2022).

Traditional irrigation systems involve the application of water to crops using gravity or pumps. Flood irrigation which is the oldest and simplest method involves flooding the fields with water and allowing it to infiltre in. However, this method is highly inefficient due to fact that much of the water is lost by evaporation and runoff (Kamwamba-Mtethiwa et al., 2016; McNabb, 2019). Furrow irrigation is also similar to flood irrigation, but water is applied through trenches or furrows. Sprinkler irrigation involves the use of sprinklers to distribute water over the crops. This method is more efficient than flood and furrow irrigation but still suffers from high water loss due to evaporation and wind drift (Fahong et al., 2004; Fahong et al., 2004). Some irrigation systems require energy using electricity or diesel and this is quite expensive (Hassan and Kamran, 2018). In contrast, solar-powered irrigation systems use solar energy to power water pumps, which can significantly reduce operating costs and increase water availability for agriculture. These systems consist of photovoltaic (PV) panels that convert solar energy into electrical energy, which is used to power the water pumps. The PV*Sol program is a simulation software tool that can be used to design and analyze the technical and economic feasibility of such systems (Kazem et al., 2017; Ikram et al., 2021).

The PV*Sol program takes into account variables such as solar irradiation, temperature, and shading effects to predict the output power and energy yields of the PV system. This tool enables designers to assess the expected performance of PV systems under different climatic conditions geographical locations (Dondariya et al., 2018; Milosavljević et al., 2022). The PV*Sol program can be used to determine the optimal design and configuration of the PV system including the sizing and selection of PV modules, inverters, batteries, and other components (Alsadi & Khatib, 2018; Mohanty et al., 2016). Additionally, the PV*Sol program can be used to estimate the energy demand for pumping water for irrigation and the resulting cost savings compared to traditional irrigation methods. The use of solar-powered irrigation systems with the PV*Sol program has several advantages over traditional irrigation methods. Solar-powered irrigation systems are more efficient and have lower energy consumption compared to traditional methods. Additionally, these systems are environmentally friendly, as they do not produce any greenhouse gas emissions. Furthermore, solar-powered irrigation systems can reduce dependence on unreliable power sources and provide a reliable source of water for agriculture (Biberci et al., 2018; Deveci et al., 2015; Grant et al., 2022).

This study presents a techno-economic analysis of a solar-powered agricultural irrigation system using the PV*Sol program. The PV*Sol program was used to model the energy output of the solar panels, estimate the water requirements of the crop and determine the economic feasibility of the system. The results of the study provide valuable insights into the feasibility and economic benefits of solar-powered irrigation systems for farmers.

MATERIALS AND METHODS

Selection of the energy system simulator

Techno-economic analysis of a solar-powered agricultural irrigation system was made using the PV*Sol program. The design concept is important in the selection of the simulation program. Because some functional tools are needed to simulate and optimize the designed system. PV*Sol software offers many advanced structures to simulate the generated energy system. While it enables us to model the electrical and mechanical structure of the energy system in detail, it also provides detailed technical specifications. Many engineers, planners, architects, installers and technicians around the world use PV*Sol software to design and build efficient PV energy systems (Bocullo et al., 2023).

Energy system location

The place where the photovoltaic system will be installed depends on certain parameters. These parameters can be determined as solar radiation value, amount

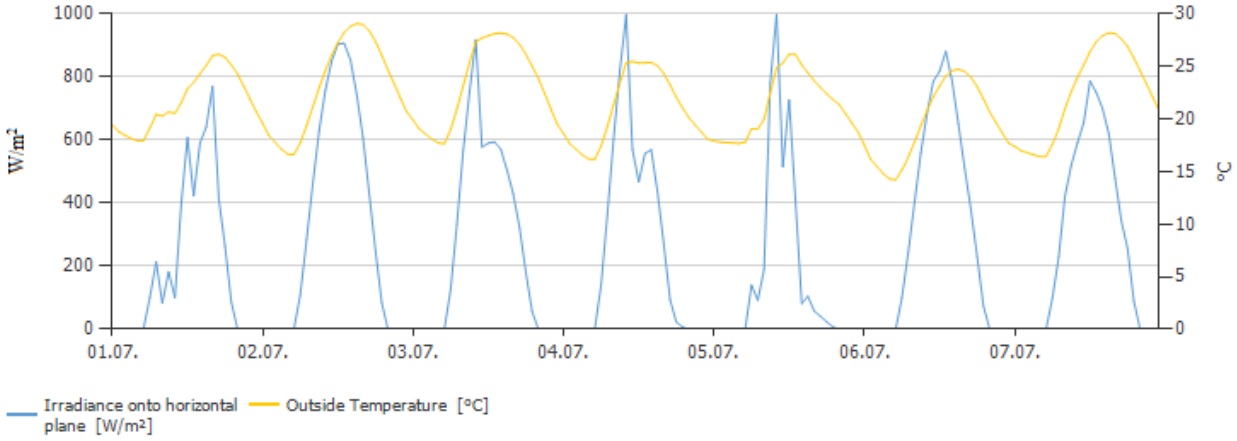


Figure 1. Horizontal radiation and outside temperature values for the first week of July for the selected location.

of shading and proximity to the consumer. For this reason, the location where the system will be installed is very important in terms of performance and efficiency (Kayhan et al., 2015).

The solar energy system to be established in this study was realized in Karatay district of Konya province. The reason for choosing this location is that it has an average temperature of 12 °C, and a high solar radiation value of 1763kWh/m² (Nkuriyingoma et al., 2022; *Climate Data (Calculation) :: PV*SOL® Help*, n.d.). At the same time, the lack of slope of the installed land is an important parameter in choosing the determined location. Horizontal radiation and outside temperature values for the first week of July for the selected location are shown in Figure 1.

Load Profile

Load profiles are usually determined by the hourly or demanded energy needs (Kamber et al., 2021). Within the scope of this study, the annual energy consumption of 6735 kWh required to irrigate an area of approximately 36 ha between April and September has been determined as the amount of consumption. The load profile distribution for the specified time interval is given in Figure 2.

Energy system configuration

Photovoltaic panels are a component that converts solar energy into direct current (Tunçer, 2022). Photovoltaic panel selection is usually made by taking into account the demanded power and losses. For this study, panel layout configuration was made using the model mounting section in the PV*Sol software, and 48 panels of ECSOLAR ECS-250P60 model with 250 W power were selected as the PV panel. The inverter, which is another component of modulation, converts the direct current (DC) coming from the PV modules to alternating current (AC) and supplies the systems working with AC current (Yinanç, 2022). Three Schneider Electric Conext RL 3000E brand inverters were selected as inverters. In Figure 3, the circuit diagram including these components as well as wiring and other electrical equipment is given in detail.

Financial analysis

Financial analysis is made to decide whether the

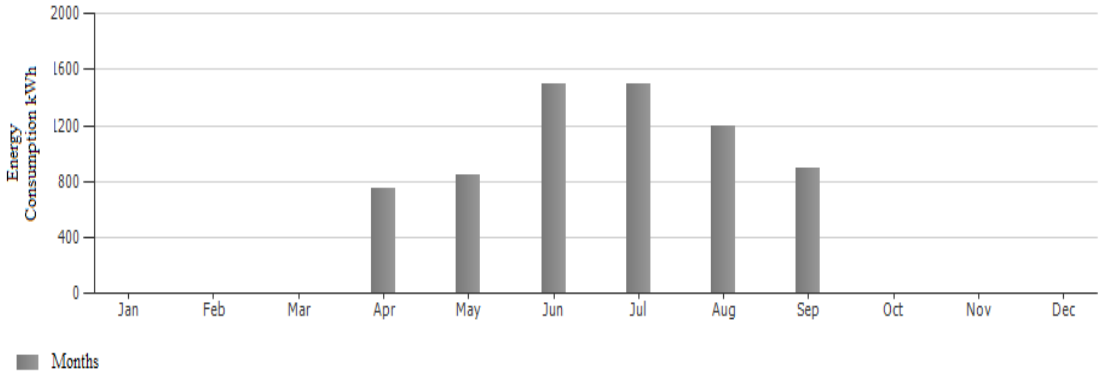


Figure 2. The load profile distribution for the specified time interval.

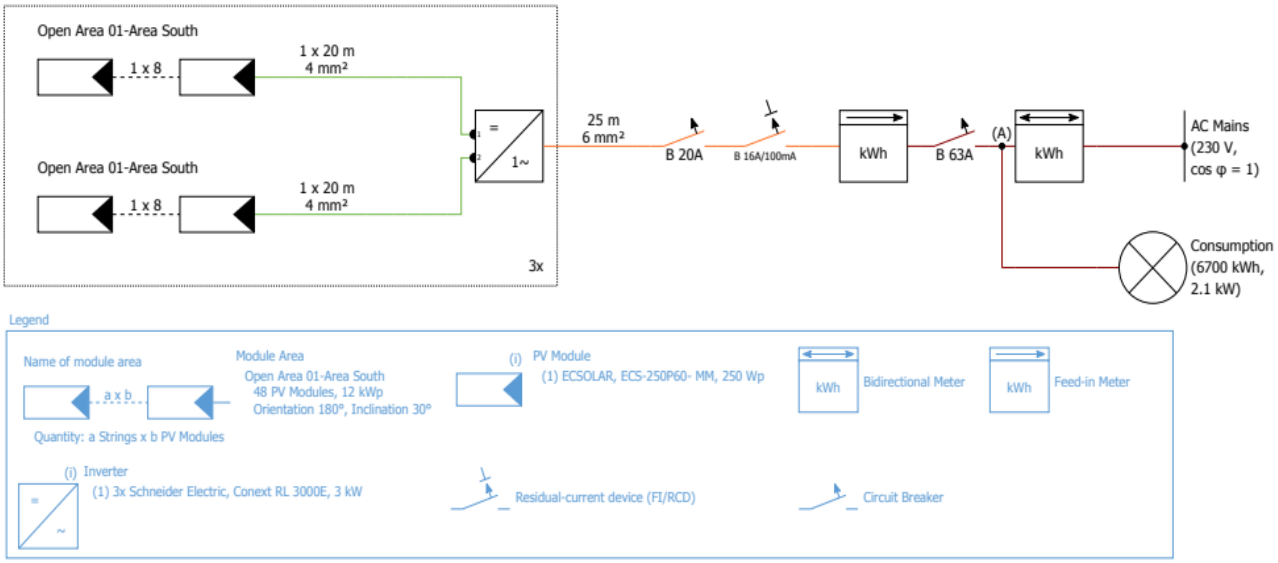


Figure 3. Circuit diagram including these components as well as wiring and other electrical equipment.

investment in the solar energy system is economically worthwhile. PV*Sol software allows financial analysis for the specified system. Financial analysis can be, thus, made by entering the price of electricity sold, net metering tariffs, inflation rate for energy price and general installation and operating costs into the software. The financial analysis parameters used in this study are given in Table 1.

Table1. Financial analysis parameters.

Economic life	20 year
Outgoing cost of system setup parts and labor	775 US$/kWp
Operation and maintenance cost	1% of investment
Net metering tariffs (energy sale price to the consumer)	Consumption type: residential (0.19 US$/kWh)
Inflation rate for energy price	2.7%/year

RESULTS AND DISCUSSION

The use of solar power for agricultural irrigation systems has several advantages, including reduced reliance on fossil fuels, lower operating costs, and reduced greenhouse gas emissions. However, the initial capital cost of setting up a solar-powered system can be a barrier for many farmers, particularly those in developing countries. The techno-economic analysis presented in this study provides useful information for farmers and policymakers in evaluating the feasibility and cost-effectiveness of a solar-powered irrigation system (Guno and Agaton, 2022).

The use of the PV*Sol program to conduct the techno-economic analysis is an effective approach, as it provides a detailed assessment of the system's performance and costs. However, the analysis could be further improved by considering additional factors, such as the variability of solar radiation, the impact of shading on the solar panels, and the availability of water resources. These factors can have a significant impact on the performance and cost-effectiveness of the system, and should be carefully considered when evaluating the feasibility of a solar-powered irrigation system.

The solar energy system is designed as Grid-connected PV System with Electrical Applications type in order to meet the energy need for agricultural irrigation and to sell the excess energy to the grid. A design configuration specific to the determined location has been created. In this direction, the system that was first installed was chosen in a place close to the consumer. When the Konya region is examined, irrigation costs increase due to the fact that agricultural irrigation is too much, in this case, it is reflected in the producer costs. Thus, the energy costs used increase the price and cost of the product considerably. For this reason, in this study, a solar energy system was modeled and simulated to meet the electrical energy needs of two 10 horsepower (hp) irrigation pumps used for irrigation of an agricultural area of 36 ha. All of the excess energy produced in this simulated system feeds the grid. A general summary of the simulation results is presented in Table 2.

Table 2. Simulation results of total system.

PV System	**Value**	**Unit**
PV Generator Output	12	kWp
Spec. Annual Yield	1,631.38	kWh/kWp
Performance Ratio (PR)	83.0	%
Yield Reduction due to Shading	1.5	%/Year
PV Generator Energy (AC grid)	19,612	kWh/Year
Down-regulation at Feed-in Point	0	kWh/Year
CO_2 Emissions Avoided	9,201	kg / year
Appliances	Value	Unit
Appliances	6,700	kWh/Year
Standby Consumption (Inverter)	35	kWh/Year
Total Consumption	6,735	kWh/Year
Power Surplus	12,876.6	kWh
Solar Fraction	291.2	%
Level of Self-sufficiency	Value	Unit
Total Consumption	6,735	kWh/Year
Covered by Grid	3,596	kWh/Year
Level of Self-sufficiency	46.6	%

Agricultural irrigation activities are carried out in the selected region in April-September. The realized PV energy system is configured to meet the amount of energy demanded by the consumer even when the sunshine duration is low and the least energy is produced. In addition, shading analyzes were made during the design and it was ensured that shading was at the minimum level. The selection of each part used in the PV energy system has been optimized and thus the best efficiency has been obtained from each part. At the same time, each of the parts used with the benefit of optimization has been selected to meet the minimum needs of the system. In this case, it allows us to avoid excessive parts costs. The parts list is given in Table 3.

Table 3. Part list of PV*Sol system.

Number	Type	Manufacturer	Name	Quantity	Unit
1	PV Module	ECSOLAR	ECS-250P60- MM	48	Piece
2	Inverter	Schneider Electric	Conext RL 3000E	3	Piece
3	Cable		String Cable 4 mm^2 Copper	120	m
4	Cable		AC cables 1-phase 6 mm^2 Copper	75	m
5	Components		Feed-in Meter	1	Piece
6	Components		Circuit Breaker B 63A	1	Piece
7	Components		Bidirectional Meter	1	Piece
8	Components		Circuit Breaker B 20A	3	Piece
9	Components		Circuit Breaker B 20A	3	Piece

In this study, the annual energy requirement for agricultural irrigation is 6,735 kWh and the peak load is 2.1 kW. In addition, when the PV system is determined as 115% in accordance with the simulation carried out, the simulation gives the most appopriate and optimum results. In line with these results, the PV system can feed the load with a self-consumption of 15.9%, a self-sufficiency level of 46.6% and a performance rate of 83% for a one-year reference period. According to the result of the financial analysis of the simulation, it could be stated that there is a return on assets of 9.14% and a depreciation period of 8.7 years. The annual energy loss of the energy system due to shading is 1.5% and the annual CO_2 emission eliminated by the system is 9,201 kg.

The amount of consumption (load) demanded by the consumer was directly requested from the energy system. The grid was fed continuously with the excess electrical energy produced by the energy system. The energy flow of the system is shown in Figure 4.

Considering the simulation results, considering that the life of the PV energy system is 20 years, the depreciation period of 8.7 years is quite appropriate. Figure 5 shows the cash flow.

The change in energy cost developments by years before the PV system was installed (in blue) and after it was installed (in yellow) is shown in Figure 6. When Figure 6 is examined, it is observed that the energy cost has increased significantly over the years before the PV system was installed. After the PV system is installed, it is seen that the increase in energy costs has increased almost negligibly over the years.

CONCLUSION

In this study, the electrical and mechanical structures of the PV solar energy system required for an agricultural irrigation system were designed and simulated with PV*Sol software. For the system created, Karatay district of Konya province was selected. This location was chosen because of the high need for agricultural irrigation in this region. The excess of agricultural irrigation in the region increases the demand for electricity and energy costs. The load profile consists of two 10 hp irrigation pumps. The electrical energy needed for the pumps is required in April-September. On the day of irrigation, the pumps operate for 10 hours a day. Therefore, the annual energy requirement for agricultural irrigation is 6,735 kWh and the peak load is 2.1 kW. According to the simulation results, the PV system can feed the load with a self-consumption of 15.9%, a self-sufficiency level of 46.6% and a performance rate of 83% when the desired rate is set as 115% for a reference period of one year. When the financial analysis of the simulation is examined, it is seen that there is a return on assets of 9.14% and a depreciation period of 8.7 years. The annual gain loss of the energy system due to shading is 1.5% and the annual CO_2 emission prevented by the system is 9,201 kg.

The results of the techno-economic analysis presented in this study demonstrate the feasibility and cost-effectiveness of a solar-powered agricultural irrigation system. The PV*Sol program provides a valuable tool for evaluating the performance and costs of such systems, and can help farmers and policymakers make informed decisions about adopting renewable energy solutions for agricultural irrigation. Further research is needed to refine the analysis and to better understand the factors that can impact the performance and cost-effectiveness of solar-powered irrigation systems. Overall, this study highlights the potential of solar power to support sustainable agriculture and reduce greenhouse gas emissions in the agricultural sector.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Alsadi, S., & Khatib, T. (2018). Photovoltaic Power Systems Optimization Research Status: A Review of Criteria, Constrains, Models, Techniques, and Software Tools. *Applied Sciences*, *8*(10), Article 10. https://doi.org/10.3390/app8101761

Arnell, N. W. (1999). Climate change and global water resources. *Global Environmental Change*, *9*, 31–S49. https://doi.org/10.1016/S0959-3780(99)00017-5

Biberci, M. A., Özhüner, E., & Çelik, M. B. (2018). Yenilenebilir Enerji Kaynaklarının Belediyelerde Kullanımı. *Şehir Düşünce Dergisi*, *11*, 80–93. (in Turkish)

Bocullo, V., Martišauskas, L., Pupeikis, D., Gatautis, R., Venčaitis, R., & Bakas, R. (2023). UAV Photogrammetry Application for Determining the Influence of Shading on Solar Photovoltaic Array Energy Efficiency. *Energies*, *16*(3), Article 3. https://doi.org/10.3390/en16031292

*Climate data (Calculation): PV*SOL® help*. (n.d.). Retrieved February 17, 2023, Retrieved from https://help.valentin-software.com/pvsol/en/calculation/irradiation/climate-data/

Deng, X.-P., Shan, L., Zhang, H., & Turner, N. C. (2006). Improving agricultural water use efficiency in arid and semiarid areas of China. *Agricultural Water Management*, *80*(1), 23–40. https://doi.org/10.1016/j.agwat.2005.07.021

Deveci, O., Onkol, M., Unver, H. O., & Ozturk, Z. (2015). Design and development of a low-cost solar powered drip irrigation system using Systems Modeling Language. *Journal of Cleaner Production*, *102*, 529–544. https://doi.org/10.1016/j.jclepro.2015.04.124

Dondariya, C., Porwal, D., Awasthi, A., Shukla, A. K., Sudhakar, K., S.r., M. M., & Bhimte, A. (2018). Performance simulation of grid-connected rooftop solar PV system for small households: A case study of Ujjain, India. *Energy Reports*, *4*, 546–553. https://doi.org/10.1016/j.egyr.2018.08.002

Fahong, W., Xuqing, W., & Sayre, K. (2004). Comparison of conventional, flood irrigated, flat planting with furrow irrigated, raised bed planting for winter wheat in China. *Field Crops Research*, *87*(1), 35–42. https://doi.org/10.1016/j.fcr.2003.09.003

Grant, F., Sheline, C., Sokol, J., Amrose, S., Brownell, E., Nangia, V., & Winter, A. G. (2022). Creating a Solar-Powered Drip Irrigation Optimal Performance model (SDrOP) to lower the cost of drip irrigation systems for smallholder farmers. *Applied Energy*, *323*, 119563. https://doi.org/10.1016/j.apenergy.2022.119563

Guno, C. S., & Agaton, C. B. (2022). Socio-economic and environmental analyses of solar irrigation systems for sustainable agricultural production. Sustainability, 14(11), 6834. https://doi.org/10.3390/su14116834

Hassan, W., & Kamran, F. (2018). A hybrid PV/utility powered irrigation water pumping system for rural agricultural areas. Cogent Engineering, 5(1), 1466383. https://doi.org/10.1080/23311916.2018.1466383

Hassanli, A. M., Ahmadirad, S., & Beecham, S. (2010). Evaluation of the influence of irrigation methods and water quality on sugar beet yield and water use efficiency. *Agricultural Water Management*, *97*(2), 357–362. https://doi.org/10.1016/j.agwat.2009.10.010

Ikram, H., Javed, A., Mehmood, M., Shah, M., Ali, M., & Waqas, A. (2021). Techno-economic evaluation of a solar PV integrated refrigeration system for a cold storage facility. *Sustainable Energy Technologies and Assessments*, *44*, 101063. https://doi.org/10.1016/j.seta.2021.101063

Jensen, M. E. (2007). Beyond irrigation efficiency. *Irrigation Science*, *25*(3), 233–245. https://doi.org/10.1007/s00271-007-0060-5

Kamber, E., Körpüz, S., Melih, C. A. N., Aydoğmuş, H. Y., & Gümüş, M. (2021). Yapay Sinir Ağlarina Dayali Kisa Dönemli Elektrik Yükü Tahmini. *Endüstri Mühendisliği*, *32*(2), 364–379.

Kamwamba-Mtethiwa, J., Weatherhead, K., & Knox, J. (2016). Assessing Performance of Small-Scale Pumped Irrigation Systems in sub-Saharan Africa: Evidence from a Systematic Review. *Irrigation and Drainage*, *65*(3), 308–318. https://doi.org/10.1002/ird.1950

Kayhan, V. A., Ulker, F., & Elma, O. (2015). Photovoltaic system design, feasibility and financial outcomes for different regions in Turkey. *2015 4th International Conference on Electric Power and Energy Conversion Systems (EPECS)*, 1–6. https://doi.org/10.1109/EPECS.2015.7368518

Kazem, H. A., Albadi, M. H., Al-Waeli, A. H., Al-Busaidi, A. H., & Chaichan, M. T. (2017). Techno-economic feasibility analysis of 1 MW photovoltaic grid connected system in Oman. Case studies in thermal engineering, 10, 131-141. https://doi.org/10.1016/j.csite.2017.05.008

Mancosu, N., Snyder, R. L., Kyriakakis, G., & Spano, D. (2015). Water Scarcity and Future Challenges for Food Production. *Water*, *7*(3), Article 3. https://doi.org/10.3390/w7030975

McNabb, D. E. (2019). Agriculture and Inefficient Water Use. In D. E. McNabb (Ed.), *Global Pathways to Water Sustainability* (pp. 99–115). Springer International Publishing. https://doi.org/10.1007/978-3-030-04085-7_7

Milosavljević, D. D., Kevkić, T. S., & Jovanović, S. J. (2022). Review and validation of photovoltaic solar simulation tools/software based on case study. *Open Physics*, *20*(1), 431–

451. https://doi.org/10.1515/phys-2022-0042

Mohanty, P., Muneer, T., Gago, E. J., & Kotak, Y. (2016). Solar Radiation Fundamentals and PV System Components. In P. Mohanty, T. Muneer, & M. Kolhe (Eds.), Solar Photovoltaic System Applications: A Guidebook for Off-Grid Electrification (pp. 7–47). Springer International Publishing. https://doi.org/10.1007/978-3-319-14663-8_2

Nkuriyingoma, O., Özdemir, E., & Sezen, S. (2022). Techno-economic analysis of a PV system with a battery energy storage system for small households: A case study in Rwanda. *Frontiers in Energy Research*, *10*.

Özhüner, E. (2020a). Bitkilerde kuraklık stres cevabında mikroRNA'ların rolü, 3. Uluslararası Mühendislik Bilimleri Ve Multidisipliner Yaklaşımlar Kongresi. (in Turkish)

Özhüner, E. (2020b). Tarımsal ve hayvansal ürünlerde biyoteknolojik uygulamalar. 9.Uluslararası Bilimsel Araştırmalar Kongresi. Ankara. (in Turkish)

Özhüner, E., K. Özrenk (Ed.) ve E.Küçüker, (Ed.) (2022), *CRISPR/CAS genome editing: Recent advances in abiotic stress response in plants*. Current Research in Agriculture, Forestry and Aquaculture (s. 43-57). Gece Kitaplığı Yaynevi.

Tunçer, B. (2022). Şebeke bağlantılı fotovoltaik güç sistemlerinin analizi, benzetimi ve boyutlandırılması [Master's Thesis]. Pamukkale Üniversitesi Fen Bilimleri Enstitüsü. (in Turkish)

Yinanç, D. M. (2022). Ordu İlinde Fotovoltaik Sistemin Zamana Bağlı Verim Değişimi ve Su Pompalama Kapasitesi Üzerine Etkilerinin Belirlenmesi [Master's Thesis]. Fen Bilimleri Enstitüsü. (in Turkish)

Wallace, J. S. (2000). Increasing agricultural water use efficiency to meet future food production. Agriculture, ecosystems & environment, 82(1-3), 105-119. https://doi.org/10.1016/S0167-8809(00)00220-6

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 163-170
DOI: https://doi.org/10.31015/jaefs.2023.1.20

Determination of the effect of cucumber grafting on some morphological and physiological characteristics in hydroponic conditions

Ömer Faruk Coşkun[1] **Seher Toprak**[1]

[1] Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University, Hatay 31060, Türkiye

Abstract

Due to the benefits and importance of the use of grafted seedlings, the demand for high quality grafted seedlings is increasing. In this study, it was aimed to determine the effect of rootstocks on some morphological and physiological parameters of cucumber (*Cucumis sativus* L.) plant under hydroponic growing conditions. In this study, grafted on seven different rootstocks and non-grafted plant characteristics were compared. Non-grafted seedlings were used as control plants. The values measured in at least one grafting application in 9 different morphological and/or physiological parameters among the 12 parameters measured were higher than the non-grafted plants. In leaf chlorophyll content (SPAD), photosynthetic active radiation (PAR) and root dry weight/fresh weight rate measurements, the values measured in non-grafted plants were not lower than grafted plants. It was determined that Cremna and Devrim rootstocks were effective in terms of shoot length and biomass values, but rootstock use did not have a significant effect in terms of photosynthetic activity. It was concluded that grafting of cucumber plants on different rootstocks may cause significant advantages in terms of some parameters, but the effects largely depend on the rootstock selection.

Keywords: *Cucumis sativus*, Hydroponic, Photosynthetic activity, Grafting, Rootstock

Citation: Coskun, O.F., Toprak, S. (2023). Determination of the effect of cucumber grafting on some morphological and physiological characteristics in hydroponic conditions. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 163-170

Received: 06 December 2022
Revised: 25 December 2022
Accepted: 29 December 2022
Published Online: 15 March 2023

Correspondence: Omer Faruk Coskun
E-mail: omerfaruk.coskun@mku.edu.tr

INTRODUCTION

Environmental pollutants, which are one of the most important global problems today and increasing as a result of human activities, also threaten the existence of biota (Adrees et al., 2015). Some pollutants can affect plant growth, photosynthesis mechanism and biochemical activities to a degree that may decrease (Ali et al., 2013; Keller et al., 2015; Rizwan et al., 2015). The deterioration of the soil ecosystem as a result of environmental pollutants causes loss of agricultural land, yield and quality decreases in agricultural products. Due to intensive production and monoculture cultivation in greenhouses, soil-related problems can cause yield and quality losses in aquaculture (Vural et al., 2000). For this reason, ways to reduce or eliminate yield and quality losses caused by pollutants are investigated.

One of the most important methods to reduce the negative effects of environmental conditions is grafting. Due to its advantages, the use of grafted plants has become a common agricultural method in many parts of the world (Turhan et al., 2011). In order to reduce the negative effects of soil-based stress factors, use of resistant/tolerant rootstocks is preferred. Grafting applications in vegetables can be evaluated to improve fruit quality under biotic or abiotic stress conditions (Sabatino et al., 2019; Singh et al., 2020; Ulas et al., 2020; Ulas,

2021; Ulas et al., 2021). The advantages of grafting in vegetables include providing tolerance to low soil and air temperatures, prolonging the economic harvest period and increasing yield accordingly, preventing damage to the environment as a result of better uptake of plant nutrients in the soil (Yetisir et al., 2004). With grafting, nutrient intake and use efficiency can also be increased (Colla et al., 2010; Ulas et al., 2021). Producer demand for grafted seedlings has increased in vegetable growing, especially in *Solanaceae* and *Cucurbitaceae* family vegetable species (Güngör and Balkaya, 2016; Karaağaç et al., 2018). Grafted seedling production in Turkey is carried out commercially mostly in tomato, watermelon, eggplant, cucumber and melon species.

Cucumber (*Cucumis sativus* L.) is one of the most economically valuable species of the *Cucurbitaceae* family. Cucumber production in the world is 91.258.272 tons, and in Turkey it is 1.926.883 tons (FAO, 2020). It is an environmentally friendly application that provides an important advantage to solve the problems encountered in cucumber growing is grafted seedlings. In some studies on cucumber, it has been determined that grafting increases plant growth, yield and water use efficiency (Günay, 2011). One of the most important factors determining the effectiveness of grafting is the determination of suitable rootstocks in grafted seedling production (Colla et al., 2012; Balkaya, 2014; Coşkun et al., 2022). Rootstock-scion interaction affects many features such as mineral uptake, fruit growth, fruit quality (Martínez-Ballesta et al., 2010). In this study, it was aimed to determine some morphological and physiological parameters of cucumbers grafted on different rootstocks in hydroponic culture medium.

MATERIALS AND METHODS

Plant material

In the study, cucumber was grafted different rootstocks. Minimix F1 was used as scion and TZ148, Devrim, Cremna, Kubai, Strong, RS841 and Maximus were used as rootstocks. The rootstocks used are *Cucurbita maxima* x *Cucurbita moschata* hybrid rootstocks and non-grafted plants were used as control.

Method

The study was carried out in the greenhouse and laboratory of Hatay Mustafa Kemal University, Agriculture Faculty, Horticulture Department. The seeds were sown in mixture of peat:perlite (3:1) with an EC of 0.4 and the seedlings were grown until they reached the first true leaf stage. Cucumber variety used as a scion was planted four days before the rootstocks and grafting was done when the first true leaves started to form. As the grafting method, single cotyledon grafting method was used. The disinfection of the equipment to be used in grafting was provided by using 75% ethyl alcohol. Grafted plants were stored for 7 days in grafting care unit with a relative humidity of 95% and a temperature of 27 °C. After the grafting had taken place, the plants were gradually acclimated to the normal growing medium, then the roots were washed and transferred to aerated water culture in 8 liter pots. The experiment was carried out according to the randomized trial plots design with 3 replications. Hydrophonic medium was changed every 7 days and 4 plants were used in each replication. The plants are grown in a nutrient solution containing 1125 µM $Ca(N0_3)_2$, 375 µM $(NH_4)_2SO_4$, 750 µM K_2SO_4, 650 µM $MgSO_4$, 500 µM KH_2PO_4, 10 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.4 µM $CuSO_4$, 0.4 µM $MoNa_2O$ and 80 µM Fe. The electrical conductivity (EC) of the growing solution was kept at 1.50 dS/m and the pH between 6.5-7. On the 15th day, shoot length, stem diameter, leaf number, leaf proportional water content, SPAD (leaf chlorophyll content), PAR (photosynthetic active radiation), shoot weights and root weights were measured in non-grafted and grafted plants. In order to determine the relative water content of the leaves, the fresh weight, turgor weight and dry weights of the samples taken from the 3rd and 4th leaves of the plants were calculated. For turgor weight, leaf samples were kept in water for 4 hours and weighed. For dry weight was determined after 48 hours at 65 °C. Leaf relative water content values were calculated with the formula "RWC=(Fresh Weight-Dry Weight)/(Turgor Weight-Dry Weight)x100". One-way ANOVA test was used to determine the statistical significance of the difference between the groups. The post-hoc Duncan test was used to compare the mean values of each group. All statistical analyzes were performed using the SPSS 16.0 package program.

RESULTS AND DISCUSSION

Shoot length (cm)

In grafted plants, mean shoot length values varied between 101.33±2.91 cm plant^{-1} and 174.53±1.18 cm plant^{-1}. Shoot length values in non-grafted plants were determined as 113.00±9.45 cm. It was concluded that some rootstock-scion grafting may be more effective in increasing shoot length. The significantly highest shoot length values were measured in Cremna-Minimix grafting. The significantly lowest shoot length values were measured in non-grafted plants and when Strong rootstock was used. In the case of Cremna, Devrim, Kubai and RS841 rootstocks, it was determined that the shoot length increased significantly compared to the non-grafted plants. The average shoot length values were measured as 136.45 cm plant^{-1} in all grafted plants, and 133.52 cm plant^{-1} in all applications with non-grafted plants (Table 1).

Shoot length is one of the most important parameters affecting yield. In optimum growing conditions, some rootstocks have advantages in terms of shoot length. In this study, shoot length values measured in four different rootstock applications (Cremna, Devrim, Kubai

Table 1. Shoot length values in different grafting combinations

	Mean±S.E.	Minimum	Maximum
Non-grafted	113.00±9.45 ef	99	131
TZ148-Minimix	119.33±1.07 e	117.6	121.3
Devrim-Minimix	156.33±5.04 b	149	166
Cremna-Minimix	174.53±1.18 a	172.5	176.6
Kubai-Minimix	146.37±1.27 bc	144.2	148.6
Strong-Minimix	101.33±2.91 f	96	106
RS841-Minimix	135.93±6.14 cd	124.6	145.7
Maximus-Minimix	121.33±6.39 de	111	133
Mean	133.52±5.00	96	176.6

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

Table 2. Stem diameter values in different grafting combinations

	Mean±S.E.	Minimum	Maximum
Non-grafted	5.14±0.01 d	5.12	5.17
TZ148-Minimix	3.66±0.01 f	3.64	3.67
Devrim-Minimix	5.72±0.01 a	5.68	5.74
Cremna-Minimix	5.48±0.02 b	5.45	5.51
Kubai-Minimix	5.70±0.06 a	5.61	5.86
Strong-Minimix	5.37±0.01 c	5.32	5.39
RS841-Minimix	3.63±0.01 f	3.58	3.64
Maximus-Minimix	4.94±0.01 e	4.89	4.96
Mean	4.96±0.17	3.58	5.86

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

and RS841) were higher than the non-grafted plants. In some studies, it has been determined that grafting is effective on the vegetative development of plants (Salehi-Mohammedi et al., 2009; Ulas, 2021). Similarly, in this study, shoot length values of some grafting applications were determined higher in the control application. Aktaş and Üre (2019) determined grafted on TZ148 plants produced significantly higher shoot lenght than non-grafted plants. amar and Solmaz (2020) determined in their study that non-grafted plants are in the shortest group. Similarly, in this study, non-grafted plants had lower shoot length values compared to some grafting combinations. The findings of this study are different from the findings of the study of Kacjan Maršić and Jakše (2010), who reported that the stem length of the cucumber was not affected by grafting in the hydroponic system. In this study, which was carried out in a hydroponic environment, a significant variation in shoot length was detected between grafted and non-grafted plants. Similar to the results of the study carried out by Heidari et al (2010) and Ban et al (2014), it was determined that the stem length of the stem increased in cucumbers grafted on some interspecies hybrid rootstocks. It is evaluated that different rootstock-scion combinations may produce different responses in terms of shoot length.

Stem diameter (mm)

Stem diameter values varied between 5.12 mm plant^{-1} and 5.17 mm plant^{-1} in non-grafted plants. The average of the stem diameter was 5.14 mm in non-grafted plants. Stem diameter values in grafted plants ranged from 3.58 mm plant^{-1} to 5.86 mm plant^{-1}, with an average of 4.93 mm plant^{-1}. An average of 4.96 mm plant^{-1} stem diameter values were obtained in all grafted and non-grafted plants. It was determined that some rootstock applications were higher than the non-grafted plants in terms of stem diameter values, while some were lower. The stem diameter values obtained in the combinations using Devrim and Kubai rootstocks are significantly higher than the non-grafted plants and the plants using other rootstocks (Table 2).

In grafted plants, the stem diameter depends on the rootstock variety (Kurum, 2010; Aktaş and Topçu, 2020). In this study, the mean stem diameter values were determined as 4.96 mm. The minimum and maximum values ranged from 3.58 mm to 5.86 mm, resulting in a wide variation. In this study, stem diameter increased significantly in Devrim and Kubai rootstocks. Damar and Solmaz (2020) found the stem diameter to be higher in the TZ148-Solo grafting combination than the non-grafted plants. In this study, the stem diameter values obtained using TZ148 and Maximus rootstocks were found to be lower than the non-grafted plants. It can be evaluated that the reason for the differences depends on the rootstock types used, and rootstock-scion interaction may cause significant differences.

Leaf Number

The number of leaves in non-grafted plants ranged from 8 to 12 LN plant^{-1}, with an average of 10.0 LN plant^{-1}. The

Table 3. Leaf number values in different grafting combinations

	Mean±S.E.	Minimum	Maximum
Non-grafted	10.0±0.58 c	8	12
TZ148-Minimix	14.2±0.57 b	13	15
Devrim-Minimix	20.3±0.88 a	19	22
Cremna-Minimix	15.6±1.12 b	13	17
Kubai-Minimix	15.1±1.11 b	13	17
Strong-Minimix	13.3±1.09 b	11	15
RS841-Minimix	15.0±1.15 b	13	17
Maximus-Minimix	12.7±0.58 bc	10	14
Mean	14.52±0.65	8	22

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

Table 4. Leaf relative water content values in different grafting combinations

	Mean±S.E.	Minimum	Maximum
Non-grafted	68.06±0.04 d	67.99	68.11
TZ148-Minimix	77.25±0.24 c	76.85	77.69
Devrim-Minimix	82.67±4.43 ab	75.18	90.51
Cremna-Minimix	76.23±0.01 c	76.23	76.23
Kubai-Minimix	86.77±0.28 a	86.23	87.18
Strong-Minimix	82.97±0.07 ab	82.86	83.11
RS841-Minimix	78.79±0.16 bc	78.48	79.01
Maximus-Minimix	83.29±0.19 ab	82.95	83.64
Mean	79.51±1.22	67.99	90.51

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

number of leaves in grafted plants varied between 10-22 LN plant^{-1} and the average was determined as 15.17 LN plant^{-1}. The number of leaves of all grafted and non-grafted plants was found to be between 8-22 LN plant^{-1} and an average of 14.52 LN plant^{-1}. The highest leaf number values (20.3±0.88) were obtained from Devrim-Minimix rootstock-scion combination. The leaf number values obtained in plants using Devrim rootstock were higher than both non-grafted plants and other rootstock applications. The leaf number values measured in non-grafted plants were lower than all graft combinations except the plants in which Maximus used as rootstock (Table 3).

Variation in the number of leaves is high in non-grafted plants, grafted plants and between different rootstock uses. The number of leaves detected in all rootstocks except the plants in which Maximus rootstock is used is higher than the non-grafted plants. Heidari et al. (2010) and Ban et al. (2014) determined that the use of hybrid rootstocks between species increased the leaf number values. Similar results were also found in this study. The increase in the number of leaves is directly proportional to the increase in the vegetative part and may cause high photosynthetic activity. In this case, it can related to yield and is advantageous in terms of hydrophonic culture.

Relative Water Content (RWC) (%)

The relative water content values of leaves in non-grafted plants were determined between 67.99% and 68.11% and an average of 68.06%. In the grafted plants, the relative water content of the leaves increased. The leaf relative water content values obtained as a result of all rootstock applications were statistically significantly higher than the non-grafted plants. Significant differences were also detected between different rootstock-scion combinations. Leaf relative water content values obtained in plants using Kubai rootstock are higher than TZ148, Cremna and RS841 rootstock applications (Table 4).

Leaf relative water content, which was determined as 79.51% on average, was measured at the lowest values in non-grafted plants. Although there were different results between rootstocks, the highest values were obtained in the use of Kubai rootstock.

Leaf chlorophyll content (SPAD) and Photosythetic Active Radiation (PAR) Values (µmol/m^2/s)

The SPAD values were found to be between 26.4-34.2 in non-grafted cucumber plants, with an average of 29.72±1.44. In grafted plants, these values showed a wider variation (14.5-37.7) and averaged 28.92. When all plants were examined, the SPAD averages were 28.77. Statistically, no significant differences were detected between grafted and non-grafted plants, but differences were detected between some rootstock applications. SPAD values obtained in plants applied Maximus, Strong and Kubai rootstocks were statistically significantly higher than TZ148 and Cremna rootstock applications

(Table 5).

The PAR values in grafted and non-grafted plants varied between 18.12-74.33 and were calculated as 46.33 on average. Average PAR values are 50.67 for non-grafted plants and 45.49 for grafted plants. The PAR values obtained in the Kubai-Minimix rootstock combinations among the graft combinations were found to be lower than the non-grafted plants. At the same time, the PAR values obtained from Devrim and Cremna rootstock applications are higher than the Kubai-Minimix combination. No statistically significant difference was found between the other groups (Table 5).

Table 5. SPAD and PAR values in different grafting combinations

	SPAD	PAR
Non-grafted	29.72±1.44 a-c	50.67±8.99 a
TZ148-Minimix	24.67±2.077 c	49.75±4.38 a
Devrim-Minimix	26.07±2.32 bc	48.83±5.09 a
Cremna-Minimix	24.6±1.6 c	49.67±3.63 a
Kubai-Minimix	30.6±2.02 ab	30.2±5.87 b
Strong-Minimix	32.23±1.12 a	46.5±3.30 ab
RS841-Minimix	30.02±2.41 a-c	46.3±6.77 ab
Maximus-Minimix	34.28±0.75 a	47.17±4.61 ab
Mean	28.77±0.79	46.33±2.06

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

Depending on the rootstock variety, the parameters affecting the photosynthesis metabolism, which have an important effect on plant growth and development, may change (Öztekin, 2009). In this study, SPAD and PAR analyzes were performed to determine photosynthetic activity. SPAD values have very close values in grafted and non-grafted plants. Although there are no statistically significant differences between grafted and non-grafted plants, it has been determined that some rootstocks have higher values than other rootstocks. It was determined that the PAR values determined in the non-grafted plants were not lower than the grafted plants, and even higher than the Kubai-Minimix grafting combinations. Uysal (2010) determined leaf chlorophyll contents in grafted and non-grafted cucumber plants and found that the difference between grafted and non-grafted plants was insignificant in terms of chlorophyll a, chlorophyll b and total chlorophyll. Similarly, in this study, the difference in SPAD values between grafted and non-grafted plants is insignificant. Differently, in this study, it was determined that SPAD values varied between different rootstocks. In this study, it is evaluated that the low variation between grafted and non-grafted plants in terms of photosynthetic parameters may be due to the absence of a stress cause. The difference between rootstocks is an indication that different rootstock-scion interactions can produce different responses under the same conditions.

Shoot Fresh Weight and Dry Weight (g plant^{-1})

The average of shoot fresh weight values of all plants were 43.81±2.37 g plant^{-1}, shoot dry weight values were 4.16±0.32 g plant^{-1}, and shoot dry weight/shoot fresh weight ratio was determined as 0.093±0.003 g plant^{-1}. Significantly higher results were obtained when rootstock was used in terms of shoot fresh weight values. A significant variation was also obtained among rootstocks, the highest shoot fresh weight values were obtained in Cremna and Devrim rootstock usage cases. Significantly higher results were obtained when rootstock was used in terms of shoot dry weight values. A significant variation was also obtained between rootstocks, the highest shoot dry weight values were obtained in the use cases of

Table 6. Shoot fresh-dry weight values in different grafting combinations

	Shoot Fresh Weight	Shoot Dry Weight	Dry W./ Fresh W.
Non-grafted	29.41±0.27 g	2.84±0.01 f	0.097±0.001 c
TZ148-Minimix	36.60±0.12 e	2.62±0.01 h	0.072±0.001 f
Devrim-Minimix	54.94±0.27 b	6.82±0.03 a	0.124±0.001 a
Cremna-Minimix	66.80±0.05 a	6.38±0.03 b	0.096±0.001 c
Kubai-Minimix	43.75±0.16 c	3.56±0.01 e	0.082±0.001 e
Strong-Minimix	44.12±0.17 c	4.34±0.02 c	0.098±0.001 b
RS841-Minimix	41.79±0.59 d	3.98±0.02 d	0.095±0.001 c
Maximus-Minimix	33.08±0.16 f	2.75±0.04 g	0.083±0.001 d
Mean	43.81±2.37	4.16±0.32	0.093±0.003

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

Devrim and Cremna rootstock. When the ratios of shoot dry weight to shoot fresh weight were examined, it was determined that non-grafted plants had higher values than some rootstock plants. The highest values were determined for Devrim rootstocks, the lowest values for TZ148 rootstocks (Table 6).

Table 7. Root fresh-dry weight values in different grafting combinations

	Root Fresh Weight	Root Dry Weight	Dry W./ Fresh W.
Non-grafted	7.98±0.01 h	0.92±0.01 b	0.115±0.001 a
TZ148-Minimix	8.92±0.01 g	0.72±0.01 c	0.081±0.002 b
Devrim-Minimix	14.58±0.12 d	1.23±0.01 a	0.084±0.001 b
Cremna-Minimix	16.85±0.02 c	1.20±0.06 a	0.071±0.003 c
Kubai-Minimix	20.47±0.08 a	1.20±0.03 a	0.059±0.001 d
Strong-Minimix	9.64±0.06 f	0.65±0.01 c	0.067±0.001 c
RS841-Minimix	18.00±0.27 b	0.99±0.02 b	0.055±0.001 d
Maximus-Minimix	10.61±0.16 e	0.50±0.02 d	0.047±0.001 e
Mean	13.38±0.92	0.93±0.05	0.073±0.004

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

Root Fresh Weight and Dry Weight (g)

Average root fresh weight values were 13.38±0.92 g plant^{-1}, root dry weight values were 0.93±0.05 g plant^{-1} and root dry weight/shoot fresh weight ratio was determined as 0.073±0.004 g plant^{-1} in all plants. Significantly higher results were obtained when rootstock was used in terms of root fresh weight values. A significant variation was also obtained between rootstocks, with the highest root fresh weight values obtained in the Kubai and RS841 rootstock use cases. In terms of root dry weight values, the values obtained in non-grafted plants were found to be higher than some rootstocks used. The lowest root dry weight values belong to Maximus-Minimix grafting combination. When the ratios of root dry weight to root fresh weight were examined, it was determined that non-grafted plants had higher values than all rootstocks used (Table 7).

Biomass values vary according to rootstocks. In terms of shoot weight measurements, all rootstock applications have higher values than non-grafted plants in terms of both fresh and dry weight values. In this study, shoot fresh and dry weights were determined by Yarşi et al. (2008) was found to be higher in grafted plants, similar to the our findings. In the study of Uysal (2010), while the highest stem fresh and dry weights were obtained in Maximus rootstock grafted plants, the highest shoot fresh and dry weights were obtained from other rootstocks in this study. The reason for the difference may be the difference in the type of scion used and the cultivation in the hydroponic environment. It has been determined in other studies that the biomass values increase with the use of rootstock, and plant growth is encouraged depending on the rootstock (Khah, 2005). Although biomass production is determined higher in grafted plants (Ulas et al., 2020; Ulas, 2021), the rate of change varies according to rootstocks (Öztekin, 2009). Similarly, in this study, it was concluded that rootstock-scion combinations were effective in determining biomass.

CONCLUSION

The values measured in at least one grafting application in 9 different morphological and/or physiological parameters among the 12 parameters measured were higher than the non-grafted plants. In SPAD, PAR and root dry weight/fresh weight ratio measurements, the values measured in non-grafted plants were not lower than grafted plants. In root dry weight/fresh weight measurements, the values measured in non-grafted plants are significantly higher than all grafted plant groups. This shows that grafting in cucumber has no significant effect on photosynthetic activity under optimum conditions, but it has an effect on other parameters. While no significant differences were detected between rootstocks in some parameters, significant differences were detected in some parameters. TZ148 was in the group with the highest measurements in one parameter (PAR), RS841 in two parameters (SPAD and PAR), Maximus in three parameters (RWC, SPAD, PAR), Strong in three parameters (RWC, SPAD, PAR). Cremna rootstock in four parameters (shoot length, PAR, shoot fresh weight and root dry weight), Kubai in five parameters (stem diameter, RWC, SPAD, root fresh weight and root dry weight), and Devrim rootstock in seven parameters (stem diameter, number of leaves, RWC, PAR, shoot dry weight, shoot dry weight/fresh weight and root dry weight) was in the group with the highest measurements. It was concluded that Cremna and Devrim rootstocks stand out in terms of shoot length and biomass values, and rootstock use did not have a significant effect in terms of photosynthetic activity.

It is important to take measures to minimize the risks of factors that adversely affect soil, vegetation, water and atmosphere. Grafting and hydroponic media culture in vegetables are environmentally friendly applications that can be used for this purpose. Due to the benefits of using grafted seedlings, the demand for high quality

grafted seedlings is increasing. In grafting technique, it is important to select the appropriate rootstock and scion for superior yield and quality. In addition, rootstock-scion interactions and growing environment factors affect the yield and quality of grafted vegetables. In this study, it was determined that grafting of cucumber plants on different rootstocks had a positive effect on some morphological and physiological parameters in hydroponic medium cultivation, but the effects could vary greatly with rootstock selection. In order to better understand the efficacy of grafted species of the *Cucurbitaceae* family, a scientific approach to genomics, proteomics and/or metabolomics can be performed in addition to the current study.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., Rehman, M.Z., Irshad, M.K., and Bharwana, S.A. (2015). The Effect of Excess Copper on Growth and Physiology of Important Food Crops: A Review. Environmental Science and Pollution Research, 2, 8148-8162.

Aktaş, H., ve Topçu, T. (2020). Domateste Kullanılan Farklı Anaçların Bitki Büyümesi, Verim ve Meyve Kalitesi Üzerine Etkilerinin Belirlenmesi. Ziraat Fakültesi Dergisi, 15(1), 27-40. (in Turkish)

Aktaş, H., ve Üre, H.S. (2019). Farklı Anaçlar Üzerine Aşılamanın Hıyarlarda Bitki Büyümesi, Verim ve Kalite Üzerine Etkileri. Türk Bilim ve Mühendislik Dergisi, 1(1), 17-22. (in Turkish)

Ali, S., Farooq, M.A., Yasmeen, T., Hussain, S., Arif, M.S., Abbas, F., Bharwana, S.A., and Zhang, G.P. (2013). The Influence of Silicon on Barley Growth, Photosynthesis and Ultra- Structure Under Chromium Stress. Ecotoxicology and Environmental Safety, 89, 66-72.

Balkaya, A. (2014). Aşılı Sebze Üretiminde Kullanılan Anaçlar. TÜRKTOB Türkiye Tohumcular Birliği Dergisi, 3(10): 4-7. (in Turkish)

Ban, S.G., Žanić, K., Dumičić, G., Raspudić, E., Selak, G.V., and Ban, D. (2014). Growth and Yield of Grafted Cucumbers in Soil Infested with Root-Knot Nematodes. Chilean Journal of Agricultural Research, 74: 29-34.

Colla, G., Suãrez, C.M.C., Cardarelli, M., and Rouphael, Y. (2010). Improving Nitrogen Use Efficiency in Melon by Grafting. HortScience, 45, 559–565.

Colla, G., Rouphael, Y., Rea, E., and Cardarelli, M., (2012). Grafting Cucumber Plants Enhance Tolerance to Sodium Chloride and Sulfate Salinization. Scientia Horticulturae, 135: 177–185.

Coşkun, Ö.F., Toprak, S., Aydın, A., Başak, H., ve Mavi, K. 2022. Tuz Stresi Koşullarında Hıyarın Bazı Büyüme Parametreleri Üzerinde Farklı Anaçların Etkinliğinin Belirlenmesi. Presented at the XIII. Sebze Tarımı Sempozyumu, İzmir. (in Turkish)

Damar, E., ve Solmaz, İ. (2020). Farklı Anaçların Aşılı Hıyarlarda (*Cucumis sativus* L.) Bitki Gelişimi, Verim ve Meyve Özelliklerine Etkileri. Çukurova J. Agric. Food Sci., 35(2): 99-106.

FAO, (2020). World cucumber production list. http://www.fao.org/faostat/en/#data/QC. Access date: 06.03.2022.

Günay, B. (2011). Menderes İlçesinde Sera Hıyar Yetiştiriciliğinde Aşılı Fide Kullanımının Etkileri. Ege Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi. (in Turkish)

Güngör, B., ve Balkaya, A. (2016). Yerli Kabak Anaç Çeşit Adaylarının Aşılı Mini Karpuzun Vejetatif Büyümesi Üzerine Kantitatif Etkilerinin İncelenmesi. Bahçe Özel Sayı, VII. Ulusal Bahçe Bitkileri Kongresi Bildirileri, 2: 21-26. (in Turkish)

Heidari, A.A., Kashi, A., Saffari, Z., and Kalatejari, S. (2010). Effect of Different Cucurbita Tootstocks on Survival Rate, Yield and Quality of Greenhouse Cucumber cv. Khassib. Plant Ecophysiology, 2:115-120.

Kacjan Marsic, N., and Jakse, M. (2010). Growth and Yield of Grafted Cucumber (*Cucumis sativus* L.) on Different Soilless Substrates. Journal of Food, Agriculture & Environment, 8:654-658.

Karaağaç, O., Balkaya, A., ve Kafkas, N.E. (2018). Karpuzda (*Citrullus lanatus*) Meyve Kalitesi ve Aroma Özellikleri Üzerine Anaçların Etkisi. Anadolu Tarım Bilimleri Dergisi, 33: 92-104. (in Turkish)

Keller, C., Rizwan, M., Davidian, J.C., Pokrovsky, O.S., Bovet, N., Chaurand, P., and Meunier, J.D. (2015). Effect of Silicon on Wheat Seedlings (*Triticum turgidum* L.) Grown in Hydroponics Under Cu Stress. Planta, 241, 847-860.

Khah, E.M. (2005). Effects Grafting on Growth, Performance and Yield of Aubergine (*Solanum melongena* L.) in the Field and Greenhouse. Journal of Food, Agriculture & Environment, 3:92-94.

Kurum, R. (2010). Hıyar (Cucumis sativus L.) Yetiştiriciliğinde Farklı Anaç/Çeşit Kombinasyonlarının Bitki Gelişimi, Verim ve Bitki Besin Elementleri Kapsamları Üzerine Etkilerinin Araştırılması. Süleyman Demirel

Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi. (in Turkish)

Martínez-Ballesta, M.C., Alcaraz-López, C., Muries, B., Mota-Cadenas, C., and Carvajal, M. (2010). Physiological Aspects of Rootstock–Scion Interactions. Scientia Horticulturae,127, 112–118.

Öztekin, G.B. (2009). Aşılı Domates Bitkilerinde Tuz Stresine Karşı Anaçların Etkisi. Ege Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi. (in Turkish)

Rizwan, M., Meunier, J.D., Davidian, J.C., Pokrovsky, O.S., Bovet, N., and Keller, C. (2015). Silicon Alleviates Cd Stress of Wheat Seedlings (*Triticum turgidum* L. cv. Claudio) Grown in Hydroponics. Environmental Science and Pollution Research, 23(2):1414-27.

Sabatino, L., Ntatsi, G., Iapichino, G., D'Anna, F., and De Pasquale, C. (2019). Effect of Selenium Enrichment and Type of Application on Yield, Functional Quality and Mineral Composition of Curly Endive Grown in a Hydroponic System. Agronomy, 9, 207.

Salehi-Mohammadi, R., Kashi, A., Lee, S.Y., Huh, YC., Lee, J.M., Babalar, M., and Delshad, M. (2009). Assessing The Survival and Growth Performance of Iranian Melon to Grafting on to Cucurbita Rootstocks. Korean Journal of Horticultural Science & Technology, 27(1), 1-6.

Singh, H., Kumar, P., Kumar, A., Kyriacou, M.C., Colla, G., and Rouphael, Y. (2020). Grafting Tomato as a Tool to Improve Salt Tolerance. Agronomy, 10, 263.

Turhan, A., Ozmen, N., Serbeci, M.S., and Seniz, V. (2011). Effects of Grafting on Different Rootstocks on Tomato Fruit Yield and Quality. Scientia Horticulturae, 38, 142-149.

Ulas, A., Aydin, A., Ulas, F., Yetisir, H., and Miano, T.F. (2020). Cucurbita Rootstocks Improve Salt Tolerance of Melon Scions by Inducing Physiological, Biochemical and Nutritional Responses. Horticulturae, 6, 66.

Ulas, F. (2021). Effects of Grafting on Growth, Root Morphology and Leaf Physiology of Pepino (*Solanum muricatum* Ait.) as Affected by Salt Stress under Hydroponic Conditions. International Journal of Agriculture, Environment and Food Sciences, 5(2):203-212.

Ulas, F., Aydın, A., Ulas, A., and Yetişir, H. (2021). The Efficacy of Grafting on Alkali StressedWatermelon Cultivars Under Hydroponic Conditions. Gesunde Pflanzen, 73:345–357.

Uysal, N. (2010). Farklı Anaçların Sera Hıyar Yetiştiriciliğinde Bitki Gelişimi, Verim ve Meyve Kalitesine Etkileri. Ege Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi. (in Turkish)

Vural, H., Eşiyok, D., ve Duman, İ. (2000). Kültür Sebzeleri (Sebze Yetiştirme). E.Ü.Ziraat Fakültesi Bahçe Bitkileri Bölümü, E.Ü Basımevi, s: 440, Bornova. (in Turkish)

Yarşi, G., Rad, S., ve Çelik, Y. (2008). Farklı Anaçların Kybele F1 Hıyar Çeşidinde Verim, Kalite ve Bitki Gelişimine Etkisi. Akdeniz Üniversitesiz Ziraat Fakültesi Dergisi, 21(1), 27-34. (in Turkish)

Yetişir, H., Yarsi, G., ve Sarı, N. (2004). Sebzelerde Aşılama. Bahçe, 33 (1- 2): 27- 37. (in Turkish)

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 171-181
DOI: https://doi.org/10.31015/jaefs.2023.1.21

Subscale mapping of animal waste-based biogas potential and its equivalent energies using GIS: Canakkale, Türkiye

Melis Inalpulat[1]

[1] Computer-Agriculture-Environment-Planning (ComAgEnPlan) Study Group M.- Agricultural Remote Sensing Laboratory (AGRESEL), Department of Agricultural Structures and Irrigation, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye

Citation: Inalpulat, M. (2023). Subscale mapping of animal waste-based biogas potential and its equivalent energies using GIS: Canakkale, Türkiye. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 171-181

Received: 29 November 2022
Revised: 23 January 20223
Accepted: 26 January 2023
Published Online: 21 March 2023

Correspondence: Melis Inalpulat
E-mail: melissacan@comu.edu.tr

Abstract

The study presents the first attempt of determination and mapping of recent biogas potentials (BP) at different scales from province to village level in Çanakkale using Geographic Information Systems (GIS). The BP of different scales was calculated based on animal waste amounts from bovine, ovine and poultry farming. The study area covers the ten districts of Çanakkale province with the exception of Imbros and Tenedos Islands. The inventory records of different animal types were obtained from of Republic of Turkey Ministry of Agriculture and Forestry Çanakkale Directorate of Provincial Agriculture and Forestry. GIS procedures are conducted in ArcGIS (10.3) software. Findings revealed that the annual biogas production potential of the whole province is almost 6.4×10^7 m^3. Biga district seemed to include 39 % of overall BP whereas Eceabat district presented a slight percentage of the potential production with the value of approximately 1 %. Moreover, the highest and lowest subscale-level potentials have found in Yukarıdemirci (Biga) and Bahçedere (Ayvacık), with approximately 154×10^4 m^3 and 137 m^3 BP, respectively. The overall BP of the province have concluded to be promising, and present study believed to serve as a baseline for future studies related to determination of new biogas plant suitable lands.

Keywords: Animal waste, Biogas potential, Çanakkale, GIS, Mapping, Subscale level

INTRODUCTION

The worldwide rapid population growth together with improvements in the living standards of people has led to decline in available resources. Among these resources, one of the most important requirements of growing population is majored on energy sources such as fossil fuels, which have also several adverse effects on the environment in addition to being depletable. The continuous processes have triggered the consumption of currently insufficient sources. Moreover, the global energy consumption is predicted to be increased by approximately 30 % in the next two decades (Gülşen-Akbay, 2020). As it is cited in Ignaciuk and Sulewski (2021), initiatives such as clean energy and sustainable agriculture, plays a key role for promoting assumptions in European Green Dealt strategy, which intends use of environment-friendly approaches, and decrease of emissions from generation and use of energy (European Comission, 2019). The same report has denoted that one of the most important challenges in application of European Green Deal is considered as energy transformation since over 75 % of greenhouse gas emissions known to be sourced from energy sector. Therefore, the search for different energies instead of depletable ones, particularly, renewable sources has accelerated (Atelge et. al., 2020). Agricultural production has great importance and potential in terms of providing substance

for such kind of energy. Particularly, procurement of different fuel types from agricultural biomass, which are mainly assigned to biogas production, seems significant for increasing renewable energy sources (Mirosz et al., 2015). The importance of biogas energy has arised from different points of view in terms of environment and bioeconomy, since it presents an environmental-friendly and relatively economic alternative due to related investments within in the last 20 years in different countries.

Acquisition of biogas is dependent to anaerobic decomposition of organic components in an oxygen-free medium and with existence of diverse microorganisms (Yılmaz and Gonbe, 2021). Moreover, as it is cited in Yılmaz and Gonbe (2021), biogas is referred as converted energy due to the acquirement procedure, whereas it can be obtained from not only agricultural biomass from plants and animals, but also domestic or industrial wastes (Şenol et. al, 2017; Demir-Yetiş et. al., 2019). In agricultural point of view, Turkey has significant potential of animal wastes that can be obtained from great number of different types of animals according to 2021 records of Turkish Statistical Institute (TUİK) (Figure 1) (TUİK, 2021), and Turkey known to be standing within the first twenty countries in European Continent with respect to number of installed biogas plants. However, even though there are numerous biogas plants in different regions of the country, it was denoted that there is an enormous amount of plant and animal wastes are preferred to be used as fertilizers or directly burned (Nacar-Koçer et. al, 2006; Atılgan et. al, 2021). Utilization of these amounts for biogas production would prevent the negative effects, namely, odors, diseases and different kind of pollutions, while conserving natural resources Erdal et. al, 2007; İnci et al., 2016). On the other hand, along with the total capacities of the constructed biogas plants, Marmara region takes the first place by composing approximately 40 % of total installed power (Anonymous, 2021). Rapid and reliable evaluation of the needs for new biogas plants together with determination of their locations has enabled by Geographic Information Systems (GIS), which have long been successfully used in the solutions of diverse environmental and ecological problems, as well as management and planning strategies (Bharti et al., 2021).

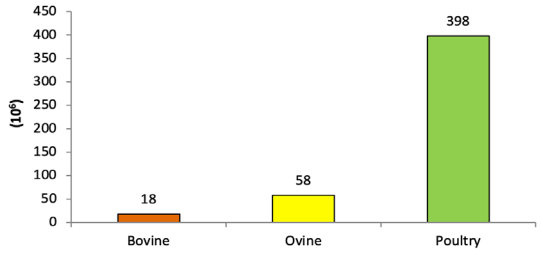


Figure 1. Number of different types of animals (NoA) in Turkey according to 2021 year TÜİK reports.

Previous studies have dealt with determination of biogas potentials (BP) from different types of animals in national (Onurbaş-Avcıoğlu and Türker, 2012; Ertop et. al., 2022), and regional (Ayhan, 2016; Tınmaz-Köse, 2017; Akyürek and Coşkun, 2019) levels in Turkey using the NoA inventories of different year. Moreover, there are numerous studies conducted in local level in different regions of our country. For instance; a study was conducted in Adıyaman province by Baran et. al (2017) to determine animal waste energy potential; Polat-Bulut and Topal-Canbaz (2019) identified the BP for Sivas province, and energy potential of Samsun province from animal manure has been studied by Zenk (2019) . Furthermore, there is a limited number of studies that was conducted in Çanakkale Province (Ilgar, 2016; Özpınar, 2018), which is located within in the Marmara Region that remarked to involve major part of presently installed power. However, mentioned studies have investigated the BP of Çanakkale at upper scales of province and district level, respectively.

The study presents the first attempt of evaluation and mapping animal waste sourced BP of Çanakkale Province from province to subscale level by integrating town- and village-based inventory records into GIS, whereby up-to-date calculations were also served at district level. It was aimed to determine and designate the BP in different scales considering bovine, ovine, and poultry productions. Investigation of BP in smaller scales believed to give more precise results and may provide a strong basis particularly for site-selection studies of new biogas plant locations since determination of optimal distances to main production centers plays a key role in such studies. Moreover, differently from many biogas studies conducted in our country, use of GIS tools enabled visualization of potential production amounts at sub-scale level for manifestation of the production hotspots, whereby the production maps can be easily updated in regular intervals in respect to data from the latest reports or databases in the future.

MATERIALS AND METHODS

Study area

The study area is consisted of ten district of Çanakkale province including; the Provincial Center (PC), Ayvacık, Bayramiç, Biga, Çan, Eceabat, Ezine, Gelibolu, Lapseki, and Yenice. The province is located between the coordinates of 25° 40´- 27° 30´ E and 39° 27´- 40° 45 N. The survey area of the province is approximately 9.5 km^2 with the exceptions of Imbros and Tenedos islands. Moreover, there are over 500 subscales of townships and villages within the study area. Figure 2 represents the locations of the districts and the belonging subscales within Çanakkale Province, and Turkey. The area is mainly covered by forests, and it is followed by agricultural lands, where agricultural production has great importance for the local and regional economy since the climate, soil

and topographic conditions are highly suitable. Among the different agricultural activities, animal production is known to be one of the most significant economic incomes for the area, whereby Ezine cheese presents probably the most popular product. However, the statistical reports that the majority of animal production is located in Biga district, which is located in the North-eastern part of the study area.

types, respectively. Furthermore, among these waste productions, utilizable part (UM) of each farming type denoted to be 65 %, 13 %, and 99 %, with the same order. Finally, the BP productions were calculated depending on the assumption that demonstrates 33 m^3, 58 m^3, and 78 m^3 of BP can be obtained per ton of utilizable manure (UMBP) from bovine to poultry farming type (Kaya et al., 2005; Altıkat and Çelik, 2012). The equations

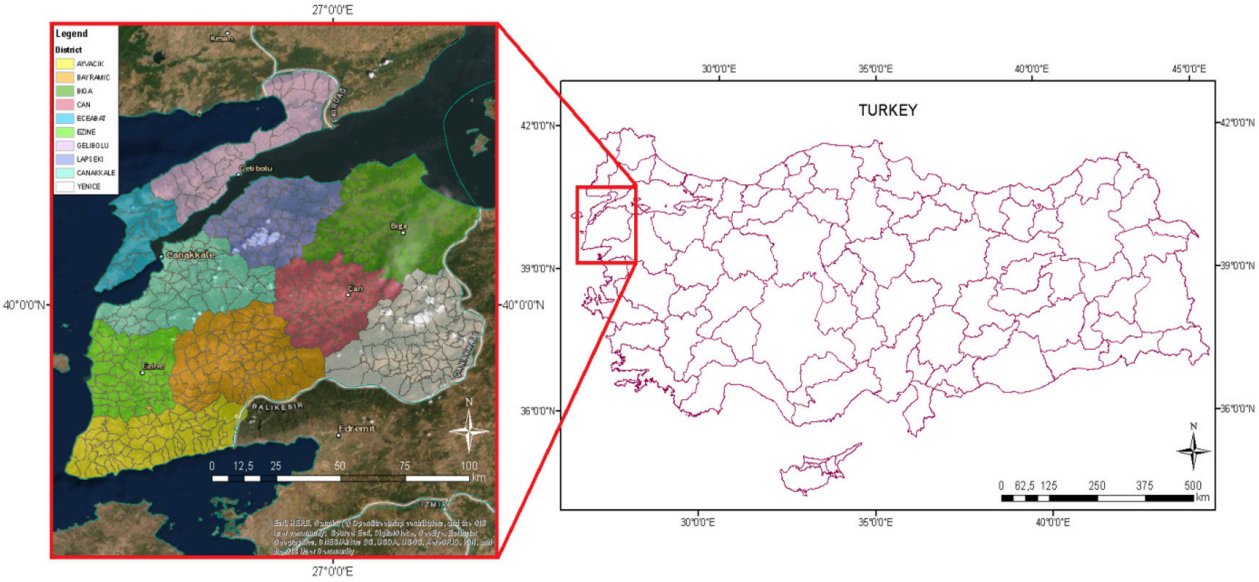


Figure 2. District-based locations of the subscales in Çanakkale and Turkey

Inventory of animal counts

Inventory records covering the number of animals in bovine, ovine and poultry farming types of all subscales are obtained from Republic of Turkey Ministry of Agriculture and Forestry Çanakkale Provincial Directorate in 2020 for preceding year of 2019, which integrated to attribute tables of created GIS layers latter.

Determination steps of BP

As it was reported by Salihoğlu et. al. (2019), BP can be calculated using different assumptions and constants depending on the formulations in the literature. Using different aspects and formulations would result in varied amounts of obtainable and utilizable manure, and thus, different BPs. In this study, the calculation procedures of Agro-Waste project have adopted. Identification of BP regarding to Agro-Waste Project includes implementation of three main steps, as it was explained in Kaya et al. (2005). The first step presents determination of hypothetically produced manure from different animal types, whereas the second step designates the calculation of utilizable manure amounts dependently to hypothetically produced manure. On this account, some assumptions were considered on prediction of these manure amounts (Table 1). Accordingly, 9.94 ton, 0.82 ton, and 0.029 ton wastes (PM) per year are assumed to be produced by bovine, ovine and poultry farming

implemented to each rural settlement for calculating total BP from bovine, ovine, poultry, and are given below (Equation 1-4).

$$\sum BP_{Bovine}(m^3 year^{-1}) = NoA_{Bovine} \times UM_{Bovine} \times UMBP_{Bovine} \quad \text{(Eq. 1)}$$

$$\sum BP_{Ovine}(m^3 year^{-1}) = NoA_{Ovine} \times UM_{Ovine} \times UMBP_{Ovine} \quad \text{(Eq. 2)}$$

$$\sum BP_{Poultry}(m^3 year^{-1}) = NoA_{Poultry} \times UM_{Ovine} \times UMBP_{Poultry} \quad \text{(Eq. 3)}$$

$$\sum BP_{Total}(m^3 year^{-1}) = \sum BP_{Bovine} + \sum BP_{Ovine} + \sum BP_{Poultry} \quad \text{(Eq. 4)}$$

Determination of equivalent energies for BP

Subsequent to determination of BP, the equivalents of biogas (m^3) in terms of fire wood (FW) (kg), gas oil (GO) (L), butane (B) (kg), electric (E) (kW h^{-1}), gasoline (G) (L), and coil (C) (kg) were calculated using the values given below (Table 2) (Akbulut and Dikici, 2004; Gümüşçü and Uyanık, 2010; Lüle, 2019).

RESULTS AND DISCUSSION

Distribution of animals in different farming systems

The inventory records have revealed that the poultry farming composes the majority of farming systems with 88 % of the total number of animals (NoA) within the province, and it is followed by ovine (9 %) and bovine (3 %) type, respectively (Figure 3). Moreover, district level distribution of total NoA, NoA_{bovine}, NoA_{ovine}, and $NoA_{poultry}$

Table 1. Considered assumptions for BP calculation.

Assumption	Bovine	Ovine	Poultry
Potential Manure (PM) (ton year^{-1})	9.94	0.82	0.029
Utilizable Manure (UM) Constant	0.65	0.13	0.99
Utilizable Manure (UM) (ton year^{-1})	6.4610	0.1066	0.0287
Unit UM BP (UMBP) (m^3 year^{-1})	33	58	78

Table 2. Equivalent values of different energy sources.

BP (m^3)	FW (kg)	GO (L)	B (kg)	E (kW h^{-1})	G (L)	C (kg)
1	3.47	0.63	0.43	4.7	0.8	1.46

types can be seen on Figure 4 a-d. Depending on Figure 4a, b and d, it was again emphasized that Biga district have a great part of total number of animals, together with bovine and poultry, whereby the highest number of ovine type is found in Ezine district (Figure 4c). The lowest NoA from all farming types have obtained from Eceabat. In fact, this situation is expected due to relatively small survey area of the district, together with the limitations arised from protection of cultural and historical heritage of the area.

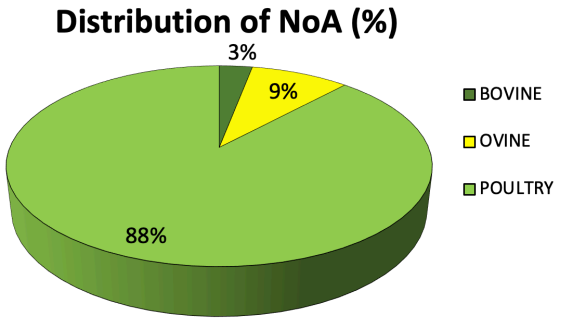


Figure 3. Percent distribution of NoA in respect to different farming systems.

Total BP of Çanakkale province

The previous studies have revealed the BPs of different years have ranged between 9.7×10^7 m^3 and 1.8×10^7 m^3 respectively by Ilgar (2016) and Özpınar (2018). The discrepancies have arised from two main reasons in these studies. Primarily, selection of different calculation methods have resulted in amended results, and secondly, consideration of less animal types and distinguishing ages of different animals led to variations in BP. The total BP of whole province was calculated as 6.4×10^7 m^3 in present study, and it was more consistent with the study conducted in 2016. The difference mainly occurred due to changes in the NoA in years, together with disregarding of the BPs in Imbros and Tenedos islands. The calculated BP is found to be respectable in comparison with different studies. The contribution of bovine, ovine and poultry farming systems to BP is another important parameter for such studies. Thereby, the amounts of BP from different farming types are given in Figure 5 in percentages (%). Accordingly, the major part of BP found to be obtained from bovine farming (71.8 %) within the whole province while the ovine and poultry have 6.2 % and 22.1 % of total potential, respectively. Even though the NoA in the ovine farming were higher than bovine, the highest BP found to be obtained from bovine farming and it was followed by poultry. The situation has arised due to the fact that the utilizable part of produced manure of ovine farming was significantly lower with a value of 13 % when compared to bovine (65 %) and poultry farming (99 %).

Moreover, the province level BP for 2020 and 2021 years were also calculated considering more recent NoA from TÜİK reports to show the latest status in the whole area due to the fact that data of 2022 is not available in the TÜİK database yet, since the sub-scale level data was covering 2019 inventory records. It was seen that total BP was 6.8×10^7 in 2020 and 6.9×10^7 in 2021. The NoA_{Bovine}, NoA_{Ovine}, and $NoA_{Poultry}$ were given as 221235, 784664 and 7030701 for 2020, and 222691, 850466, 7121413 for 2021 years, respectively. The portions of bovine, ovine, and poultry farming within total BP were calculated as 69.6 %, 7.2 %, and 23.2 % for 2020, and 69.1 %, 7.7 %, and 23.2% for 2021 years.

Equivalents of province-level BP

The equivalents of the BP in other energy sources are given in Figure 6. Dependently, it was seen that highest annual value has obtained for electrical energy with a value of over 300×10^6 kwh^{-1}. Conversely, the lowest equivalent value is calculated for butane as approximately 28×10^6 kg year^{-1}.The findings are important in terms of further calculations for cost savings on energy consumptions. On this account, a study was conducted by Demir-Yetiş et. al (2019) in Bitlis Province and it was denoted that the highest and lowest savings could be obtained from GO (L) and natural gas (m^3), respectively, considering the economic conditions and energy costs in 2017. Additionally, the equivalent values for 2020 and 2021 years are calculated and given in Table 3.

Table 3. The equivalents of BP in other energy sources based on 2020 and 2021 TUİK records

Year	BP (m^3)	FW (kg)	GO (L)	B (kg)	E ($kW\ h^{-1}$)	G (L)	C (kg)
2020	68×10^6	235×10^6	43×10^6	29×10^6	319×10^6	54×10^6	99×10^6
2021	69×10^6	238×10^6	43×10^6	30×10^6	323×10^6	55×10^6	100×10^6

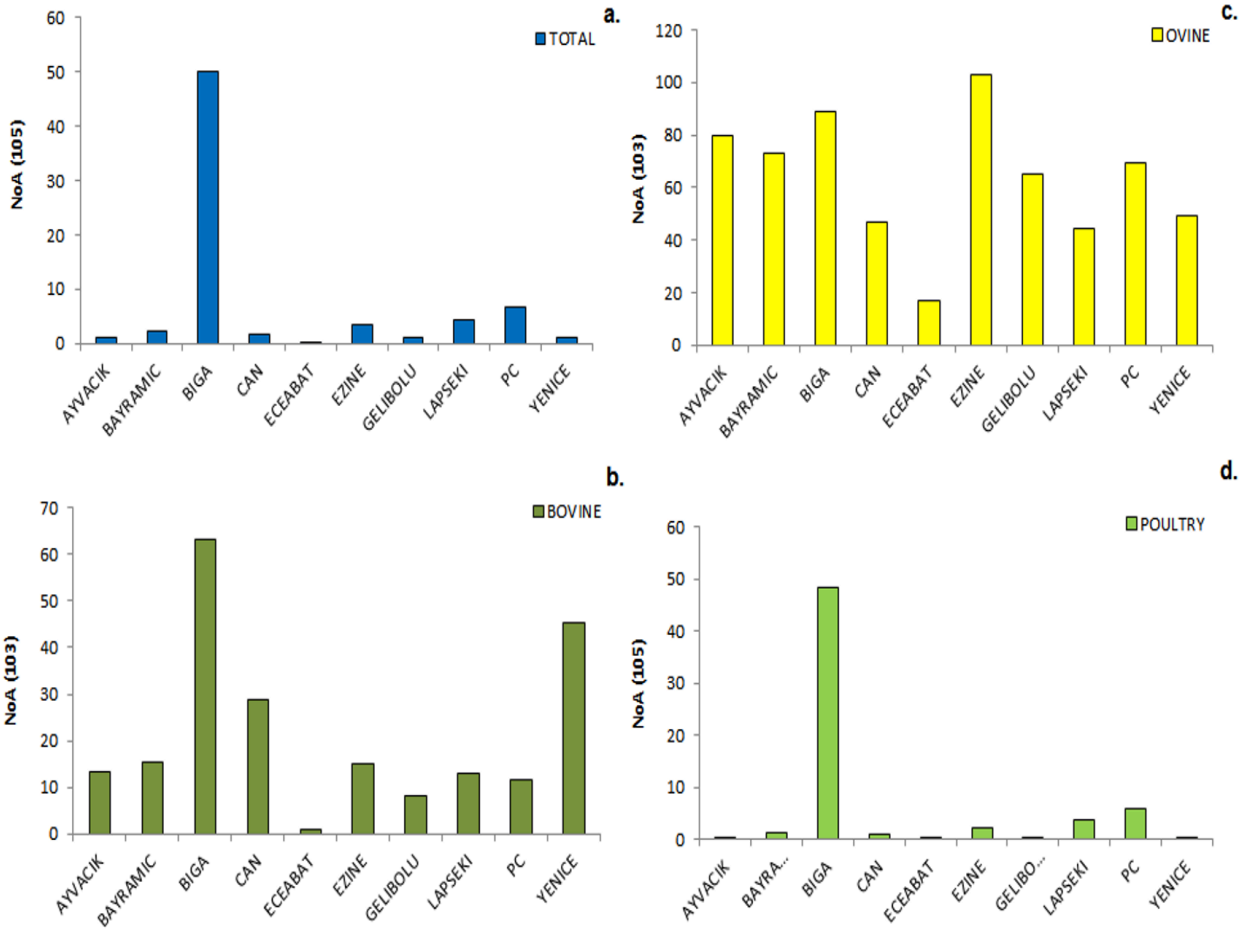


Figure 4. a. NoA_{Total}, b. district-level NoA_{Bovine}, c. district-level NoA_{Ovine}, d. district-level $NoA_{Poulrty}$ farming.

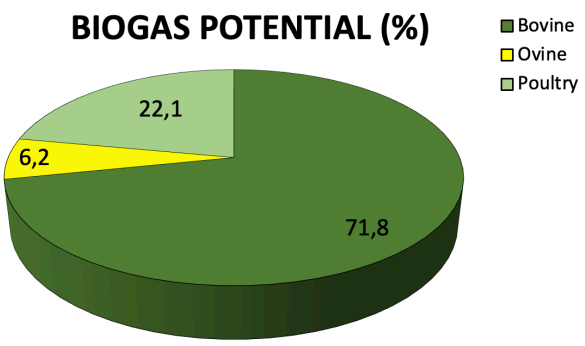


Figure 5. Distribution of total BP from bovine, ovine and poultry farming

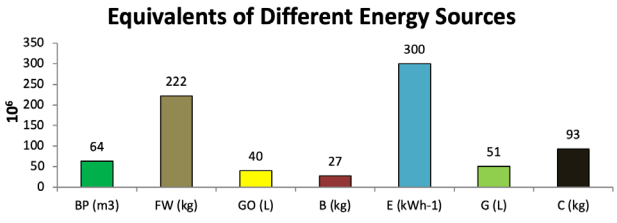


Figure 6. The equivalents of total BP in different energy sources.

District-level BP

The district based distribution of total BP (%) can be seen on Figure 7. Findings have revealed that Biga district owns the highest BP with 39 % of the total potential of

whole province, and it was followed by Yenice district with a value of 15.7 % while the lowest potential found to be obtained from Eceabat district. The respectably high proportion of Biga district in the whole province BP was an expected result, which is directly sourced from comparatively higher NoA with the exception of the relatively small difference in NoA_{ovine} of Ezine district. Similarly, the situation in Eceabat was inevitable since the district included the least NoA in terms of all farming types.

The BP values of districts were investigated dependent to farming types (Figure 8). Accordingly, BP of each district was dominantly obtained from bovine farming, and it was generally followed by ovine farming except Biga, Lapseki and Provincial Centre (PC), where the poultry farming sourced BP was higher than the BP can be obtained from ovine farming.

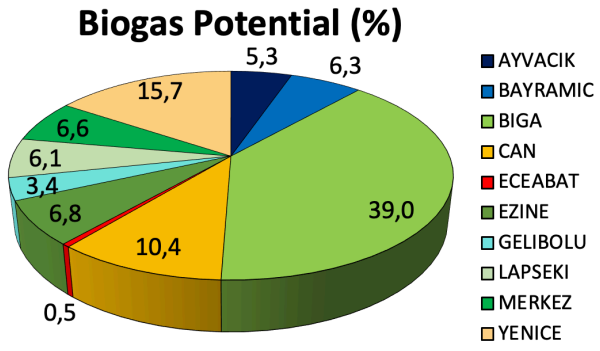


Figure 7. Proportions of total BP according to districts.

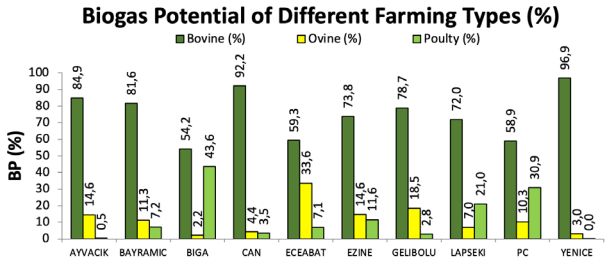


Figure 8. District level distribution of BP in terms of farming type.

Equivalents of district-level BP

The equivalents of district-level BPs can be seen on Figure 9. The distribution patterns of both potential biogas and its equivalents are similar to each other whereby highest potentials can be obtained from Biga district and corresponds to highest amount of electrical energy in all districts.

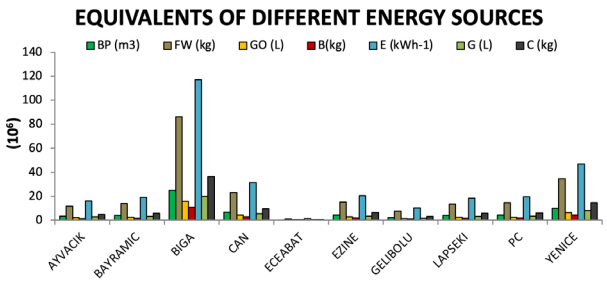


Figure 9. District-level amounts of equivalences for different energy source of BP.

Mapping distribution of subscale BP

Although, there are a few studies on BP mapping, they represented province or district potentials, for instance; Aybek et. al. (2015), and Atılgan et. al. (2020). Thence, present study differs from the previous researches conducted in other regions of Turkey. Also, it should be noticed that the records of two settlements located in the Southern part of Yenice district have included missing values, and thus, they were excluded and not considered within the frame of the study. The subscale distribution of BP can be seen on the BP map given in Figure 10. As it can be seen from the figure, majority of the subscales with higher BP is found in North-eastern part of the study area, where Biga district is located. The highest and lowest BP of each district is given in Table 4. Depending on the findings of the study, it was seen that the lowest BPs of different districts were ranged between 136.6 m^3 and 8336.0 m^3. Likewise, the highest BPs of districts has taken values between 99027.1 m^3 and 1543904.0 m^3. Namely, the lowest BP value can be obtained from Bahçedere village of Ayvacık district whereas the highest BP have found in Yukarıdemirci village of Biga. Accordingly, these values correspond to 0.0002 % and 2.4 % of total BP. Moreover the maximum value of lowest BP that can be obtained in different districts was from Güzeloba of Yenice district. On the other hand, the minimum value of highest BP has calculated as for Central Eceabat.

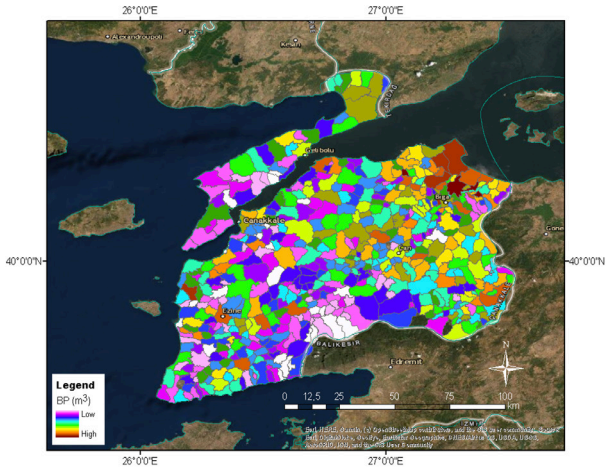


Figure 10. Digital map for distribution of subscale-level BP.

Table 4. Highest and lowest BP from the subscales of the districts.

	Lowest BP		Highest BP	
DISTRICT	**Subscale**	**BP (m^3)**	**Subscale**	**BP (m^3)**
AYVACIK	Bahçedere	136.6	Center	535614.4
BAYRAMİÇ	Osmaniye	1653.7	Türkmenli	574535.8
BİGA	Ilıcabaşı	5141.4	Yukaridemirci	1543904.0
CAN	Kazabat	6099.3	Yaykın	503326.4
ECEABAT	Bigalı	4834.0	Center	99027.1
EZİNE	Bozköy	474.8	Üvecik	737833.3
GELİBOLU	Burhanlı	2915.2	Yeniköy	192696.9
LAPSEKİ	Sındal	1658.9	Çardak	524953.7
PC	Güzelyalı	353.8	Gökçalı	344961.2
YENİCE	Güzeloba	8336.0	Pazarköy	474951.1

Table 5. The different energy equivalents of lowest BP from each district.

DISTRICT	Subscale	FW (kg)	GO (L)	B (kg)	E (kWh^{-1})	G (L)
AYVACIK	Bahçedere	474.0	86.1	58.7	642.0	109.3
BAYRAMİÇ	Osmaniye	5738.3	1041.8	711.1	7772.3	1322.9
BİGA	Ilıcabaşı	17840.8	3239.1	2210.8	24164.7	4113.2
CAN	Kazabat	21164.7	3842.6	2622.7	28666.9	4879.5
ECEABAT	Bigalı	16774.0	3045.4	2078.6	22719.9	3867.2
EZİNE	Bozköy	1647.4	299.0	204.2	2231.4	379.8
GELİBOLU	Burhanlı	10115.6	1836.6	1253.5	13701.2	2332.1
LAPSEKİ	Sindal	5756.2	1045.1	713.3	7796.6	1327.1
PC	Güzelyalı	1227.8	222.9	152.1	1663.0	283.1
YENİCE	Güzeloba	28926.1	5251.7	3584.5	39179.4	6668.8

Investigation of BP in smaller scales has several advantages, especially for selection of convenient sites using coordinates and their energy values. Such kind of analysis may be conducted by undertaking different approaches ranging from consideration of accumulated values for upper-scales to accounting of individual inclusions of each singular establishment. In this context, a study was conducted in Düzce province by Yürük and Erdoğmuş (2015) to determine optimum biogas plant locations within eight districts using coordinates of poultry farming establishments via k-means clustering algorithym. In that study, it was reported that the capacities of the establishments should be considered in addition to their proximities to obtain more precise results. Therefore, the findings of present study seemed to provide valuable information for further evaluations on selection of possible biogas plant locations using appropriate statistical analysis since the capacities are considered together with boundaries of subscales. Moreover, the advancements in BP can be captured annually by updating NoA data at the end of each year. Although there is currently biogas plant within the area, integration of latest data is expected to designate new locations for latter investments rapidly, due to the fact that the BP patterns may change in response to changes in NoA.

Mapping energy equivalents of subscale BP

Finally, the equivalents of the BP is mapped and given in Figure 11 a-e. As the result of higher BP, the patterns of hotspots for different energy sources are consistent. Even though these patterns were similar, the magnitude of collectable energy differs. On the other hand, it should be noticed that identifying the proportion of potential amounts within actual consumptions would be effective for better understanding of the necessity for collecting

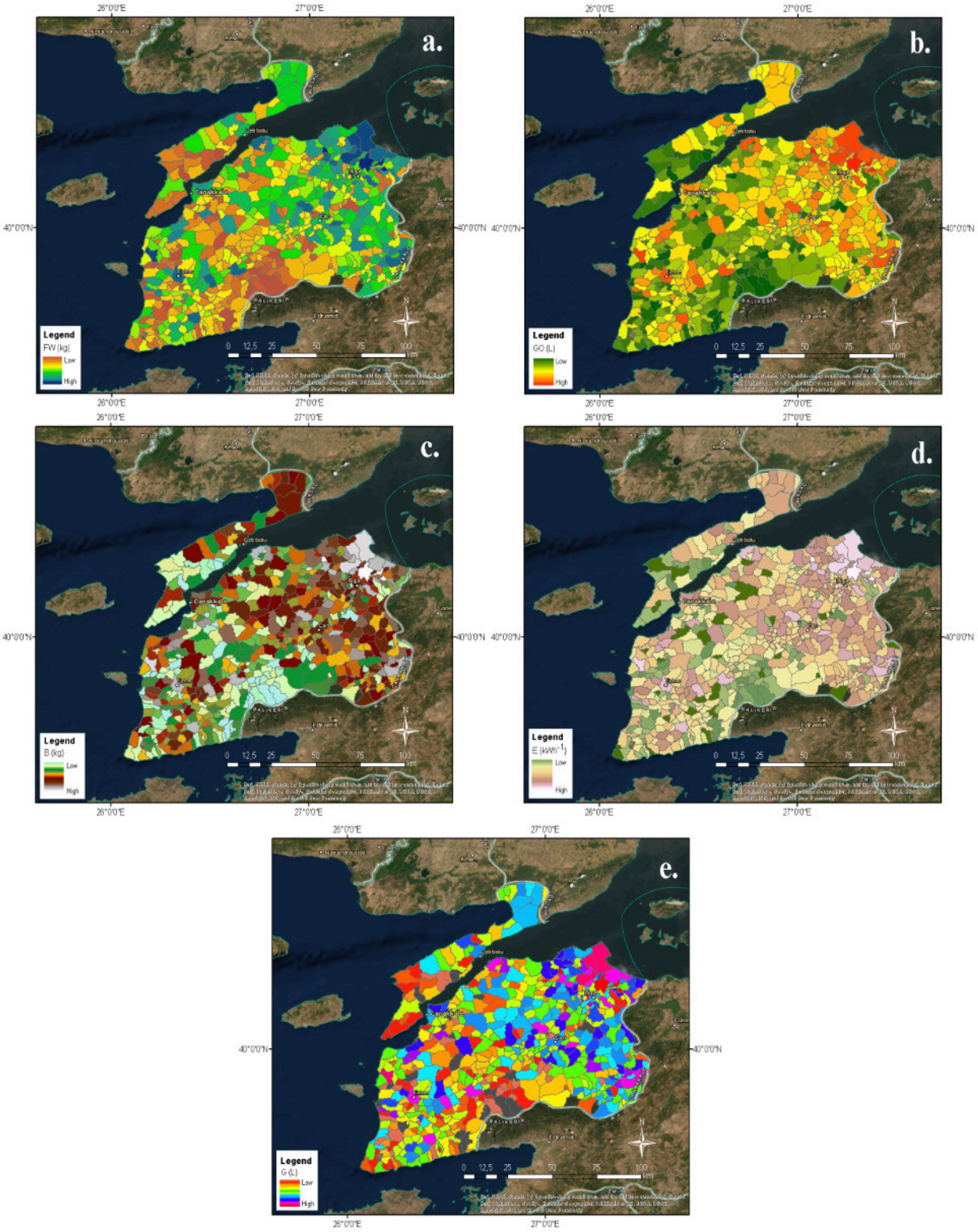


Figure 11. Subscale distribution of BP equivalents a. FW (kg), b. GO (L), c. B (kg), d. E (kWh^{-1}), and e. G (L).

the animal wastes in each settlements since the process will also contribute to avoiding from adverse effects of such wastes on natural resources like surface and ground water, as well as soil, in different ways.

The equivalents of BP in different energy sources are given in Table 5 and Table6, according to the lowest and highest BP of each district, respectively. Dependent to Table 5, the minimum and maximum values from the lowest BP of each district seemed to be obtained from Ayvacık and Yenice districts. On this account, annual

minimum and maximum values of FWs were calculated as 474 kg and 28926 kg, GOs were 86 L and 5252 L, Bs were 59 kg and 3585 kg, Es were 642 kWh^{-1} and 39179 kWh^{-1}, and G were 109 L and 6669 L.

The minimum and maximum equivalent values from the highest BPs of each district have shown that the minimum and maximum values of equivalents from highest BP of each district were calculated for Eceabat and Biga districts (Table 6). The maximum and minimum amounts of FWs were 343624 kg and 5357347 kg, GOs were 62387 L and 972660 L, Bs were 42582 kg and 663879 kg, Es were 465428 kWh^{-1} and 7256349 kWh^{-1}, Gs were 79222 L and 1235123 L.

Table 6. The different energy equivalents of highest BP from each district.

DISTRICT	Subscale	FW (kg)	GO (L)	B (kg)	E (kWh^{-1})	G (L)
AYVACIK	Center	1858584.0	337437.4	230314.4	2517390.0	428491.9
BAYRAMİÇ	Türkmenli	1993639.0	361957.5	247050.4	2700318.0	459628.6
BİGA	Yukaridemirci	5357347.0	972659.5	663878.7	7256349.0	1235123.0
CAN	Yaykın	1746543.0	317095.6	216430.4	2365634.0	402661.1
ECEABAT	Center	343624.1	62387.1	42581.7	465427.5	79221.7
EZİNE	Üvecik	2560281.0	464835.0	317268.3	3467816.0	590266.6
GELİBOLU	Yeniköy	668658.4	121399.1	82859.7	905675.6	154157.6
LAPSEKİ	Çardak	1821589.0	330720.8	225730.1	2467283.0	419963.0
PC	Gökçalı	1197015.0	217325.5	148333.3	1621318.0	275968.9
YENİCE	Pazarköy	1648080.0	299219.2	204229.0	2232270.0	379960.9

Consequently, identifying the proportions of all energy types within their consumption amounts for all considered settlements may represent a finer illustration for actualization of these potentials. Thereby, the ratio of concurrently used energy to obtainable energy believed to provide valuable information in further studies. Moreover, inclusion of population data is highly suggested to compare the percentages of a certain population's needs met through the obtainable energies. At this point, collection of subscale level socio-economic data through surveys is necessary, which is not yet publicly available. Additionally, the geographic distribution of NoA in different farming systems such as livestock or poultry would also presents vital knowledge for determining availability of biogas feedstock (Scarlat et. al., 2018; Levstek and Rozman, 2022). In this point of view, GIS tools provide simultaneously consideration of several environmental, technical, and economical phenomenon and concepts including, nature and water conservation, proximities to settlements, water resources, transportation networks, and costs (Sliz-Szkliniarz and Vogt, 2012).

CONCLUSION

As it is well known, Turkey includes a wide variety of agricultural production due to suitability of soil, topography, and climate conditions. Increments in the use of agricultural wastes, particularly manure, for producing renewable energy would provide more efficient plans, initiatives, and investments for a sustainable future. For this purpose, the potential contribution of Çanakkale Province seems promising with an amount of 6.4×10^7 m^3 BP. The district level highest and lowest BPs were predicted for Biga and Eceabat. Moreover, the study presented the first subscale biogas investigation initiative in the area, and provided highly precise results by consideration of smaller settlements such as townships and villages in addition to districts. District level results have relealed that highest BP can be obtained from Biga district, while the lowest BP was calculated for Eceabat. However, subscale level distribution patterns were quite different. On this account, even though the highest BP is found in a sublevel settlement of Biga district, namely, Yukarıdemirci village (154×10^4), the lowest BP was found to be obtained from Bahçedere village of Ayvacık district with a value of 137 m^3 instead of Eceabat. Usage of the whole potentials from each settlements would enable to overcome many environmental issues, contributes to maintanance of soil and water resources. Also, it is known that treatment of agricultural level in energy production provides the advantages of purification of anumal waste from weed seeds and undesirable levels odour before using as manure. Furthermore, such attempts will help to reduce the emissions of greenhouse gasses. In another point of view the process would led to economic and social welfare, in return. In conclusion, the BP of Çanakkale Province seemed noteworthy when compared to studies conducted in other relatively small cities of our country. Utilization from GIS tools for biomass evaluation provided

labour effective and rapid demonstration for geographic distribution of energy potentials. In addition, integration of inventories into GIS have allowed expeditious update of the data conveniently by considering the latest annual reports, due to it's dynamic structure. The results believed to serve as a baseline for further studies, particularly for site selectioion by denominating the capacities of each settlement since the transport costs of produced wastes comprise a challenge in determination of appropriate areas for biogas plant construction. Currently, a study is ongoing to identify the proper locations for optimizing financial support allocation using topographic data, soil properties, latest land use and land cover data, current status and future predictions of NoA, socio-economic data and relevant statistical analysis.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The author declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Akbulut, A., Dikici, A. (2004). Biogas potential of Elazığ province and cost analysis. Fırat University Journal of Eastern Researches, 2(2), 36-41. Retrieved from https://dergipark.org.tr/en/pub/fudad/issue/47000/590371

Akyürek, Z., Coşkun, S. (2019). Determination of biogas energy potential of Aegean Region based on animal waste. Celal Bayar University Journal of Science, 15(2), 171-174. https://doi.org/10.18466/cbayarfbe.492880

Altıkat, S, Çelik, A. (2012). Biogas potential from animal waste of Iğdır province. Iğdır Üni. Journal of the Institute of Science and Technology, 2(1), 61–66. Retrieved from https://dergipark.org.tr/en/pub/jist/issue/7928/104281

Anonymous (2021). Energy Atlas [In Turkish]. Retrieved from https://www.enerjiatlasi.com/biyogaz/

Atelge, M.R., Atabani, A.E., Rajesh-Banu, J., Krisa, D., Kaya, M., Eskicioglu, C., Kumar, G., Lee, C., Yildiz, Y.Ş., Unalan, S., Mohanasundaram, R., Duman, F. (2020). A critical review of pretreatment technologies to enhance anaerobic digestion and energy recovery. Fuel, 270, 117494. https://doi.org/10.1016/j.fuel.2020.117494

Atılgan, A., Saltuk, B., Ertop, H., Aksoy, E. (2020). Determining the biogas energy potential from greenhouse wastes and creating maps: The case of Antalya province. Euroasia Journal of Mathematics, Engineering, Natural & Medical Sciences, 7(12), 19-30. https://doi.org/10.38065/euroasiaorg.270

Atılgan, A., Saltuk, B., Ertop, H., Aksoy, E. (2021). Determination of the potential biogas energy value of animal wastes: Case of Antalya. European Journal of Science and Technology, Special Issue 22, 263-272. https://doi.org/10.31590/ejosat.844631

Aybek, A., Üçok, S., İspir, M.A., Bilgili, M.E. (2015). Digital mapping and determination of biogas energy potential of usable animal manure and cereal straw wastes in Turkey. Journal of Tekirdag Agricultural Faculty, 12(3), 109-120.

Ayhan, A. (2016). Biogas potential of animal waste in Marmara Region, Turkey. Agronomy Research, 14(3), 650-660.

Baran, M.F., Lüle, F. Gökdoğan, O. (2017). Energy potential can be produced by animal waste of Adiyaman Province. Turkish Journal of Agricultural and Natural Sciences, 4(3), 245–249. Retrieved from https://dergipark.org.tr/en/download/issue-full-file/30559

Bharti, A., Paritosh, K., Mandla, V.R., Chawade, A., Vivekanand, V. (2021). GIS application for the estimation of bioenergy potential from agriculture residues: An overview. Energies. 14(4):898. https://doi.org/10.3390/en14040898

Demir-Yetiş, A., Gazigil, L., Yetiş, R., Çelikezen, B. (2019). Biogas potential from animal waste: A case study for Bitlis province. Academic Platform Journal of Engineering and Science, 7(1), 74-78. https://doi.org/10.21541/apjes.405308

Erdal, G., Esengün, K., Erdal, H., Gündüz, O. (2007). Energy use and economical analysis of sugar beet production in Tokat province of Turkey. Energy, 32(1), 35-41. https://doi.org/10.1016/j.energy.2006.01.007

Ertop, H., Atılgan, A., Saltuk, B., Aksoy, E. (2022). Establishment of numerical maps by determining the biogas and electricity production potential obtainable from cattle waste. European Journal of Science and Technology, 35, 530-540. https://doi.org/10.31590/ejosat.1034086

European Commission, Directorate-General for Energy, Clean energy for all Europeans, Publications Office, 2019.

Gülşen-Akbay, H.E., Kumbur, H. (2020). Determination of biogas potential of livestock manure: A case study from Mersin Province. Academic Platform Journal of Engineering and Science, 8(2), 295-303. https://doi.org/10.21541/apjes.590660

Gümüşçü, M., Uyanık, S. (2010). Güneydoğu Anadolu Bölgesi hayvansal atıklarından biyogaz ve biyogübre eldesi. Tesisat Mühendisliği (MMO), 16(118), 59-65.

Ignaciuk, W., Sulewski, P. (2021), Conditions of development of the agricultural biogas industry in Poland in the context of historical experiences and challenges of the European Green Deal. Problems of Agricultural Economics. 3, 55-77. https://doi.org/10.30858/zer/140413

Ilgar, R. (2016). A study for determination of biogas potential in Çanakkale, assets by animals. Eastern Geographical Review, 35(21), 89-106. https://doi.org/10.17295/dcd.08733

İnci, H., Söğüt, B., Gökdoğan,O., Ayasan, T., Şengül, T. (2016). Determining the energy usage efficiency and economic

analysis of broiler chickens raised under organic conditions. Indian Journal of Animal Sciences, 86(11), 1323-1327.

Kaya, D., Çankakılıç, F., Dikeç, S., Baban, A., Güneş, K. (2005). Türkiye'de tarımsal atıkların değerlendirilmesi rehberi, LIFE 03 TCY/TR/000061 Project Report, Tubitak [In Turkish].

Levstek T, Rozman Č. (2022). A Model for finding a suitable location for a micro biogas plant using GIS tools. Energies. 15(20):7522. https://doi.org/10.3390/en15207522

Lüle, F. (2019). Potential energy from animal wastes in the South-Eastern Anatolia Region. ÇOMÜ Journal of Agricultural Faculty, 7(1), 145-150. https://doi.org/10.332027/comuagri.435371

Mirosz, L., Amrozy, M., Trzaski, A., Wiszniewska A. (2015). What policymakers should know about micro-scale digestion. Bioenergy Farm II–Manure, the Sustainable Fuel For the Farm. Intelligent Energy Europe Programme of the European Union. Warszawa: National Energy Conservation Agency (NAPE).

Nacar-Koçer, N., Cengiz, Ö., Sugözü, İ. (2006). Castle-dealing potential of Turkey and biogas production. Fırat University Journal of Eastern Researches, 4(2), 17-20. Retrieved from: https://dergipark.org.tr/tr/pub/fudad/issue/47077/592012

Onurbaş-Avcıoğlu, A., Türker, U. (2012). Status and potential of biogas energy from animal wastes in Turkey. Renewable and Sustainable Energy Reviews, 16(3), 1557-1561. https://doi.org/10.1016/j.rser.2011.11.006

Özpınar, S. (2018). An assessment of biogas production potential from animal manures in Çanakkale. COMU Journal of Agriculture Faculty, 6(2), 115-122.

Polat-Bulut, A., Topal-Canbaz, G. (2019). Hayvan atıklarından Sivas ili biyogaz potansiyelinin araştırılması. Karaelmas Fen ve Mühendislik Dergisi, 9(1), 1-10. https://doi.org/10.7212/zkufbd.v9i1.1010

Salihoğlu, N.K., Teksoy, A. (2019). Determination of biogas production potential from cattle and sheep wastes: Balikesir case study. Niğde Ömer Halisdemir University Journal of Engineering Sciences, 8(1), 31-47. https://doi.org/ 10.28948/ngumuh.516798

Scarlat, N., Fahl, F., Dallemand, J.F., Monforti, F., Motola, V. (2018). A spatial analysis of biogas potential from manure in Europe. Renewable Sustainsble Energy Reviews, 94, 915–930. https://doi.org/10.1016/j.rser.2018.06.035

Sliz-Szkliniarz, B., Vogt, J. (2012). A GIS-based approach for evaluating the potential of biogas production from livestock manure and crops at a regional scale: A case study for the Kujawsko-Pomorskie Voivodeship. Renewable and Sustainable Energy Reviews, 16(1), 752-763. https://doi.org/10.28948/ngumuh.516798

Şenol, H., Elibol, E.A., Açıkel, Ü., Şenol, M. (2017). Türkiye'de biyogaz üretimi için başlica biyokütle kaynaklari. Bitlis Eren Üniversitesi Fen Bilimleri Dergisi, 6(2), 81-92. https://doi.org/10.17798/bitlisfen.315118

Tınmaz-Köse, E. (2017). Determination and digital mapping of biogas energy potential of animal manures in Thrace Region. Pamukkale University Journal of Engineering Sciences, 23(6), 762-772. https://doi.org/10.5505/pajes.2016.33600

TÜİK (2021). Turkish Statistical Institute annual reports for province and district level animal production statistics. Retrieved from https://biruni.tuik.gov.tr/medas/?locale=en

Yılmaz, H.İ., Gönbe, Y. (2021). Electric energy potential that can be produced using cattle manure in the Isparta Region. International Journal of Agriculture Environment and Food Sciences, 5(3), 310-322. https://doi.org/10.31015/jaefs.2021.3.8

Yürük, F., Erdoğmuş, P. (2015). Biogas potential of Düzce which can be produced by animal waste and determination of optimum plant location with k-means clustering. Journal of Advanced Technology Sciences, 4(1), 47-56. Retrieved from https://dergipark.org.tr/en/pub/duzceitbd/issue/4817/66454

Zenk, H. (2019). Samsun ilinin hayvan gübrelerinden üretilebilecek elektrik enerji potansiyeli. Avrupa Bilim ve Teknoloji Dergisi, 17, 1307-1312. https://doi.org/10.31590/ejosat.661910

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 182-191
DOI: https://doi.org/10.31015/jaefs.2023.1.22

Morphological and pomological characterization of some genotypes Sumac (Rhus coriaria L.) obtained by selection breeding

Sumeyye Adali[1] • **Remzi Ugur**[1] • **Esra Bulunuz Palaz**[1] • **Yesim Aslan**[1]
H. Osman Ozatar[1] • **Mehmet Fatih Yilmaz**[1] • **Adem Bardak**[2]

[1] East Mediterranean Transitional Zone Agricultural Research Institute Kahramanmaras, Türkiye
[1] Kahramanmaras Sutcu Imam University Agricultural Biotechnology Department Kahramanmaras, Türkiye

Citation: Adali, S., Ugur, R., Palaz, E.B., Aslan, Y., Ozatar, H.O., Yilmaz, M.F., Bardak, A. (2023). Morphological and pomological characterization of some genotypes Sumac (Rhus coriaria L.) obtained by selection breeding. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 182-191

Received: 29 November 2022
Revised: 26 December 2022
Accepted: 31 December 2022
Published Online: 19 March 2023

Correspondence: Remzi Ugur
E-mail: remzibey@hotmail.com

Abstract

Sumac (*R. coriaria* L.) is a subtropic and temperate climate plant with medicinal and aromatic properties which has a natural distribution in many regions of Anatolia. Sumac which is used as a food additive in the food industry due to the flavoring substances has been in high demand in recent years. Besides the beneficial secondary metabolites, it is used in the field of medicine and pharmacy. Sumac (*R. coriaria*) which grows spontaneously in the natural environment without any agricultural practices is collected by the local people in the region and supplied to the local market. Since this situation, the ideas of creating modern orchards for sumac have begun to emerge. For this reason, selection breeding studies have been planned in sumac (*R. coriaria* L.). This study was carried out at the East Mediterranean Transition Zone Agricultural Research Institute during 2019-2021 years. Approximately 300 different sumac genotypes were observed and botanically 92 genotypes were found positive in this study. It was determined that the wet weight values of the clusters in the examined sumac genotypes varied between 5.63-87.74 g as a result of the statistical analysis. The highest cluster weight varied between 87.74, 78.92, and 70.81 g in GN26, GN86, and GN90 genotypes respectively. The lowest cluster wet weight was found in GN28 (5.63 g) and GN41 (6.00 g) genotypes in the study. It was found in the study that the cluster powder efficiency values varied between 30.62% and 72.49% and the average cluster powder efficiency was 49.15%. It was determined that the results obtained from about 20 sumac genotypes were found to be positive in the statistical analyzes made on the characteristics examined in this study. It is aimed to use this sumac (*R. coriaria* L.) genotypes in the modern sumac cultivation planned for the future.

Keywords: Characteristic, Fruit, Medicinal and aromatic, *Rhus coriaria*, Spice

INTRODUCTION

Sumac (*Rhus coriaria* L.) belongs to the *Rhus* genus of the *Anacardiaceae* family and contains approximately 250 species. The name sumac is derived from the Syriac word "sumaga" meaning "red" (Wetherill and Pala, 1994). *R. coriaria* has a natural distribution in all subtropical and temperate climate zones especially in Iran, Southern Europe, North Africa, Western Asia, and the Middle East. These plants which are studied as a non-forest wood products in botanical form grow naturally in almost every region, especially in western and southern Anatolia (Basoglu and Cemeroglu 1984; Kurucu et al., 1993). It is reported that Turkey has a natural distribution area of primarily *R. coriaria* L. *Cotinus coggyria* and *Rhus chinensis* species (Eminagaoglu and Ozcan 2018; Sutyemez et al., 2019). Among these, *R. coriaria* L. is the species with the most widely growing area and medicinal aromatic usage area (Yucedag et al., 2010). In the survey studies

conducted that this species naturally spreads in arid and semi-arid regions calcareous soils non-agricultural lands and forests in the Kahramanmaraş region (Guvenc et al., 2017). In addition, it can be said that this plant can grow from sea level up to altitudes of 2000 m (Yucedag et al., 2010). Sumac (*R. coriaria* L.) is traditionally used as a food additive due to its acid content and intense sour taste (Fereidoonfar et al., 2018). On the other hand, due to the biochemicals it contains it is used in medicine and pharmacy (Elagbar et al., 2020; Alsamri et al., 2021), the leather industry (Davis, 1885; Tiryaki 2010; Pipinis et al., 2017), veterinary (Langroodi et al., 2018), landscaping (Corbaci et al., 2011) and erosion control studies (Gokturk et al., 2006). It has been reported to have antioxidant, anticancer, analgesic, antidiabetic, antihistamine, antiviral, antibacterial and antifungal effects (Bloshenko and Letchamo, 1996; Kizil and Turk, 2010; Kurt et al., 2014; Yuksel, 2018; Under and Saltan, 2019). Sumac (*R. coriaria L.*) can be propagated by seed generatively. Due to the secretion of a resin-like substance in the base of the cutting vegetative propagation is difficult (Muhamed and Dawodet, 2017; Karakuzulu, 2018). However, these problems are less common in micropropagation applications (Edwards and Thomas, 1980; Amiri and Mohammadi, 2021). The endocarp structure of the sumac seed which is extremely hard makes it difficult to germinate the physical and physiological dormancy state of the embryo in propagation with seeds (Uzun, 2016).

Due to the commercial importance of sumac (*R. coriaria* L.) establishment of a modern orchards have started. Therefore, it was necessary to carry out selection breeding studies in order to establish more homogeneous and productive modern orchards. Various articles on the phytochemical contents of Turkish sumac have been published (Gezici 2019; Ozcan et al., 2021; Caliskan et al., 2022). But studies on morphological and pomological data have been very limited (Yilmaz, 2021). This study was carried out to determine the morphological and pomological characteristics of some sumac (*R. coriaria* L.) genotypes naturally distributed in the Kahramanmaraş region. It is aimed to obtain 20 different sumac (*R. coriaria* L.) genotypes with different characteristics and commercial importance in the following stages of the study.

MATERIALS AND METHOTS

Materials

The material of this study consisted of 92 different sumac (*R. coriaria* L.) genotypes in terms of botanical characteristics obtained by selection breeding from the Eastern Mediterranean Region of Turkey. The pomological analysis of the study was carried out in the Eastern Mediterranean Transition Zone Agricultural Research Institute laboratories.

Methods

Fruit samples were taken and labeled at the appropriate ripening time in order to perform morphological and pomological analyzes of plants in different locations. The measurement of each fruit characteristic was made over 3 replications and the average values were used (Fereidoonfar et al., 2018). Qualitative features such as yield, fruit density in the cluster, and presence of waxy layer on the fruit were evaluated according to coding and scoring (Fereidoonfar et al., 2018). Selected 92 sumac (*R.coriaria*) genotypes were examined in terms of 15 different morphological and pomological features.

Pomologic and Morphologic Observations

The selected 92 sumac genotypes were evaluated in terms of 15 different characteristics both qualitative and quantitative (Table 2).

Yield

The yield calculations were taken into account the number of clusters per tree (Yilmaz, 2021; Fereidoonfar et al., 2018). Yield values were calculated in accordance with the 1-4 criteria (1: Low yield, 4: High yield)

Bunch width, length, bunch diameter and wet-dry bunch weight

The width, length and diameter of the bunch stem were measured with the help of a 0.01 mm sensitive digital caliper (0.01 mm Gomax GMX1017020), and the weight of the bunch was measured with the help of an electronic balance with 0.01 g precision (Fereidoonfar et al., 2018). The fresh weight of the bunch was measured immediately after the harvest and the dry weight of the bunch was measured after the bunches were dried in an oven at 60°C for 24 hours.

Color of fruit

Sumac fruits are small, tightly packed spheres that form dense clusters of reddish seeds called sumac bobs (Sakhr and Khatib 2020). The fruit color of sumac fruits was presented to the observers during the harvest period and determined by the survey methods. Observers were asked to choose one of 4 color alternatives: red, reddish brown, brown, and burgundy (Fereidoonfar et al., 2018).

Average fruit weight, fruit width, fruit length and fruit thickness, 100 fruit fresh and dry weights

100 pieces fresh fruit pieces weight was measured with the help of sensitive electronic scales. After the fruit pieces were dried in an oven at 60°C for 24 hours and measured. The width, length and thickness of the fruit pieces were measured randomly in millimeters with the help of a digital caliper (0.01 mm Gomax GMX1017020) (Ozcan and Haciseferogullari, 2004).

Fruit pieces density in bunch

The bunches were presented to the observers and determined by the questionnaire method and they

were asked to give points from 1 to 5. (1: very sparsely, 2: sparsely, 3: moderately dense, 4: dense, 5: very dense) (Fereidoonfar et al., 2018).

Waxy cuticle on fruits

Sumac fruits leave color and sticky feeling on the hands when touched. Fruits were offered to the observers and determined by the questionnaire method and they were asked to give points from 1 to 5 (Sezgin et al., 2015). (1: nonstick, 2: slightly sticky, 3: medium sticky, 4: sticky, 5: very sticky).

Fruit flesh powder weight per bunch

Harvested fruits were dried and separated from their flesh and the flesh of the fruit was ground into fresh powder then the weight of sumac fresh powder coming out of a bunch was measured with the help of a precision scale (0,01 g precision).

Evaluation of data

After the fruit and bunch samples were analyzed in three parallels and the weighted grading method modified by Ugur and Kargı (2018), was applied to the results [Table 1 (a), Table 1 (b), Table 1 (c)]. Weighing grading is a method frequently used in fruit breeding studies. After adding the scores of selected sumac genotypes for each trait total scores were obtained.

Findings and Discussion

15 different characteristics were investigated in selected sumac (*R. coriaria*) genotypes in the study. It is seen in Table (3a-b-c) that there are quite different distributions among genotypes in terms of these characteristics. The wet weight values of the bunch showed a very different distribution between 5.63 - 87.74 g. It is seen that about 36% of the genotypes are above the average value (31.80 g) and the majority of the genotypes (64%) are below. It was determined that the highest wet bunch weight varied between 87.74-71.79, and 70.81 g in GN-26, GN-90, and GN-3 genotypes, respectively. The lowest values were measured in GN28 (5.63g), GN41 (6.00 g) and GN70 (9.28 g) genotypes. Fereidoonfar et al., (2018) determined that the cluster weight of sumac plants was between 0.55-6.67 in their study on 136 sumac plants. Yilmaz (2021) reported that the bunch weights of 394 sumac genotypes he investigated in the Kahramanmaraş province Dulkadiroğlu region ranged from 7.22 g to 37.48 g. It is seen that the bunch weights obtained from our study are quite high considering these values. It is thought that this situation is caused by the cultivation of some genotypes in their environment. However, bunch weight values were found to be high in our study. It is known that bunch characteristics are among the most important quality criteria in sumac breeding studies. It was determined in the study that the average dry weight of the cluster was 24.15 g. The highest dry weight of the bunch was recorded as GN-3 with 67.82 g and the lowest dry weight of the cluster was recorded as GN-46 with 3.15 g. Fereeidonfar et al. (2018) reported that bunch weight stands out as the feature with the highest coefficient of variation (CV=69.00%) among the examined features. Similarly, it was concluded that the fresh and dry weights of clusters showed a wide variation in our study. It was observed that some genotypes with high wet weight of the bunch lost more water when dried and their weight decreased. GN-27 lost 64.72% of its weightand, GN-42, 61.12%, and GN46 68.18% after drying. The high dry weight of the cluster is very important in the yield criteria of sumac plants. The yield values varied between 1.80 and 3.60 according to the 1-4 criteria. It was observed that the average yield value was 2.71 in our study. The highest yield was determined in GN-34 (3.60), GN-

Table 1 (a). Value ranges based on the scores (Ugur and Kargı, 2017)

Yield (1/10)			Bunch wet weight (g)			Bunch dried weight (g)			Fruit fleshpowder (FPP) (g)			(FPP) efficiency (%)		
Min.	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**
1,8	3,6	0,18	5,63	87,74	8,21	3,15	67,82	6,47	2,05	28,98	2,69	30,63	72,49	4,19
Points			**Points**			**Points**			**Points**			**Points**		
1	1,8	1,98	1	5,63	13,84	1	3,15	9,62	1	2,05	4,74	1	30,63	34,82
2	1,99	2,17	2	13,85	22,06	2	9,63	16,09	2	4,75	7,45	2	34,83	39,01
3	2,18	2,36	3	22,07	30,28	3	16,10	22,57	3	7,46	10,15	3	39,02	43,21
4	2,37	2,55	4	30,29	38,50	4	22,58	29,05	4	10,16	12,85	4	43,22	47,40
5	2,56	2,74	5	38,51	46,73	5	29,06	35,53	5	12,86	15,56	5	47,41	51,60
6	2,75	2,93	6	46,74	54,95	6	35,54	42,00	6	15,57	18,26	6	51,61	55,80
7	2,94	3,12	7	54,96	63,17	7	42,01	48,48	7	18,27	20,96	7	55,81	59,99
8	3,13	3,31	8	63,18	71,39	8	48,49	54,96	8	20,97	23,66	8	60,00	64,19
9	3,32	3,50	9	71,40	79,61	9	54,97	61,43	9	23,67	26,37	9	64,20	68,38
10	3,51	<	10	79,62	<	10	61,44	<	10	26,38	<	10	68,39	<

Table 1 (b). Value ranges based on the scores (Ugur and Kargı,2017)

Bunch width (mm)			Bunch size (mm)			Bunch stem diameter (mm)			Density of fruit pieces (1/5)			Weight of fruit piece (g)		
Min.	Max.	Mean	Min.	Max.	Mean	Min	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
26,93	96,77	6,98	9,5	29,2	1,97	2,67	10,59	0,79	1,5	5	0,35	0,87	6,18	0,53
Points			Points			Points			Points			Points		
1	26,93	33,91	1	9,5	11,47	1	2,67	3,46	1	1,5	1,85	1	0,87	1,40
2	33,92	40,91	2	11,48	13,45	2	3,47	4,26	2	1,86	2,21	2	1,41	1,94
3	40,92	47,90	3	13,46	15,43	3	4,27	5,07	3	2,22	2,57	3	1,95	2,48
4	47,91	54,90	4	15,44	17,41	4	5,08	5,87	4	2,58	2,93	4	2,49	3,02
5	54,91	61,89	5	17,42	19,39	5	5,88	6,67	5	2,94	3,29	5	3,03	3,57
6	61,90	68,88	6	19,40	21,37	6	6,68	7,47	6	3,30	3,65	6	3,58	4,11
7	68,89	75,88	7	21,38	23,35	7	7,48	8,27	7	3,66	4,01	7	4,12	4,65
8	75,89	82,87	8	23,36	25,33	8	8,28	9,08	8	4,02	4,37	8	4,66	5,19
9	82,88	89,87	9	25,34	27,31	9	9,09	9,88	9	4,38	4,73	9	5,20	5,73
10	89,88	<	10	27,32	<	10	9,89	<	10	4,74	<	10	5,74	<

Table 1 (c). Value ranges based on the scores (Uğur and Kargı,2017)

Weight of fruit piece (g)			Fruit width (mm)			Fruit size (mm)			Thickness of peri-carp (mm)			Waxy cuticle on fruit (1/5)		
Min.	Max.	Mean	Min.	Max.	Mean	Min	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
0,3	5,18	0,49	3,56	7,14	0,36	3,53	7,71	0,42	0,97	5,4	0,44	2,33	4,85	0,25
Points			Points			Points			Points			Points		
1	0,3	0,79	1	3,56	3,92	1	3,53	3,95	1	0,97	1,41	1	2,33	2,58
2	0,80	7,78	2	3,93	4,29	2	3,96	4,38	2	1,42	1,87	2	2,59	2,84
3	7,79	14,78	3	4,30	4,65	3	4,39	4,80	3	1,88	2,32	3	2,85	3,11
4	14,79	21,77	4	4,66	5,02	4	4,81	5,23	4	2,33	2,77	4	3,12	3,37
5	21,78	28,76	5	5,03	5,39	5	5,24	5,66	5	2,78	3,23	5	3,38	3,63
6	28,77	35,76	6	5,40	5,76	6	5,67	6,09	6	3,24	3,68	6	3,64	3,89
7	35,77	42,75	7	5,77	6,13	7	6,10	6,52	7	3,69	4,13	7	3,90	4,15
8	42,76	49,75	8	6,14	6,49	8	6,53	6,94	8	4,14	4,58	8	4,16	4,42
9	49,76	56,74	9	6,50	6,86	9	6,95	7,37	9	4,59	5,04	9	4,43	4,68
10	56,75	<	10	6,87	<	10	7,38	<	10	5,05	<	10	4,69	<

105(3.50) and GN-33(3.40) genotypes, the lowest yield value was found in GN-59 (1.80), and GN-8 (1.80) sumac genotypes in the study. It was determined that about 37% of sumac (*R. coriaria*) genotypes had yield values below the average. However, it was determined that the yield in 63% of the genotypes could be considered high. On the other hands yield values were found to be high in sumac (*R. coriaria*) genotypes. . Fereidoonfar et al. (2018) grouped the yields of 136 sumac plants as low (27.90%), medium (45.60%) and high (26.50%). It was observed that the diameter of the bunch stem varied between 2.67 and 10.59 mm and the mean diameter of the bunch stem was measured as 5.57 mm in our study. Fereidoonfar et al. (2018), measured that the diameter of the bunch stem diameter of the sumacs in the Iran region as the highest at 5.27 mm and lowest at 1.43 mm. Yilmaz (2021) observed that the cluster stem diameter values between 3.03 mm and 7.02 mm in 25 promising sumac genotypes in the Kahramanmaraş Dulkadiroğlu district. The density of fruit on the bunch was presented to the observers by the questionnaire method and they were asked to give a score between 1 and 5. According to the results, the average density of fruit in the bunch was calculated as 3.88. Fereidoonfar et al. (2018) calculated the average density of fruit on the bunch of 136 sumac genotypes as 3.29. Fereidoonfar et al. (2018) stated that 136 sumac genotypes had low density (22.10%), medium density (41.20%) and high density (36.80%) fruit densities in the

Table 2. Relative scores used in weighted grading applied to selected sumac genotypes

Pomological Feature	Relative point (Total 100)
Bunch wet weight (g)	7
Yield	15
Bunch dried weight (g)	8
Fruit fleshpowder (FPP) (g)	11
FPP efficiency (%)	16
Bunch width (mm)	5
Bunch size (mm)	4
Diameter of bunch stem (mm)	2
Density of fruit on the bunch	8
Weight of 100 wet fruit pieces (g)	6
Weight of 100 dried fruit pieces (g)	9
Fruit width (mm)	2
Fruit size (mm)	3
Thickness of pericarp (mm)	1
Waxy cuticle on fruit (1/5)	3

cluster. Yilmaz (2021) grouped the 25 promising sumac genotypes he observed 202 genotype as rare (8%), moderate (36%), dense (40%) and very dense (16%) in the Kahramanmaras region. The results of our study seem to be compatible with the literature. Fruit flesh powder yield is an important quality criterion in sumac (*R. coriaria*). Because in the food industry where sumac is widely used in many parts of the world the fruits of flesh powder are generally used as a spice and an additive in foods. For this purpose the efficiency of the fruit flesh powder of the bunch was calculated for the first time in this study. The fruit flesh powder yield in sumac (*R. coriaria*) genotypes varied between 72.49% and 30.63% and the average sumac fruit flesh powder yield was 49.15%. It was determined that the powder yield was below the average in approximately 51% of the 92 sumac (*R. coriaria*) genotypes and more promising results were obtained in 49% of them. The highest fruit flesh powder yield were in GN-113 (69.88%), GN-43 (68.80%) and GN-42 (67.33%) genotypes and the lowest was in GN-62 (30.63%) and GN-114 (33.73%) genotypes. This situation may have occurred due to genetic differences or soil conditions where the plant is located. Therefore, this feature should be reviewed under equal soil conditions. It was conducted that the average bunch length was 186.2 mm in sumac (*R. coriaria*) genotypes with bunch lengths ranging from 95 to 292 mm. The highest cluster lengths were found in GN103 (292 mm), GN54 (290 mm), and GN83 (284 mm) genotypes. The lowest bunch lengths were found in GN46 (95 mm), GN80 (116 mm), and GN25 (135 mm) genotypes.

Fereidoonfar et al. (2018) observed that the sumac bunch lengths in the Iran region ranged from 54.71 mm to 142.42 mm. Yilmaz (2021), in his study with sumacs in Kahramanmaraş Dulkadiroğlu district reported that the length of the bunch was between 91.80 mm and 302.50 mm. Accordingly, it can be said that the bunch of sumacs in Turkey is much longer. The bunch widths ranged from 26.93 mm to 96.77 mm with an average of 50.24 mm in our study. Feredidoonfar et al. (2018), stated that the bunch widths of Iranian sumacs vary between 13.65 and 35.55 mm. It can be said that the reason why the

Figure 1. Tree, leaf, flower and fruit of the studied edible *Rhus coriaria* genotypes

Table 3(a). Pomological analyzes of selected sumac (*R. coriaria* L.) genotypes

NO	GNTP	BWW	Y	BDW	FPP	FPPE	BW	BS	DBS	DFB	WWFP	WDFP	FW	FS	TP	WCF	TOTAL
1	GN105	53,26	3,50	46,64	24,68	52,91	96,77	159,00	6,82	4,50	3,50	2,68	6,00	5,11	2,95	3,75	760
2	GN111	50,23	3,20	45,11	20,46	45,36	95,36	262,5	8,12	4,60	3,84	2,90	6,78	6,88	3,22	4,00	738
3	GN58	69,84	3,20	52,24	23,82	45,60	76,82	178,0	6,30	4,50	4,54	3,54	6,10	5,31	2,15	3,00	646
4	GN33	49,45	3,40	43,67	19,18	43,91	60,96	236,0	5,23	4,50	4,96	3,02	5,51	5,64	2,51	4,75	638
5	GN26	87,74	2,50	58,96	25,75	43,68	61,01	201,0	6,74	4,70	4,58	2,94	5,95	6,06	2,07	3,00	602
6	GN109	61,48	3,20	38,11	19,37	50,83	47,94	228,0	6,28	3,80	4,44	2,63	5,41	5,72	2,55	3,00	591
7	GN94	60,03	2,80	57,01	21,37	37,48	68,74	238,0	7,98	4,60	3,40	3,63	6,35	4,69	2,97	4,80	587
8	GN34	38,94	3,60	36,40	14,25	39,15	64,14	257,0	6,36	4,30	2,70	2,76	6,16	5,72	2,48	4,20	580
9	GN90	71,79	2,90	43,84	23,92	54,57	61,45	130,0	4,88	5,00	4,20	3,42	6,84	6,62	2,45	3,74	579
10	GN61	46,66	2,50	42,31	20,27	47,91	74,00	238,0	6,06	4,50	3,72	3,48	6,14	5,36	2,40	3,75	564
11	GN3	70,81	2,50	67,82	28,98	42,73	75,57	245,0	5,87	4,85	3,66	2,76	6,33	5,89	2,71	4,50	562
12	GN85	59,35	2,80	52,03	25,42	48,86	54,26	258,0	6,68	4,85	3,38	3,24	5,13	5,40	2,25	3,74	556
13	GN87	54,22	2,50	31,10	15,87	51,03	52,19	215,0	7,04	4,50	5,10	3,00	5,91	7,03	2,66	3,74	549
14	GN106	29,64	3,60	27,44	12,74	46,43	80,00	157,0	5,85	3,80	3,90	3,60	6,54	5,69	3,45	4,00	548
15	GN60	28,35	3,20	26,13	14,89	56,98	40,29	174,0	5,40	4,50	3,46	3,32	5,42	5,26	2,44	3,74	543
16	GN89	53,91	2,70	36,56	18,88	51,64	57,93	245,0	9,42	4,85	3,97	2,56	5,74	5,96	2,16	3,74	530
17	GN54	66,92	2,50	41,47	14,80	35,68	60,06	290,0	8,02	4,60	5,06	3,45	6,07	6,49	2,56	3,74	523
18	GN76	24,93	3,20	24,81	11,88	47,88	49,78	157,0	5,01	4,60	3,07	2,90	5,33	6,19	2,90	3,74	513
19	GN51	30,76	3,00	28,98	13,49	46,55	55,60	168,0	4,95	4,00	4,91	4,76	6,22	6,70	2,75	3,40	512
20	GN115	35,52	3,30	22,78	9,84	43,20	60,67	247,0	6,36	3,75	4,82	1,50	5,79	6,69	3,34	3,74	512
21	GN17	58,25	3,00	38,85	13,76	35,42	61,64	192,0	4,65	4,20	3,91	2,64	5,76	6,02	2,15	3,74	509
22	GN101	26,07	3,00	22,63	12,78	56,47	47,95	160,0	4,67	4,00	2,86	3,26	4,74	5,96	2,47	4,10	505
23	GN62	67,22	3,00	35,52	10,88	30,63	68,18	248,0	7,02	4,30	3,88	2,46	5,17	6,00	2,26	3,74	502
24	GN31	45,00	2,50	41,19	19,66	47,73	39,71	142,5	4,77	4,60	3,54	3,14	5,26	5,30	2,87	4,00	499
25	GN110	29,68	3,00	28,79	14,23	49,42	61,33	222,0	6,70	3,75	2,56	2,42	6,49	5,68	3,54	2,50	499
26	GN108	44,39	3,00	37,12	18,08	48,71	55,38	164,0	6,10	5,00	3,16	3,19	5,22	5,24	2,22	4,00	497
27	GN25	29,78	3,00	13,60	8,95	65,81	36,39	135,0	4,74	4,00	3,53	2,78	5,31	4,92	2,14	3,74	494
28	GN27	38,44	2,40	13,56	9,83	72,49	56,81	210,0	6,42	4,60	2,18	0,87	3,56	4,13	2,57	3,74	494
29	GN18	35,14	2,70	15,02	9,90	65,91	48,71	151,0	5,92	4,20	3,56	2,76	4,91	5,12	2,46	3,74	492
30	GN113	15,27	2,50	13,18	9,21	69,88	40,40	189,0	3,53	3,50	4,24	4,04	7,14	6,84	2,89	3,70	492
31	GN83	40,94	1,80	27,00	15,31	56,70	69,89	284,0	5,95	4,00	3,70	2,74	5,27	5,78	2,19	3,74	491
32	GN116	29,85	2,80	24,65	12,54	50,86	47,61	209,0	5,39	4,20	3,54	3,49	5,41	5,76	2,78	4,85	490
33	GN91	22,73	2,80	17,96	9,44	52,56	49,06	260,0	6,02	3,50	3,94	3,38	5,50	6,28	2,49	3,74	487
34	GN40	36,55	3,10	20,00	10,81	54,05	50,38	210,0	5,79	3,70	5,82	3,64	6,23	6,23	2,29	3,74	486
35	GN20	37,71	2,80	22,18	11,16	50,31	47,61	175,0	6,96	3,80	4,30	3,10	5,68	5,84	2,61	3,74	481

GNTP= Genotype code; BWW=Bunch wet weight (g); Y= Yield (1/4); BDW=Bunch dried weight (g); FPP=Fruit flesh powder (g); FPPE= Fruit flesh powder efficiency (%); BW=Bunch width (mm); BS=Bunch size (mm); DBS=Diameter of bunch stem (mm); DFB=Density of fruit on the bunch (1/3); WWFP=Weight of 100 wet fruit pieces (g); WDFP=Weight of 100 dried fruit pieces (g); FW=Fruit width (mm); FS=Fruit Size (mm); TP=Thickness of pericarp (mm); WCF=Waxy cuticle on fruit (1/5)

length and width of the bunch is higher than the Iranian sumacs are the climatic factors, soil structure, altitude between the two countries. Yilmaz (2021) reported that the width of the bunch varied between 27.95 mm and 73.91 mm in his study. The average fruit width was 5.57 mm and it varied between the lowest 3.56 mm (GN-27) and the highest 7.14 mm (GN-113). The average value of fruit length was measured as 5.55 and it was calculated between the lowest 3.53 mm (GN-41) and the highest 7.71 mm (GN-98). Fereidonfaar et al. (2018) measured that the fruit lengths of 136 sumac genotypes between 2.98 -4.54 mm and fruit width between 3.20-4.49 mm. Yilmaz (2021), fruit lengths in the range of 7.24-5.45 mm in 25 promising sumac genotypes. Fruit thicknesses were measured between 0.97 mm (GN-28) - 5.40 mm (GN-2). Mazaheri et al. (2017) recorded that the sizes of sumac fruits from Iran's Gonabad, Ferdows and Zohk regions were 3.84 mm, 3.58 mm, and 3.6 mm respectively. The diameter of the sumac fruit sample taken from Turkey was measured as 3.64 mm. Özcan and Hacıseferogullari (2004) reported that average fruit length and fruit width as 4.72 mm and 3.90 mm in Turkey. In addition, the fruit sizes are relatively larger and compatible with the literature in our study. It is thought that the reason for the larger fruit sizes may be related to the geographical, climate and soil structure of Kahramanmaraş. 100 fruits weight the highest GN-50 (6.18 g) and the lowest GN-28 (0.87 g) average was 2.76 g. Fereidoonfar et al. (2018) observed that the weight of 10 fruits in Iranian sumac ranged between 0.06 g and 0.21 g with an average of 0.14 grams. Yilmaz (2021) stated that the weight of 100 fruits varied between 2.40 g and 4.14 g in 25 promising sumac genotypes. It is understood that the sumac (*R. coriaria*) genotypes differ from each other in terms of all characteristics and show a wide distribution.

Table 3(b). Pomological analyzes of selected sumac (*R. coriaria* L.) genotypes

NO	GNTP	BWW	Y	BDW	FPP	FPPE	BW	BS	DBS	DFB	WWFP	WDFP	FW	FS	TP	WCF	TOTAL
36	GN53	42,87	2,75	27,53	11,23	40,79	65,07	208,0	5,53	4,70	3,68	2,96	5,18	4,77	2,76	4,00	481
37	GN30	52,63	2,50	41,12	15,26	37,11	46,38	200,0	7,13	4,70	4,86	3,40	5,83	6,44	2,85	3,20	478
38	GN37	39,17	2,90	23,41	9,74	41,61	70,19	197,0	4,48	4,00	4,92	1,38	6,32	5,39	2,22	3,74	477
39	GN84	37,95	2,50	21,56	12,22	56,68	48,24	143,0	4,96	4,70	3,34	1,92	5,75	5,62	2,27	3,74	474
40	GN75	27,78	3,00	27,67	12,34	44,60	59,42	153,0	4,00	4,00	3,20	3,16	6,07	5,32	3,06	4,30	469
41	GN39	21,76	3,40	15,20	7,76	51,05	37,82	150,0	4,56	4,10	3,50	2,84	4,99	5,05	2,37	3,74	460
42	GN42	29,91	2,00	11,63	7,83	67,33	79,12	262,0	5,78	3,50	2,70	1,18	4,47	5,53	1,97	3,74	460
43	GN24	22,85	3,00	17,62	8,40	47,68	45,56	165,0	4,47	4,30	3,54	3,02	5,17	5,64	2,10	3,74	458
44	GN86	78,92	2,00	37,55	17,54	46,71	70,61	122,0	6,43	5,00	4,64	2,64	6,70	6,20	2,54	3,74	457
45	GN59	45,27	1,80	31,96	16,27	50,91	59,92	218,0	5,67	4,00	4,06	2,76	4,90	5,68	2,35	3,75	455
46	GN48	37,47	2,00	18,25	9,81	53,75	46,07	155,0	5,47	4,50	4,88	2,90	6,69	5,87	2,35	3,74	446
47	GN56	20,03	3,00	16,55	8,02	48,46	33,09	190,0	4,12	4,10	2,98	2,57	5,23	5,22	2,40	4,60	444
48	GN50	22,41	2,70	16,49	10,09	61,19	45,62	150,0	5,50	3,60	6,18	3,54	6,59	5,39	2,53	3,74	442
49	GN2	26,93	2,71	13,99	7,27	51,97	64,50	146,0	5,34	4,30	3,64	1,98	6,04	5,52	5,40	2,33	440
50	GN23	23,85	2,80	18,09	8,88	49,09	41,13	230,0	4,78	3,50	3,12	2,14	5,19	5,19	2,23	3,74	436
51	GN98	19,45	3,00	15,14	8,71	57,53	33,29	130,0	5,12	3,50	5,78	5,18	6,23	7,71	2,75	3,20	434
52	GN43	15,52	2,50	7,34	5,05	68,80	42,75	132,0	5,18	3,90	2,33	1,25	4,84	5,76	1,99	3,74	434
53	GN104	37,55	2,50	25,75	11,15	43,31	55,45	200,0	6,74	4,40	3,32	2,42	5,09	4,37	1,87	2,80	434
54	GN92	22,00	2,90	15,77	7,81	49,52	37,79	189,0	8,22	3,40	3,14	2,16	5,97	5,82	2,00	3,74	426
55	GN57	26,77	2,50	24,71	12,19	49,32	40,41	202,0	7,13	3,30	3,00	2,74	5,92	5,33	2,89	3,74	425
56	GN47	14,45	2,80	11,50	5,85	50,87	36,97	122,5	4,54	3,90	4,28	3,48	5,68	6,28	2,38	3,74	417
57	GN88	36,96	2,25	33,46	16,78	50,15	52,61	107,0	4,78	4,80	4,64	3,82	6,67	5,34	2,49	3,74	416
58	GN99	28,88	2,70	26,90	12,78	47,51	45,36	214,0	5,64	2,80	3,18	3,06	5,70	5,21	2,07	2,80	416
59	GN22	23,08	2,80	19,07	8,92	46,78	38,63	200,0	4,57	3,60	3,06	2,44	5,37	5,65	2,22	3,74	414
60	GN72	27,82	2,50	27,09	11,10	40,97	51,03	167,0	5,35	4,30	3,47	3,39	6,21	5,60	2,77	3,74	414
61	GN81	15,00	3,30	14,38	7,53	52,36	48,10	139,0	5,31	2,40	1,50	1,44	4,96	5,04	1,86	3,74	413
62	GN35	23,23	2,90	21,75	8,55	39,31	46,58	227,0	4,33	2,80	3,62	3,92	6,19	6,04	2,84	4,00	411
63	GN77	20,50	2,70	20,26	9,45	46,64	39,03	176,0	4,39	4,00	4,06	3,92	6,13	5,48	3,20	4,00	411
64	GN100	30,68	2,50	27,71	12,88	46,48	53,75	147,0	5,23	4,00	2,36	1,95	4,70	5,91	2,64	3,00	410
65	GN112	13,04	3,00	11,59	5,47	47,16	37,02	173,0	5,92	3,50	4,78	4,49	4,96	5,32	2,59	3,74	409
66	GN21	10,00	2,80	9,33	4,97	53,30	26,93	165,0	4,51	4,50	2,14	1,56	5,71	5,02	2,98	4,00	408
67	GN36	23,10	2,80	22,18	9,20	41,48	36,98	186,0	4,47	3,70	3,22	3,10	5,85	5,52	2,51	3,80	407
68	GN95	15,78	2,50	15,28	8,03	52,55	42,04	180,0	4,34	4,00	3,10	2,30	4,85	4,15	2,48	4,20	406
69	GN29	13,78	3,00	8,76	4,26	48,63	36,56	170,0	5,10	4,00	2,60	2,40	5,17	5,54	2,00	4,50	398
70	GN67	12,84	2,50	11,52	6,54	56,77	34,06	18,10	5,12	3,00	3,30	3,10	6,29	5,92	2,48	3,30	393
71	GN49	19,31	2,50	17,98	8,52	47,39	44,22	14,30	4,95	3,80	4,06	3,90	5,36	6,46	2,58	3,74	391

GNTP= Genotype code; BWW=Bunch wet weight (g); Y= Yield (1/4); BDW=Bunch dried weight (g); FPP=Fruit flesh powder (g); FPPE= Fruit flesh powder efficiency (%); BW=Bunch width (mm); BS=Bunch size (mm); DBS=Diameter of bunch stem (mm); DFB=Density of fruit on the bunch (1/3); WWFP=Weight of 100 wet fruit pieces (g); WDFP=Weight of 100 dried fruit pieces (g); FW=Fruit width (mm); FS=Fruit Size (mm); TP=Thickness of pericarp (mm); WCF=Waxy cuticle on fruit (1/5)

CONCLUSION

It has been revealed that there are sumac genotypes with different botanical characteristics (*R. coriaria*) which have a natural distribution in the Eastern Mediterranean Transition Region and these genotypes might be used in modern fruit growing. It was understood that these genotypes showed wide variations in terms of pomological characteristics and it is concluded that 20 genotypes showed more promising characteristics and it is important to use them in future studies. In addition, the molecular identification of 20 botanically defined genotypes will be used for future hybridizations.

Table 3(c). Pomological analyzes of selected Sumac (*R.coriaria* L) genotypes

NO	GNTP	BWW	Y	BDW	FPP	FPPE	BW	BS	DBS	DFB	WWFP	WDFP	FW	FS	TP	WCF	TOTAL
72	GN114	32,79	2,50	25,38	8,56	33,73	50,67	243,0	6,56	4,00	3,30	2,76	5,67	5,22	2,92	4,50	391
73	GN80	17,58	2,50	17,51	8,20	46,83	45,77	116,0	4,53	3,80	3,06	1,17	6,59	5,76	2,69	3,74	386
74	GN8	41,58	1,80	26,44	11,14	42,13	53,80	225,0	10,59	4,00	4,08	3,44	5,58	5,93	2,50	3,00	380
75	GN66	15,13	2,75	14,13	6,67	47,20	33,88	160,0	4,45	3,80	2,20	1,95	6,14	5,04	2,71	3,74	375
76	GN74	12,15	2,75	12,09	6,01	49,71	32,01	135,0	3,44	4,00	2,90	2,78	5,05	5,27	3,17	3,20	374
77	GN44	16,03	2,75	13,50	5,96	44,15	46,13	203,0	4,31	3,00	2,38	2,07	4,59	5,11	2,40	3,74	367
78	GN97	17,34	2,00	14,77	7,52	50,90	65,67	186,0	4,56	3,50	3,44	3,12	5,47	5,25	2,45	3,00	365
79	GN55	16,11	2,40	15,25	7,39	48,46	39,54	153,0	5,90	4,00	2,84	2,90	4,73	5,10	2,08	3,74	363
80	GN71	11,23	2,00	10,67	5,35	50,14	42,31	151,0	2,67	3,80	4,12	3,76	5,12	6,16	3,01	4,50	363
81	GN69	18,72	3,00	18,49	7,21	38,99	32,86	175,0	5,63	3,70	2,47	2,35	4,34	5,06	2,23	3,74	361
82	GN78	13,37	3,00	12,33	5,75	46,63	32,09	184,0	5,15	2,50	1,80	1,74	5,62	6,16	2,09	3,74	355
83	GN38	22,89	2,50	16,29	7,25	44,51	43,52	183,0	5,01	3,80	1,85	1,43	4,31	3,69	1,99	3,74	348
84	GN52	21,15	2,00	19,61	7,55	38,50	45,64	186,0	7,07	3,80	4,02	4,00	6,66	5,43	2,74	3,74	345
85	GN82	10,01	2,25	24,15	11,42	49,15	32,51	162,0	6,47	2,30	2,35	2,76	4,63	5,28	2,41	3,74	342
86	GN46	9,90	2,75	3,15	2,05	65,08	28,94	95,0	3,38	1,50	0,92	0,82	4,92	4,25	2,52	3,74	339
87	GN103	26,92	2,00	16,83	7,25	43,08	66,62	292,0	5,12	2,70	1,83	1,48	5,66	5,65	2,34	4,00	335
88	GN41	6,00	3,20	5,93	3,44	58,01	29,02	153,0	5,26	1,70	1,04	0,30	3,65	3,53	1,73	3,74	331
89	GN73	10,63	2,50	10,14	4,86	47,93	32,91	200,0	5,48	2,20	2,49	4,86	5,75	5,11	2,63	3,74	326
90	GN70	9,28	3,00	8,91	4,39	49,27	28,41	150,0	3,21	2,30	1,68	1,47	4,76	4,65	1,97	3,74	322
91	GN79	10,83	2,00	10,30	4,77	46,31	34,01	168,0	5,15	3,10	2,04	1,98	4,54	4,80	2,40	3,74	286
92	GN28	5,63	2,40	4,98	2,80	56,22	38,53	134,0	5,01	2,00	0,87	0,71	4,35	4,28	0,97	3,74	284
Minimum		**5,63**	**1,80**	**3,15**	**2,05**	**30,63**	**26,93**	**95,0**	**2,67**	**1,50**	**0,87**	**0,30**	**3,56**	**3,53**	**0,97**	**2,33**	**284**
Maximum		**87,74**	**3,60**	**67,82**	**28,98**	**72,49**	**96,77**	**292,0**	**10,59**	**5,00**	**6,18**	**5,18**	**7,14**	**7,71**	**5,40**	**4,85**	**760**
Mean		**31,80**	**2,71**	**24,15**	**11,42**	**49,15**	**50,24**	**186,2**	**5,57**	**3,88**	**3,45**	**2,76**	**5,57**	**5,55**	**2,52**	**3,74**	**456**
Stand. deviation		±18,14	±0,40	±13,07	±5,69	±7,83	±14,60	±4,34	±1,27	±0,74	±1,05	±0,92	±0,71	±0,69	±0,49	±0,43	±89

GNTP= Genotype code; BWW=Bunch wet weight (g); Y= Yield (1/4); BDW=Bunch dried weight (g); FPP=Fruit flesh powder (g);FPPE= Fruit flesh powder efficiency (%);BW=Bunch width (mm);BS=Bunch size (mm);DBS=Diameter of bunch stem (mm);DFB=Density of fruit on the bunch (1/3);WWFP=Weight of 100 wet fruit pieces (g);WDFP=Weight of 100 dried fruit pieces (g);FW=Fruit width (mm);FS=Fruit Size (mm); TP=Thickness of pericarp (mm); WCF=Waxy cuticle on fruit (1/5)

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

This article is produced from the data obtained within the scope of Project TAGEM/TBAD/B/20/A7/P6/1737 supported by TAGEM.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgments

The Authors are thankful to TAGEM.

REFERENCES

Alsamri H., Athamneh K., Pintus G., Eid A. H., Iratni R. (2021). Pharmacological and antioxidant activities of *Rhus coriaria L.* (Sumac), Antioxidants. 10(73):1-27. https://doi.org/10.3390/antiox10010073

Amiri S., Mohammadi R. 2021. Establishment of an efficient in vitro propagation protocol for Sumac (*Rhus coriaria L.*)

and confirmation of genetic homogeneity. Scientific Reports, 11(1): 1-9. https://doi.org/10.1038/s41598-020-80550-4

Basoglu F., Cemeroglu B. (1984). Research the chemical composition of sumac (in Turkish) . Food, 9(3): 161-172. https://dergipark.org.tr/tr/download/issue-full-file/20894

Bloshenko E. K., Letchamo W. (1995). Characterization of natural distribution and some biological traits of Sumach (*Rhus coriaria*) in central Asia. In International Symposium on Medicinal and Aromatic Plants, 426: 113-122. https://doi.org/10.17660/ActaHortic.1996.426.12

Caliskan R., Polat S., Buyukkilic Altinbasak B., Dinc H. O.,

Yuksel Mayda P., İsaa G. (2022). Bioactive components and antioxidant and antimicrobial activity of *Rhus coriaria* species of sumac in Turkey. 4.th International Congress of Muldispilinary Studies on Medicinal Science Antalya, Turkey. 10-11.

Corbaci O. L., Ertekin M., Ozyavuz M. 2011. Landscape architecture applications in arid and semi-arid areas . Management Workshop of Arid and Semi-Arid Area . Workshop proceeding. Nevsehir, Turkey. 5-8.

Davis C. T. (1885). The Manufacture of leather: Description of all of the processes for the tanning, tawing, currying and finishing. HC Baird & Co.

Edwards R.A., Thomas M.B. (1980). Observations on physical barriers to root formation in cuttings. Plant Propagation, 26: 6-8.

Elagbar, Z.A., Shakya A.K., Barhoumi L.M., Al-Jaber H.I. (2020). Phytochemical diversity and pharmacological properties of *Rhus coriaria*. Chemistry Biodiversity, 6: 1-17. https://doi.org/10.1002/cbdv.201900561

Eminagaoglu, O., Ozcan M. 2018. Morphological and anatomical studies of the newly recorded *Rhus chinensis* Mill.(Anacardiaceae) from Turkey. Bangladesh Journal of Plant Taxonomy, 25(1): 71-78. https://doi.org/10.1002/cbdv.201900561

Fereidoonfar H., Salehi-Arjmand H., Khadivi A., Akramian M. (2018). Morphological variability of sumac (*R.coriaria* L.) germplasm using multivariate analysis Industrial Crops and Products, 162-170. https://doi.org/10.1016/j.indcrop.2018.04.034

Gezici, S.(2019). The neuroprotective effect, antimicrobial and antioxidant potentials of sumac (*Rhus coriaria* L.) fruit extracts. Hacettepe Journal of Biology and Chemistry, 47(2): 165-170. https://doi.org/10.15671/HJBC.621202

Gokturk A., Olmez Z., Temel, F. (2006). Some native plants for erosion control efforts in coruh river valley, Artvin, Turkey. Pakistan Journal of Biological Sciences, 9(4): 667-673. https://10.3923/pjbs.2006.667.673

Guvenc G., Ozcan A., Bukucu, S.B., Sutyemez, M. (2017). Determination of Phenological Characteristics between Some Elm-Leaved Sumac (*Rhus Coriaria L.*) Genotypes. Turkish Journal of Agricultural and Natural Sciences, 4(4): 477-483.

Karakuzulu H. (2018). Determination of mineral content and antimicrobial activities of sumac (*Rhus coriaria L.*) grown under Kilis ecological conditions. Kilis 7 Aralık University. 50 s.

Khaula Sakhr, Sami El Khatib. (2020). Physiochemical properties and medicinal, nutritional and industrial applications of Lebanese Sumac (Syrian Sumac - *Rhus coriaria*): A review. Heliyon, 6: 1-9. https://doi.org/10.1016/j.heliyon.2020.e03207

Kheiri F., Rahimian Y., Nasr, J. (2015). Application of sumac and dried whey in female broiler feed. Archives Animal Breeding, 58(1): 205-210. https://doi.org/10.5194/aab-58-205-2015

Kizil S., Turk M. (2010). Microelement contents and fatty acid compositions of *Rhus coriaria L.* and *Pistacia terebinthus L.* fruits spread commonly in the southeastern Anatolia region of Turkey. Natural Product Research, 24(1): 92-98. https://doi.org/10.1080/14786410903132555

Kurucu S., Koyuncu M., Guvenç (Koroglu) A., Baser K. H. C., Ozek T. (1993). The Essential oils of *Rhus coriaria* L. (Sumac). Journal of Essential Oil Research, 5: 481-486. https://doi.org/10.1080/10412905.1993.9698267

Kurt R., Karayilmazlar S., Cabuk, Y. (2014). Determination of Foreign Trade of Non-Wood Tanning Materials. II. National Mediterranean Forest and Environment Symposium. Isparta, Turkey, p. 22-24.

Langroodi A. M., Tajik H.,Mehdizadeh T. (2018). Preservative effects of sumac hydro-alcoholic extract and chitosan coating enriched along with zataria multiflora boiss essential oil on the quality of beef during storage. In Veterinary Research Forum, 9(2): 153. https://doi.org/10.30466/VRF.2018.30831

Mazaheri T. M., Hesarinejad M.., Seyed M. R., Mohammadian R., Poorkian S. (2017). Comparing physicochemical properties and antioxidant potential of sumac from Iran and Turkey. Food Processing & Technology, 5(2): 288-294. https://10.15406/mojfpt.2017.05.00125

Muhamed H. N., Dawod N. A. (2017). The effect of IBA and cutting diameter on inducing roots of *Cotinus Coggygria* Scop. and *Rhus Coriaria* L. (*Anacardiaceae*). Science Journal of University of Zakho, 5(2):193-197. https://doi.org/10.25271/2017.5.2.367

Ozcan A., Susluoglu Z., Nogay G., Ergun M., Sutyemez M. (2021). Phytochemical characterization of some sumac (*Rhus coriaria L.*) genotypes from southern part of Turkey. Food Chemistry, *358*: 129779. https://doi.org/10.1016/j.foodchem.2021.129779

Pipinis E., Milios E., Aslanidou M., Mavrokordopoulou O., Efthymiou E., Smiris P. (2017). Effects of sulphuric acid scarification, cold stratification and plant growth regulators on the germination of *Rhus coriaria L.* seeds. Journal of Environmental Protection and Ecology, 18(2): 544-552.

Sutyemez M., Guvenc G., Bukucu S. B., Ozcan A. (2019). The Determination of genetic diversity among some sumac (*Rhus coriaria* L.) genotypes. Erwerbs-Obstbau, 61(4), 355-361. https://doi.org/10.1007/s10341-019-00440-6

Tiryaki G. Y. (2010). Kahramanmaras province produced in a symbolic traditional product: Concentrated citrus tasting water extract of sumac (*Rhus coriaria L.*) fruit. Journal of food engineering, (31): 54-60 (in Turkish).

Ugur R., Paydas Kargi S. (2017). Investigation of growing situations of apricot varieties grafted on clonal rootstock candidate some wild plum genotypes obtained by selection. Turkish Journal of Natural and Agricultural Science, 5: 64-70. https://dergipark.org.tr/en/pub/turkjans/issue/34475/381134

Uzun, E. (2016). The effect of seed size and some pre-treatments on the germination of sumac (*Rhus coriaria L.*) seeds. Master's dissertation, Artvin Coruh University, 28 p.

Sakhr K., El Khatib S. (2020). Physiochemical properties and medicinal, nutritional and industrial applications of Lebanese Sumac (Syrian Sumac-*Rhus coriaria*): A review. Heliyon, *6*(1). https://doi.org/10.1016/j.heliyon.2020.e03207

Saltan F. Z., Under D. (2019) Sumac and Its Important Biological Effects. Çukurova J. Agric. Food Sci, 34(1): 69-78. https://dergipark.org.tr/en/pub/cutarim/issue/47199/551485

Sezgin, O. (2015). Determination of the phonological morphological and phenological characteristics of wild red currant (*Ribes rubrum L.*) found in the flora of Turkey. Master's dissertation, Namık Kemal University 49 p. https://dergipark.org.tr/tr/download/article-file/354924

Wetherilt H., Pala M. (1994). Herbs and spices indigenous to Turkey. Developments in food science, 764.

Yilmaz I. A. (2021). Determination of promising genotypes in derici sumac (*Rhus coriaria L.*) population in Dulkadiroglu, Kahramanmaras. Master's dissertation, Kahramanmaras Sutcu İmam University, 83 p.

Yucedag C., Gultekin H., Pirlak I. (2010). Effects of sowing time and covering on germinatıon of sumac (*Rhus coriaria L.*) seeds in greenhouse and fıeld . Turkish Journal of Forestry, 1(1): 9-15. https://dergipark.org.tr/en/download/article-file/195762

Yuksel, E. (2018). Investigation phytochemical components of sumac plant growing in Tunceli. Master's dissertation, Munzur University, 69 p.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 192-196
DOI: https://doi.org/10.31015/jaefs.2023.1.23

The effect of altitude on soil organic carbon content in semi-arid mediterranean climate

Tugba Simsek[1] • **Ceren Ayse Bayram**[2] • **Gokhan Buyuk**[3] • **Erhan Akca**[4] • **Nilgun Kalkanci**[5]

[1] Ministry of Agriculture and Forestry, Directorate of Pistachio Research Institute, Gaziantep, Türkiye
[2] Adıyaman University, Kahta Vocational School, Kahta, Adıyaman, Türkiye
[3] Adıyaman University, Faculty of Agriculture, Kahta, Adıyaman, Türkiye
[4] Adıyaman University, Vocational School of Technical Sciences, Adıyaman, Türkiye
[5] Ministry of Agriculture and Forestry, Directorate of Pistachio Research Institute, Gaziantep, Türkiye

Citation: Simsek, T., Bayram, C.A., Buyuk, G., Akca, E., Kalkanci, N. (2023). The effect of altitude on soil organic carbon content in semi-arid mediterranean climate. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 192-196

Received: 30 November 2022
Revised: 25 December 2022
Accepted: 30 December 2022
Published Online: 21 March 2023

Correspondence: Ceren Ayse Bayram
E-mail: cerenaysenazik@gmail.com

Abstract

One of the most effective means in the combating climate change and desertification is soil organic carbon (SOC) management. However, land use puts a high pressure to fragile SOC pools particularly in semi-arid environments where SOC decomposition rate is high due to low soil moisture. Therefore, at higher elevations of Mediterranean Basin with cooler temperature SOC is higher than the coastal plains due to the better soil moisture contents. Agricultural pressure on highlands has increased in recent years because of the relatively low water requirement of crops. The purpose of this study is to analyze and determine the SOC dynamics in relation to the variations of soil physical and chemical characteristics from different elevations, ranging from 64 meters to 756 meters at semi-arid Mediterranean climate. SOC revealed decreases versus altitude increases that varied from 24.7 to 38.7 t ha^{-1} with a correlation coefficient of 0.527. The main driver of decreasing SOC by elevation is most probably displacing of fine particles from surface horizons by accelerated erosion at sloping and cultivated lands of higher altitudes. As a result, it is necessary to focus both on the plant pattern along with land management techniques for enhancing soil organic matter in agricultural production for enhancing SOC at higher elevations.

Keywords: Soil organic carbon, Altitude, Land use, Erosion

INTRODUCTION

Soil organic carbon is an effective tool in efforts to mitigate climate change and desertification that threaten the future of humanity on a global scale, thus enhancing knowledge on its management is crucial for the future of humanity. However, as soil carbon dynamics are driven by several factors such as climate, soil physical and chemical characteristics along with land management (Ramesh et al., 2019, Büyük et al., 2020) it is challenging to manifest all processes effect on it. Many studies have revealed the effect of climate on organic carbon by studying the interaction between climate and organic carbon (Jobbágy and Jackson, 2000). The effect of altitude on soil organic matter has been studied and it has been determined that there are differences in soil organic matter due to the altitudes effect on climate (Oechel et al., 2000). In general, soils in higher altitudes with cooler temperature in Mediterranean Basin have higher organic carbon content even in cultivated soils (GDCDE, 2018). However, the low irrigation requirement led to a farming pressure in high-altitude regions recently and that necessitates concerns about the sustainable management of soils (Sharma et al., 2006). Dieleman et al., (2013) reported that SOC stocks varied from 4.8 to 19.4 kg C m^{-2} at different altitudes and increased by 5.1 kg C m^{-2} for every 1000m increase. Aside from wider altitude changes, even a few meters

make a difference in vegetation as determined by Yılmaz et al., (2020) in lagoonal areas adjacent to Mediterranean Sea in Southern Turkey. Microclimate effects of altitude on soil microbiology, C weathering and accumulation rates were studied by Matus et al., (2014). They stated that there is no single formula for the stabilization of soil organic matter at various altitudes because it is reliant on various parameters. However, although there is a positive relationship between altitude and organic matter increases in general to a certain height up to 1500 m in Garhwal Himalaya and Serbia (Manojlović et al., 2011; Sheikh et al., 2009), there are also opposite situations as Bangroo et al., (2017) determined a negative relation between soil organic carbon with increasing altitudes above 1800 m. Majority of previous studies focused on large variations in altitude and the soil organic carbon of coastal areas at sea level and mountainous lands over 2000 m (Akça et al., 2020). However, since only one crop is obtained per year in agriculture at altitudes above 1000 m in the Mediterranean, land use below 1000 m is more important in terms of agriculture and income of locals. Therefore, this study sought to determine the changes in soil's physical and chemical properties through six different altitudes varying from 64 m to 756 m within an extensively cultivated basin under Mediterranean climate. In conclusion, we suggest that the determination of the relationship between altitude and organic matter for each significant agricultural region is necessary in terms providing reference data in sustainable land management decisions.

MATERIALS AND METHODS

Site Characteristics

The study area, covering 327990 ha, is located between 35° 52'- 36° 42'E and 36° 57'- 37°45'N is located in the east of the Mediterranean Region of S. Turkey. The altitude of the basin ranges from 64 m to 756 m above sea level. Long term average monthly climate data of the lowest and highest locations are given in Table 1 (1986-2019).

Soils were sampled from six sites located between 64 m to 756 m (Toprakkale 64 m, Kadirli 95 m, Osmaniye 121 m; Düziçi 389m; Bahçe 582 m and Hasanbeyli 756m). A total of 165 (Toprakkale 10, Kadirli 83, Osmaniye 40, Düziçi 25, Bahçe 1, Hasanbeyli 6) disturbed and undisturbed samples were taken from 0-20 cm in 2018-2019.

Laboratory Analysis

Soil samples were air-dried and sieved through a 2 mm sieve for analysis. Undisturbed soil samples were collected with 100 cm^3 steel cylinders. Soil texture, bulk density, electrical conductivity and pH (saturated paste) organic matter (wet digestion), soil organic carbon (Total Organic Carbonizer), $CaCO_3$, P, Zn, Fe, Cu and Mn were determined according to methods defined in Soil Survey Staff (2014). For each site, soil C content was calculated on a hectare basis (Lee et al., 2009).

Data Analyses

Analysis of variance was performed to evaluate the data obtained from soil samples taken from regions at different heights and points representing the lands to determine the differences in the physical and chemical properties of the soil. Data analyses were done to investigate the relationship between soil bulk density, organic matter, soil organic carbon, $CaCO_3$, pH, EC, Silt, Sand, Clay, P, K, Ca, Mg, Na, B, Fe, Cu, Zn and Mn from the six different altitudes. Correlation and regression analyses were performed.

RESULTS AND DISCUSSION

In the study area, with the change of altitude, there was also a differentiation in soil properties. The texture of the soils was silty clay loam and silty loam at higher altitudes, and clay or clay-loam in lower altitudes (Table 2). The most significant effect of altitude on soil texture seems to be erosion as it most probably washed-out fine particles from surface in sloping higher elevations (Table 2 and Figure 1).

In general, as altitude increases, there was a decrease in silt and clay content while sand revealed an increase. The sand content is negatively correlated to the low altitude with -0.736 (Table 3). The lowest $CaCO_3$ was determined at 582 m by 1.67 % while the highest was determined at 95 m. These high values are most probably due to the carbonate-rich alluvial sediment deposition in Kadirli (95 m) and Toprakkale (64 m) along with exposure of eroded C horizon at 756 m. Soil pH also showed similarities with $CaCO_3$, with 6.45 at 582 m and 7.98, 7.60 and 7.44 at 64 m, 95 m and 756 m respectively.

Table 1. Long term average monthly climate data of the lowest and highest locations

	Toprakkale (64 m)	Hasanbeyli (756 m)
Average temp. (°C)	18.5	9
Aver. high temp (°C)	24.8	20.8
Aver. low temp (°C)	12.7	1
The highest temp. (°C)	43.6	32
The lowest temp. (°C)	12.7	17
Average Rainy Days	78	66.4
Average Precipitation (mm)	854.9	424

Table 2. Altitudinal variation in physical and chemical properties (means±SD) across six different sites

Parameters	Locations (m)					
	Toprakkale 64	Kadirli 95	Osmaniye 121	Düziçi 389	Bahçe 582	Hasanbeyli 756
$CaCO_3$ (%)	14.1±5.80	41.7±10.4	26.6±9.27	22.4±10.32	1.67±0.59	14.3±7.89
pH	7.98±0.09	7.60±0.20	7.91±0.19	7.49±0.37	6.45±0.28	7.44±0.47
EC (dSm^{-1})	0.74±0.14	0.91±0.35	0.85±0.41	0.63±0.26	0.94±0.08	0.67±0.13
Silt (%)	23.4±5.32	26.7±8.02	23.8±20.4	18.7±4.66	20.4±2.50	21.4±7.87
Sand (%)	42.1±9.86	36.8±12.0	36.4±13.3	51.9±13.9	66.1±8.50	52.1±18.46
Clay (%)	34.5±7.38	36.5±11.2	39.9±10.54	29.4±10.7	13.5±2.29	26.5±11.78
Texture Class	SCL	CL	C	SCL	SL	SCL

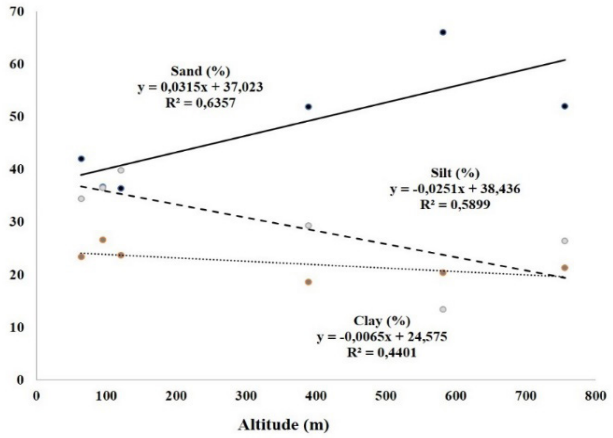


Figure 1. Effect of altitude on soil texture

Table 3. Correlation coefficient of physico-chemical properties of soil with altitude

Parameters	Correlation coefficient (r)
$CaCO_3$ (%)	0.441
pH	0.653
EC (dSm^{-1})	0.229
Silt (%)	0.757
Sand (%)	-0.736
Clay (%)	0.665
SOC (t C ha^{-1})	0.527
OM (%)	0.543

The clay content of the soils varied from 13.5 to 39.9 %. The clay in the agricultural lands located in low altitude areas is higher than that in the high-altitude areas due to deposition of clay-rich alluvial sediments (Table 2). Bulk density changed from 1.30 at Hasanbeyli at 756 m to 1.43 g cm^3 at Toprakkale at 64 m (Table 4). This variability is closely related to the organic matter and texture change (Reichert et al., 2018). Bulk density also decreased with increased altitude due to erosion of fine particles. The organic matter of the soils is decreased with the increase in altitude which varied from 1.42 to 2.21 % (Table 4) with the highest mean value of 2.21±0.80 at 64 m followed by 2.10±0.09 at 95 m, 1.95±0.7 at 121 m, 1.88±0.91 at 389 m, 1.56±0.11 at 582 m and 1.4±0.15 at 756 m (Table 4). This may be attributed to a lack of clay at higher altitudes, which holds more water and enhances aggregation that both have positive effect on soil carbon sequestration (Oades 1988; Wagner 2007). Moreover, the decreasing precipitation with altitude seems to be another parameter effective on the SOC content of the studied soils.

Climate characteristics along with altitude affect soil organic matter dynamics. Göl (2017) and Yao et al., (2019) reported that altitude changes in the ecosystem led to changes in both the quantity and quality of soil organic matter. The organic matter content is positively correlated to the decreasing altitude with 0.543 in the study site. However, while Kidanemariam et al., (2012) found increasing SOC with elevation, Bangroo et al., (2017) discussed the negative affect of decreasing

Table 4. Soil Bulk Density, OM, Carbon and SOC (means±SD) stock (0-20 cm) of six sampling sites

Site	Altitude	Bulk density	Organic matter	C	SOC
		g/cm^3	%	%	t ha^{-1}
Toprakkale	64	1.41±0.10	2.21±0.80	1.27±0.46	38.4±12.8
Kadirli	95	1.38±0.10	2.10±0.09	1.19±0.46	36.5±12.0
Osmaniye	121	1.37±0.11	1.95±0.7	1.10±0.41	33.0±11.8
Düziçi	389	1.43±0.08	1.88±0.91	1.13±0.53	32.7±14.9
Bahçe	582	1.30±0.02	1.56±0.11	0.84±0.07	27.1±1.20
Hasanbeyli	756	1.30±0.15	1.42±1.01	0.77±0.58	24.7±14.7

altitude on SOC sequestration. Thus, altitude does not seem to be the sole parameter effecting SOC dynamics.

SOC decreased as altitude increases that varied from 24.7 to 38.7 t ha^{-1} (Table 4) with a correlation coefficient of 0.527 (Table 3).

Along with effect of lowerclay, higher altitudes are known to decrease leaf surface area, number of stomas along with low shoot development, which all cause reduced biomass development and this in turn negatively affect soil organic matter (Pirlak et al., 2003; Aslantaş and Karakurt, 2007).

Micro-macro nutrient of soils revealed some significant correlation with the altitude. There was a positive correlation between soil P (0.667), K (0.3798), Ca (0.292), B (0.215), Cu (0.675), Zn (0.441) and altitude, while Mg (-0.266), Na (-0.374), Fe (-0.484) and Mn (-0.579) revealed a negative correlation (Table 5). Smectite has been reported (Akça et al., 2009) as the most common clay type in the region, it is likely that the decrease in Mg and Fe content with height is related to the decrease in clay content. Thus, decreases in micro-macro element after 582 m is another indicator of transported surface clayey soil rich in plant nutrients.

Table 5. Correlation coefficient and concentration of some macro and micro nutrients

Parameters	Altitude (m)						Correlation coefficient (r)
	64	95	121	389	582	756	
P (mg kg^{-1})	6.95	12.1	15.2	20.2	15.6	17.9	0.667
K (mg kg^{-1})	492	221	364.2	461.8	475.7	418.9	0.379
Ca (mg kg^{-1})	7287.8	410	5052.2	5010.2	7171	5115.3	0.292
Mg (mg kg^{-1})	1266.2	95	1666.8	778.3	815.7	637.8	-0.266
Na (mg kg^{-1})	370.7	277.8	362	175.7	441.6	147.6	-0.374
B (mg kg^{-1})	1.6	0.9	1.2	1.4	1.3	1.4	0.215
Fe (mg kg^{-1})	1.15	11.8	2.1	0.9	0.6	0.7	-0.484
Cu (mg kg^{-1})	0.56	0.2	0.8	1.1	1.1	0.9	0.675
Zn (mg kg^{-1})	1.16	0.3	0.9	0.9	0.8	1.3	0.441
Mn (mg kg^{-1})	26.6	10.9	44.9	13.8	10.7	10.4	-0.579

CONCLUSION

Organic matter is expected to increase as altitude increases in the Mediterranean Basin, according to common findings. As there is less evaporation at high altitudes, there is more available water in the soil profile, which leads to more biomass development in cultivation. In the case of natural regions, this is a sound argument. And it was investigated in this research whether the same judgment could be applied to agricultural areas. One of the major findings was the negative relationship between increased altitude and organic matter in sloping cultivated areas. Along with organic matter, the clay content also decreased with the increased altitude. The change in clay content is most probably due to the transport of fine material by erosion at high altitudes from sloping cultivated lands as in sampling points C horizon were exposed to the surface. Although shifting crops to higher altitudes to prevent rising temperature and drought induced by climate change can be a type of climate adaptation strategy. However, at high elevations with sloping topography erosion is severe risk at cultivated lands which is one of driver of soil degradation, So, this study revealed that priority should be given to combating erosion in land-use to increase soil organic matter at altitudes from 60 m to 1000 m in a semi-arid Mediterranean climate. The necessity to focus on the plant pattern for increasing the soil organic matter as well as combating erosion in highlands of Mediterranean basin is crucial as no land for cultivation is available beyond higher altitudes.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Akça, E., Arocena, J., Kelling, G., Nagano, T., Degryse, P., Poblome, J., Çambel, H., Buyuk, G., Tümay, T., S. Kapur. (2009). Firing temperatures and raw material sources of ancient Hittite ceramics of Asia Minor. *Transactions of the Indian Ceramic Society*. 68(1): 35-40. https://doi.org/10.1080/037175

0X.2009.11082160

Akça, E., Berberoğlu, S., Nagano, T., Kapur, S. (2020). Mediterranean anthroscapes. The Bioeconomy Approach: Constraints and Opportunities for Sustainable Development. 149.

Aslantaş, R., Karakurt, H. (2007). Rakimin Meyve Yetiştiriciliğinde Önemi ve Etkileri. *Alınteri ZBD*., 12(2), 31-37. (in Turkish)

Bangroo, S. A., Najar, G. R., Rasool, A. (2017). Effect of altitude and aspect on soil organic carbon and nitrogen stocks in the Himalayan Mawer Forest Range. *Catena*, 158, 63-68 (in Turkish). https://doi.org/10.1016/j.catena.2017.06.017

Büyük, G., Akça, E., Kume, T., Nagano, T. (2020). Biomass Effect on Soil Organic Carbon in Semi-Arid Continental Conditions in Central Turkey. *Pol. J. Environ. Stud*., 29(5), 3525-3533. https://doi.org/10.15244/pjoes/112619

Dieleman, W. I., Venter, M., Ramachandra, A., Krockenberger, A. K., Bird, M. I. (2013). Soil carbon stocks vary predictably with altitude in tropical forests: Implications for soil carbon storage. *Geoderma*, 204, 59-67. https://doi.org/10.1016/j.geoderma.2013.04.005

GDCDE. (2018). Soil Organic Carbon Project, Technical Summary", with the General Directorate of Combating Desertification and Soil Erosion (GDCDE), Ankara, Turkey (in Turkish).

Göl, C. (2017). Assessing the amount of soil organic matter and soil properties in high mountain forests in Central Anatolia and the effects of climate and altitude. *J. For. Sci*., 63(5), 199-205. https://doi.org/10.17221/70/2016-JFS

Jobbágy, E. G., and Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol Appl*., 10(2), 423-436. https://doi.org/10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2

Kidanemariam, A., Gebrekidan, H., Mamo, T., Kibret, K. (2012). Impact of altitude and land use type on some physical and chemical properties of acidic soils in Tsegede Highlands, Northern Ethiopia. *Open J. Soil Sci*., 2(03), 223. https://doi.org/10.4236/ojss.2012.23027

Lee J, Hopmans JW, Rolston DE, Baer SG, Six J. (2009). Determining soil carbon stock changes: simple bulk density corrections fail. *Agric Ecosyst Environ.* 134:251–256. https://doi.org/10.1016/j.agee.2009.07.006

Manojlović, M., Čabilovski, R., Sitaula, B. (2011). Soil Organic Carbon in Serbian Mountain Soils: Effects of Land Use and Altitude. *Pol. J. Environ. Stud*., 20(4).

Matus, F., Rumpel, C., Neculman, R., Panichini, M., Mora, M.L. (2014). Soil carbon storage and stabilization in a aridic soils: a review, *Catena*. 120,102-110. https://doi.org/10.1016/j.catena.2014.04.008

Oades J.M. (1988). The retention of organic-matter in soils. *Biogeochemistry*. 5(1):35–70.

Oechel, W. C., Vourlitis, G. L., Hastings, S. J., Zulueta, R.C., Hinzman, L., Kane, D. (2000). Acclimation of ecosystem CO_2 exchange in the Alaskan Arctic in response to decadal climate warming. *Nature*. 406, 978-981.

Pirlak, L., Güleryüz, M., Aslantaş, R., Eşitken, A. (2003). Promising native summer apple (Malus domestica) cultivars from north-eastern Anatolia, Turkey. *N.Z.J. Crop Hortic. Sci*., 31(4), 311-314. https://doi.org/10.1080/01140671.2003.9514266

Ramesh, T., Bolan, N. S., Kirkham, M. B., Wijesekara, H., Kanchikerimath, M., Rao, C. S., Freeman II, O. W. (2019). Soil organic carbon dynamics: Impact of land use changes and management practices: A review. *Adv. Agron*., 156, 1-107. https://doi.org/10.1016/bs.agron.2019.02.001

Reichert, J. M., Mentges, M. I., Rodrigues, M. F., Cavalli, J. P., Awe, G. O., Mentges, L. R. (2018). Compressibility and elasticity of subtropical no-till soils varying in granulometry organic matter, bulk density and moisture. *Catena*. 165, 345-357. https://doi.org/10.1016/j.catena.2018.02.014

Sharma, V. K., Dwivedi, K. S., Tripathi. D., Ahmed, Z. (2006). Status of available major and micro-nutrients in the soils of different blocks of Leh district of cold arid region of Ladakh in relation to soil characteristics. *J. Indian Soc. Soil Sci*., 54, 248-250.

Sheikh, M.A., Kumar, M., Bussmann R.W. (2009). Altitudinal variation in soil organic carbon stock in coniferous subtropical and broadleaf temperate forests in Garhwal Himalaya. *Carbon Balance and Manag*., 4:6. https://doi.org/10.1186/1750-0680-4-6

Soil Survey Staff. (2014). Keys to Soil Taxonomy. 12th ed. USDA National Resources Conservation Services, Washington DC. Retrieved from: https://www.nrcs.usda.gov/resources/guides-and-instructions/keys-to-soil-taxonomy

Wagner S., Cattle S.R., Scholten, T. (2007). Soil-aggregate formation as influenced by clay content and organic-matter amendment. *J. Plant Nutr. Soil Sci.* 170(1):173–180. https://doi.org/10.1002/jpln.200521732

Yao, L., Guo, N., He, Y., Xiao, Y., Li, Y., Gao, J., Guo, Y. (2019). Variations of soil organic matters and plant cuticular waxes along an altitude gradient in Qinghai-Tibet Plateau. *Plant Soil.* 1-18. https://doi.org/10.1007/s11104-019-04304-6

Yılmaz, K. T., Alphan, H., Kosztolányi, A., Ünlükaplan, Y., Derse, M. A. (2020). Coastal Wetland Monitoring and Mapping along the Turkish Mediterranean: Determining the Impact of Habitat Inundation on Breeding Bird Species. *J. Coast. Res*. 36(5), 961-972. https://doi.org/10.2112/JCOASTRES-D-19-00091.1

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 197-205
DOI: https://doi.org/10.31015/jaefs.2023.1.24

Bioactive compounds of hawthorn powders produced by convectional and lyophilized foam mat drying method

Bilge Ertekin Filiz[1]

[1] Food Engineering Department, Faculty of Engineering, Suleyman Demirel University, Isparta, Türkiye

Citation: Ertekin Filiz, B. (2023). Bioactive compounds of hawthorn powders produced by convectional and lyophilized foam mat drying method. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 197-205

Received: 11 January 2023
Revised: 13 February 2023
Accepted: 16 February 2023
Published Online: 22 March 2023

Correspondence: Bilge Ertekin Filiz
E-mail: bilgefiliz@sdu.edu.tr

Abstract

Fruit powders produced with drying technologies have a wide range of uses in the food industry. The fruit powders have the potential to be used as a food supplement or natural colorant thanks to their health-promoting functional properties. Hawthorn is one of the fruits that has attracted attention in recent years with its positive effects on health. In this study, hawthorn powder was produced by convective (C) and lyophilized (L) foam mat drying methods. In preliminary experiments, the best foam properties were obtained with 1% egg white powder. The foams were dried until their moisture content decreased to 4±0.5%. Three different temperatures (60-65-70°C) were used in convective foam mat drying. Total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (ABTS and DPPH) and phenolic composition were determined in the powders. The convective foam mat dried at 60 °C (C-60) and lyophilized foam mat dried (L) samples exhibited higher TPC and ABTS values than other samples. In powder and fresh samples, gallic acid, protocatechuic acid, chlorogenic acid, epicatechin, and catechin were detected with the liquid chromatographic method. Epicatechin and chlorogenic acid were the most abundant phenolic compounds in the samples. In the C-70 sample, epicatechin and protocatechuic acid were significantly lower ($p<0.05$). According to the results of the study, it was determined that the samples that applied the foam mat drying technique at 60 °C showed similar results with lyophilized foam mat drying. The foam mat drying method at 60 °C can be recommended as a preferred method in hawthorn powder production due to the reduction in drying time, low investment and operating costs.

Keywords: Hawthorn, Powder, Foam Mat Drying, Lyophilization, Phenolic compounds, Antioxidant

INTRODUCTION

Many fruit species are grown wild or culturally in Turkey, which is very rich in plant diversity. One of these fruits, the Hawthorn fruit, belongs to the genus *Crataegus* of the *Rosaceae* family. The hawthorn grows in the Northern Hemisphere, and it mainly grows in Western and Southern Anatolia in our country; it generally grows on slopes overlooking streams, in bushes, in rocky and stony places, in forests, or spread over mountainous areas (González-Jiménez et al., 2018).

Hawthorn contains high amounts of pectin (~9.94%), sugar compounds (~5.50%), and phenolic compounds such as anthocyanins (~0.03%) and flavonoids (~1.93%) (Cuevas-Bernardino et al., 2016; He et al., 2013). Among the phenolic compounds (+) catechin and (-)-epicatechin, procyanidin B2, procyanidin B5, procyanidin C1, and procyanidin D1, hyperoside, apigenin, quercetin, chlorogenic acid, gallic acid, vitexin, hesperetin, coumaric acid, caffeic acid, naringenin, cratenacin were

detected in hawthorn fruit, which has high antioxidant activity due to the phenolic compounds it contains (Coklar & Akbulut, 2016).

The awareness of hawthorn fruit is increasing day by day all over the world, especially because it has been used for therapeutic purposes for many years for medical purposes. Different parts of the plant (leaves, flowers, shoots, roots) and medicines prepared from them are used in traditional and complementary medicine practices and have been treated as medicine since ancient times (Chang et al., 2002). It is reported that it is widely used as a herbal medicine in various parts of the world for the treatment of gastrointestinal complaints, high blood pressure, sore throat, cough, flu, asthma, colds, skin diseases, nephritis, hemorrhoids, especially cardiovascular diseases (Dahmer & Scott, 2010; Nazhand et al., 2020). Many of which are wild, have recently been under the spotlight worldwide due to growing requests for natural and sustainable eco-compatible remedies for pathological conditions with beneficial health effects that are able to support/supplement a daily diet or to support and/or replace conventional pharmacological therapy. The main requests for these products are: safety, minimum adverse unwanted effects, better efficacy, greater bioavailability, and lower cost when compared with synthetic medications available on the market. One of these popular herbs is hawthorn (Crataegus spp.). These positive health effects are also associated with the flavonoids in hawthorn (Rigelsky & Sweet, 2002) pharmacology, clinical efficacy, dosage and administration, adverse effects, and drug interactions of hawthorn are discussed. Hawthorn (Crataegus oxyacantha) in animal studies, hawthorn has been shown to have positive effects on blood pressure, and blood lipids; antioxidant and anti-inflammatory effects. In addition, it has been reported that it may have protective effects on heart health and can be listed as a functional food against cardiovascular diseases (Kisioglu & Nergiz-Unal, 2018).

Despite the stated health benefits, fruits and vegetables, are easily perishable products with a moisture content of over 80%. It can be difficult to implement low-temperature storage techniques, which are the best way to keep the product fresh, during the distribution chain. Drying can be applied as the most appropriate post-harvest processing technique, especially in regions where cold chain applications and processing facilities are insufficient (Dev & Raghavan, 2012). In addition, uncultivated hawthorn-like fruits are offered for sale in small local markets in a short period of a year, especially for people living in big cities which are not easy to reach.

Physical and biochemical changes occur with the reduction and the mobility of water from food during the dehydration process. In addition to physical changes such as shape and structure changes, encrustation, and regional dry matter accumulation in the fruit structure (Cemeroğlu, 2003), changes occur in the color, flavor, and nutritional properties of the dried product (Nijhuis et al., 1998). The changes uniquely occur in each product, and the temperature intensity applied in the drying process significantly affects the level of these changes (Cemeroğlu, 2003).

In the dried foods group, dry powder foods have become preferred by consumers and manufacturers in recent years due to their various advantages. With the increasingly changing living conditions, consumers expect a single product they buy to meet all their requirements and provide ease of use. On the other hand, food manufacturers have expectations from the product such as long shelf life, easy processing, reduced packaging, storage, and transportation costs due to reduced volume/weight. For this reason, food manufacturers and consumers especially tend to foods in powder form. For powder food production, spray drying can be carried out by vacuum drying, freeze-drying and foam mat drying methods (Koç & Ertekin, 2016). However, various difficulties the use of spray drying poses due to the adhesiveness problem in drying foods such as fruit juices with low glass transition temperatures due to components such as sugars and organic acids. In vacuum drying and freeze-drying methods, due to drying at low temperatures, a sticky structure is formed with the removal of the vacuum, as well as preserving the nutrient content (Jiang et al., 2013). Also, freeze-drying and vacuum-drying methods have additional disadvantages such as high installation and operating costs due to low temperature and high vacuum applications long drying times, working in batch systems, low temperature and high vacuum applications (Sangamithra et al., 2015; Türker et al., 2018).

Foam mat drying is the process of converting a liquid or semi-liquid product into a stable foam and then drying it generally convectively. This drying technique gives good results especially in drying foods with high sugar content, viscous and low glass transition temperature. It is stated as an economical and practical alternative to other drying methods for food powder production (Qadri et al., 2020). The foam mat drying method has additional advantages of increasing food quality such as preservation of nutritional quality and bioactive components as a result of drying at a lower temperature and in a shorter time, and easy reconstitution (Sangamithra et al., 2015). Despite the advantages mentioned, in some products, low-quality products can be obtained compared to traditional powder product production methods such as spray drying with FMD. Therefore, research is carried out on hybrid drying methods that are alternatives to hot air drying methods in FMD. Hybrid methods are developed by applying FMD together with other drying methods such as vacuum drying, freeze drying and microwave drying (Qadri et al., 2020). Compared to other conventional drying methods, freeze-drying (FD) is

accepted as the method that minimizes the loss of taste, aroma and nutritive elements. However, due to the very high cost of production, the use of FD is restricted to high-value-added and sensitive products (Ratti, 2013). It is thought that the use of FD in combination with FMD as a hybrid method may provide an additional advantage as it can reduce the drying time of foamed and frozen products. There is a limited number of studies conducted with Foam Mat Freeze Drying (FMFD). This method has been applied in previous studies for drying egg white (Muthukumaran et al., 2008), apple juice (Raharitsifa & Ratti, 2010), dates (Seerangurayar et al., 2018), and blueberry juice (Darniadi et al., 2018).

In line with all these objectives, it was aimed to produce hawthorn powder by convectional foam mat drying (C) and lyophilized foam mat drying (L), and to examine the bioactive properties which provide antioxidant effects of the produced hawthorn powders.

MATERIALS AND METHODS

Foam Preparation and Drying

The hawthorn fruit used was obtained from the local market in October 2020 and 2021. The fruits were kept in the refrigerator at 4 ± 2 °C until used in the experiments. According to the results of preliminary experiments, the conditions to obtain stable foams were determined. After washing and sorting, boiled water at a ratio of 1:2 (w:v) was added to the fruits and left for 30 minutes. The fruit flesh obtained after the sorting process was crushed with a blender for about 1 minute. To minimize possible bioactive component losses, the amount of water-soluble dry matter (Brix) was adjusted to 3.5 by adding its boiling water. As a foaming agent, 1% (w/v) egg white powder (Gusto, İstanbul-Turkey) was used. After the foaming agent was added, a hand mixer (Arzum Ar1017 Prostick 1000 W, Turkey) was mixed at maximum speed for 5 minutes and foam was obtained. The foam obtained was homogeneously spread on 20 cm diameter stainless steel trays with a thickness of 5 mm.

Laboratory type digital tray convective dryer was used for drying (Dalle Lt-10, China). Three temperatures, 60, 65 and 70 °C (C-60, C-65, C-70) were chosen as the drying temperature. The foams obtained for freeze-drying (L) were frozen and then lyophilized in a freeze-drying device (Xianou-12 N Freeze Dryer) under a vacuum of 100 Pa. The drying was continued until the moisture content of the powders was 4 ± 0.5%. The samples that reached the desired humidity level were removed from the dryer and scraped with the help of a plastic spatula and homogeneous particle size was obtained by using a grinder (Fakir Hausgeräte, Germany). The production flow chart is given in Figure 1.

Determination of moisture and Water activity (aw)

An IR moisture analyzer (Kern & Sohn Gmbh, Germany) was used in the moisture analysis of powder samples. Temperature mode was selected as step (60°C, 70°C, 80°C). The water activity values of the powder samples were determined with a digital water activity meter (Novasina Lachen, Switzerland).

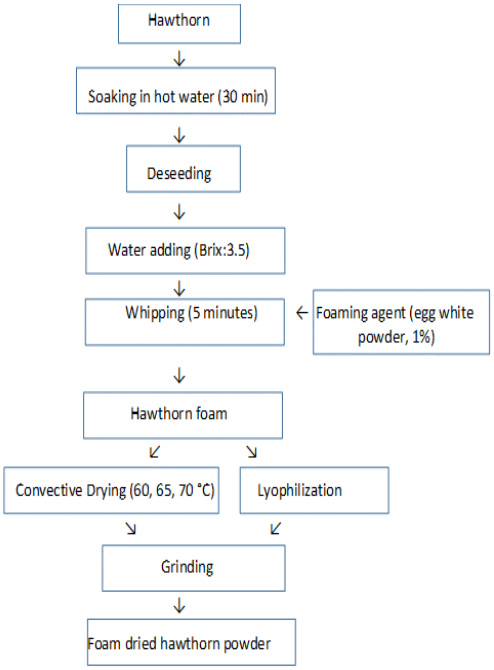


Figure 1. Flow chart for production of foam mat dried hawthorn powder

Total phenolic content (TPC) analysis

Total phenolic content (TPC) was determined according to the colorimetric Folin-Ciocalteu method (Singleton et al., 1999). After adding sodium carbonate solution (%20) and foline reagent (2 N) to the diluted sample, the tubes were kept in the dark for 120 minutes. The total amount of phenolic substance in the samples was calculated as gallic acid equivalent (GAE) from the gallic acid standard curve. The absorbance values were measured at 760 nm (Shimadzu Scientific Instruments, Inc., Tokyo, Japan). The concentration of total phenolic compounds in samples was calculated as gallic acid equivalent (GAE) using an equation obtained from the standard gallic acid curve (R^2 =0.993).

The total flavonoid content (TFC) analysis

The TFC of samples was determined using the spectrophotometric method as reported by (Zhishen et al., 1999). The absorbance of the mixture was measured at 510 nm after the addition of sodium nitrite (NaNO2), aluminum chloride (AlCl3) and NaOH solutions in the samples diluted with distilled water at appropriate ratios. A catechin calibration curve was created using a catechin solution in the range of 0-400 mg/L. Total flavonoid content was calculated from the calibration curve as catechin equivalent.

Determination of Antioxidant Activity (ABTS$^+$ and DPPH)

ABTS$^+$ (2,2'-azinobis (3-ethlybenzthiazolin-6-sulfonic acid) diammonium salt) radical scavenging activity was spectrophotometrically measured at 734 nm (Re et al., 1999). The diluted and homogenized sample at appropriate concentrations was added to the absorbance-adjusted ABTS solution. The absorbance value after completion of the reaction at 30 °C for 6 minutes. ABTS$^+$ values were expressed as Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) equivalents (mmol TE/100 g).

To measure the DPPH radical scavenging activity, the diluted samples were added to the DPPH solution and kept in the dark for 30 minutes. The absorbance was measured at 517 nm. The linear regression equation was obtained from the standard curve prepared from Trolox concentrations. The value was given as mmol TE/100 g (Brand-Williams et al., 1995)

Liquid Chromatographic Analysis of Phenolic compounds

To determine the effects of different foam mat drying methods on phenolic compounds, liquid chromatographic analysis (HPLC) was used (Shimadzu SCL-10A, Scientific Instruments, Inc., Tokyo, Japan). Phenolic compounds including quercetin (R^2=0.995), catechin (R^2=0.993), chlorogenic acid (R^2=0.997), caffeic acid (R^2=0.988), rutin (R^2=0.986), p-coumaric acid (R^2=0.999), gallic acid (R^2=0.990), protocatechuic (R^2=0.998), epicatechin (R^2=0.989) were separated. The compound amounts were calculated by the external standard method and shown as µg/g dry weight (d.w.). min flow rate, column temperature 30°C and injection volume 20 µl. The gradient procedure started at 7% of solvent B for 10 min and increased linearly during 90 minutes of analysis time.

Statistical analysis

Experimental data were analyzed with OneWay ANOVA of $p<0.05$ significance level. Duncan's multiple comparison tests were used to assess differences between samples. Statistical evaluation was carried out using SPSS 22.00 statistical package program (SPSS Inc., Chicago, IL). All the experiments were carried out in duplicate and repeated three times.

RESULTS AND DISCUSSION

In food powders, the moisture content is critical in processing and storage. It is especially associated with physical properties such as cohesiveness, and caking problems (Barbosa-Cánovas et al., 2019). Water activity is related to food stability and the aw values of dried products are relatively low (Perera, 2005).

The moisture value of fresh hawthorn samples was 73.25 ±1.9 % (Table 1). After the drying process, the moisture content of the samples was between 3.97-4.58%. The water activity values of the samples were 0.129-0.162.

In the literature, there is no study on foam drying of the hawthorn. Therefore moisture and aw values of different fruit powders produced with foam mat drying methods have been reviewed. The moisture content of Spray dried and FMFD blueberry powders were 2.2-4 % (Darniadi et al., 2018). Water content and water activity of freeze-dry, convectional, microwave and lyophilized FMD apple juices were reported as 1.81-3.81% and 0.097-0.171 (Jakubczyka et al., 2011).

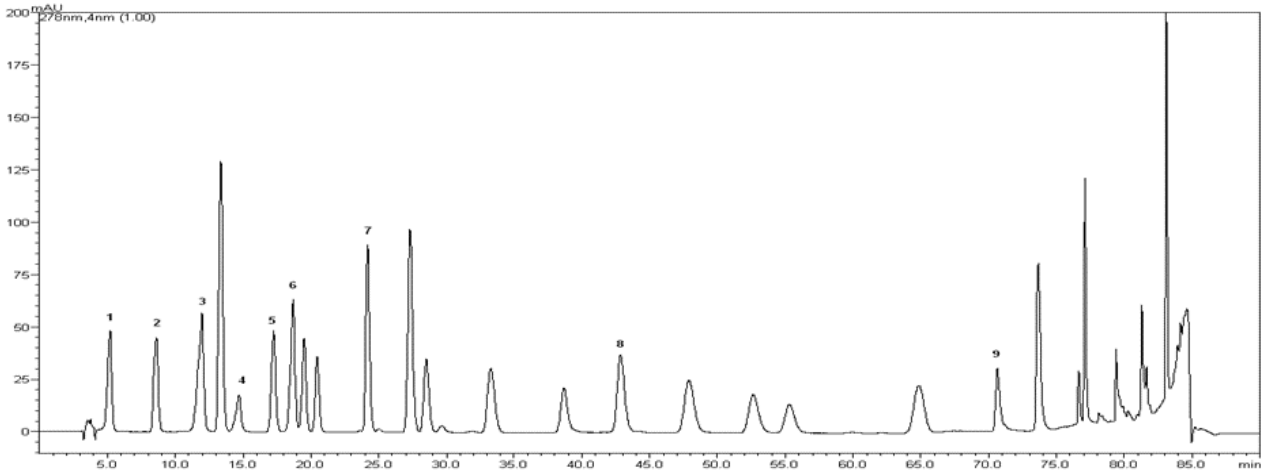


Figure 2. Standard chromatogram of phenolic compounds (1:gallic acid, 2:protocatechuic acid, 3:catechin, 4:chlorogenic acid, 5:caffeic acid, 6:epicatechin, 7:p-coumaric acid, 8:rutin, 9:quercetin)

The standard chromatogram was given in Figure 2. Agilent Eclipse XDB-C18 (250x4,60 mm, 5µm) was used as column and chromatographic conditions were gradient phase (A: %3 acetic acid, B: Methanol) 0,8 ml/

Fruits and vegetables show positive effects on health because they contain high amounts of phenolic compounds with bioactive properties. They are the most important sources of phenolic substances, including

flavonoids and anthocyanins (Lin & Tang, 2007).

TPC and TFC of fresh samples were 23975 mg GAE/kg and 13062 mg CE/kg, respectively. TPC and TFC of powder samples are shown in Figure 3. The highest total phenolic content was found in the samples produced by foam mat drying and C-60 and L samples. The total phenolic content of the convective FMD samples decreased with increasing temperature. The TPC content of the C-70 sample was found to be significantly lower than the other samples ($p<0.05$). Total flavonoid content (TFC) was between 26999-29224 CE mg/kg. TFC was the highest in the L sample, however, differences were not statistically significant.

Table 1. Moisture and water activity values (aw) values of hawthorn powder samples

Powder samples	Moisture (%)	aw
C-60	3.97±0.58	0.162±0.01
C-65	4.12±0.33	0.127±0.01
C-70	4.13±0.55	0.129±0.01
L	4.58±0.68	0.139±0.01

C-60: Convective foam mat dried hawthorn powder at 60 °C, C-65: Convective foam mat dried hawthorn powder at 65 °C, C-70: Convective foam mat dried hawthorn powder at 70 °C, L:Lyophilized foam mat dried hawthorn powder

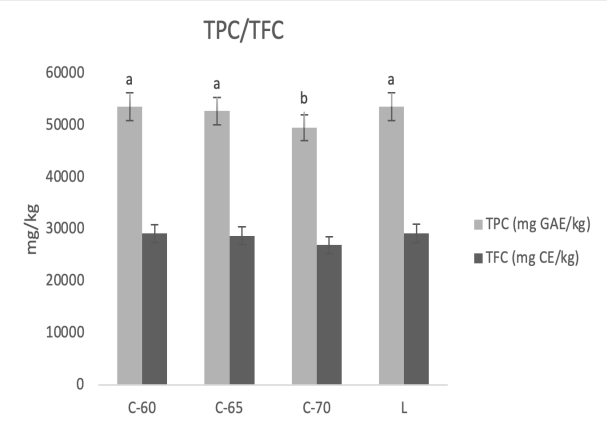


Figure 3. Total phenolic content (TPC) and total flavonoid content (TFC) of foam mat dried hawthorn powders
Different lowercase letters indicate a significant difference among different samples ($p<0.05$)

González-Jiménez et al. (2018) reported that the amount of TPC and TFC in fresh hawthorn (*Crataegus pubescens*) was 168.6 mg GAE/g and 55.89 g Quercetin Equivalent/g. In hawthorn slices dried by different methods, TPC was found the highest in lyophilized and dried at 60°C samples, while the flavonoid content was highest in the lyophilized sample In hawthorn slices, TPC increased in the FD sample and samples applied hot air drying at 60 °C. However, TPC decreased with increasing drying temperatures. TFC values of freeze-dried hawthorn were higher than fresh and hot air-dried samples (Liu et al., 2019). Li et al. (2020) found the amount of TPC and TFC in convective dried (60 C-16 hours) hawthorn were 2982 mg GAE/100 g and 51 mg RT/100 g and they stated that TPC and TFC were 3158 mg GAE/100 g and 79 mg RT/100 g in lyophilized samples, respectively. They emphasized that thermal treatment caused a reduction in soluble and total phenolic components of hawthorn. Darniadi, (2017) dried blueberry juice with foam mat drying method using Maltodextrin and Whey Protein Isolate, and he reported higher TPC and Total Monomeric Anthocyanin amounts in dried powders than in spray drying.

Flavonoids, flavone-C-glycosides, catechins, triterpene saponins, and oligomeric procyanidins in hawthorn have antioxidant effects (Rigelsky & Sweet, 2002). The antioxidant activity of such nutrient-derived antioxidant molecules found in fruits can be measured by various methods. Antioxidant activity values of hawthorn powders were determined by ABTS and DPPH methods (Figure 4). ABTS values were between 1,345-1,605 mmol TE/100 g and L and C-60 samples exhibited higher ABTS radical scavenging activity than powders produced at 65 and 70 C temperatures ($p<0.05$). DPPH radical scavenging activities of samples were in the range 5.88-6.37 mmol TE/100 g. Although L and C-60 showed high radical scavenging activity, no statistical difference was found. The DPPH values of soluble and insoluble phenolics in dried hawthorn were found to be 0.15 and 5.10 mg/ml loss, respectively, while ABTS values were reported as 0.06 and 4.46 mg/ml loss for soluble and insoluble phenolics, respectively (Li et al., 2020). Liu et al., (2019) reported that DPPH values decreased significantly in hot air-dried hawthorn slices.

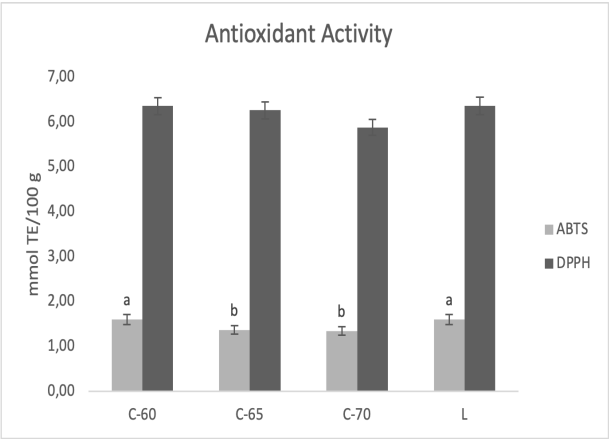


Figure 4. Antioxidant activity (ABTS and DPPH) values of fam mat dried hawthorn powders
Different lowercase letters indicate a significant difference among different samples ($p<0.05$)

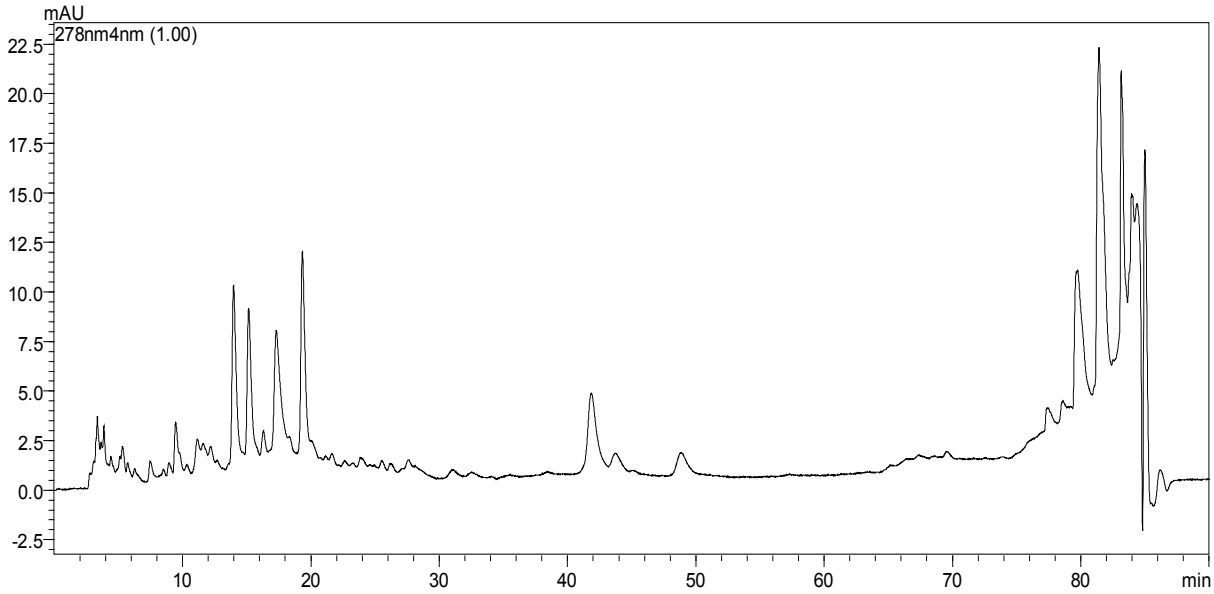


Figure 5. A sample phenolic chromatogram of the hawthorn powder (C-70)

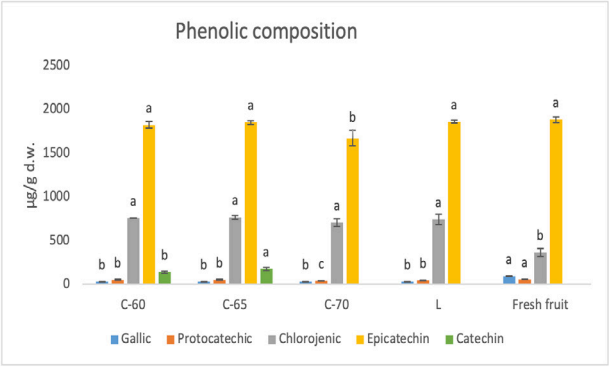


Figure 6. Phenolic compound profile of hawthorn powders and fresh fruit
Different lowercase letters indicate a significant difference among different samples ($p<0.05$)

Figure 5 shows a sample chromatogram of the C-70 sample. Five phenolic compounds in FMD hawthorn powders were separated. Major phenolic compounds in hawthorn powders were chlorogenic acid and epicatechin (Figure 6). Similar to total phenolic matter results, epicatechin and protocatechuic acid content of C-70 was statistically lower than other powder samples.

Gallic acid content significantly decreased in dried samples and gallic acid content of the fresh sample was higher than in dried samples ($p<0.05$). In another study examining the thermal stability of gallic acid, it was reported that it decreased by 15% and 25% with the application of 60 and 80C temperatures for 4 hours (Volf et al., 2014).

All powder samples contained higher chlorogenic acid content than fresh samples. The protocatechuic acid content of the fresh sample was the highest ($p<0.05$). Catechin was only found in C-60 and C-65 samples.

Procyanidin B2, Epicatechin, and Rutin, among 11 phenolic compounds detected in ripe hawthorn fruit, were reported as three major phenolic compounds (Coklar & Akbulut, 2016). In another study, catechin, chlorogenic acid, procyanidin B2, epicatechin, quercetin, paracoumaric acid, hyperoside, and isoquercitrin phenolics were identified in fresh hawthorn (Zhang et al., 2020). In dried hawthorn slices, amounts of Procyanidin B2; epicatechin and chlorogenic acid, which were major phenolic compounds, were 2.67; 1.93; 1.34 mg/g, respectively. Other detected phenolics were vitexin rhamnoside, rutin, hyperoside and isoquercetin. It was found that the decrease in the amount of epicatechin and chlorogenic acid in hawthorn, which was applied to hot air drying at 60 °C for 16 hours, was significant (Li et al., 2020). Liu et al., (2019) dried hawthorn slices with various methods and reported that the amounts of chlorogenic acid, epicatechin, rutin, ellagic acid and protocatechuic acid in freeze-dried samples were 482, 517, 143, 21 and 32 mg/100 d.w., respectively. In this study, epicatechin was determined as the most abundant phenolic component, which was consistent with our results. The amount of chlorogenic acid increased in the FD sample compared to the fresh sample, similar to our results. The difference between the chlorogenic acid content of the samples dried at 60 and 80 °C and the chlorogenic acid content of the fresh sample was not found significant. The amount of epicatechin decreased with increasing drying temperature. While protocatechuic acid decreased in the lyophilized sample, it increased in the hot air-dried (60 and 80°C) samples and decreased at higher temperatures (100-120°C).

Generally, fresh plant products are thought to have a higher phenolic content than dried products due to the degradation of phenolics during drying. However,

it has been stated that dried products such as tomatoes and shiitake mushrooms have higher phenolic content compared to fresh ones (Suvarnakuta et al., 2011). Heat treatment can lead to the release of phenolic acids accumulating in vacuoles due to the breakdown of cellular components (Dewanto et al., 2002). Most antioxidant compounds in plants are covalently bound to insoluble polymers. It is suggested that heat treatment breaks down the cell wall, liberates the antioxidant components from the insoluble part, and increases the number of bioavailable antioxidant components (Choi et al., 2006). In our study, it is thought that the reason why the amount of chlorogenic acid in the powder samples was higher than in the fresh sample may be that the heat treatment liberates the phenolics, which are bound in the plant cell walls, which are complex, porous polysaccharide structure.

In the study examining the phenolic stability in apple pomace, apple pomace was dried at temperatures of 50, 60, 70 and 80 °C. In the samples dried at 80 °C, the decrease in the amount of epicatechin was found to be significant compared to the samples dried at 50 C, the change in the amount of chlorogenic acid was found to be insignificant for all four drying temperatures. The amounts of chlorogenic acid, epicatechin and catechin in the dried samples increased compared to the pre-drying sample (Heras-Ramírez et al., 2012).

Phenolic compounds are significantly affected by foam mat drying conditions. It has been reported in some studies that high foam mat drying temperature reduces phenolic compounds. On the other hand, the type and concentration of the foaming agent can be effective in the loss or preservation of the bioactive components. Depending on the foaming and stabilizing agent and the type of dried product, variable results can be seen promoting either the preservation of phenolic substances or their degradation (Reis et al., 2021)

CONCLUSION

Foam mat drying is a drying method applied after foaming liquid or semi-liquid foods using various foaming and stabilizing agents. It provides an advantage because it is an easy-to-apply method with low investment and operating costs. Another advantage of this method is that the drying time is shortened and the thermal degradation of bioactive components in foods is less. To increase product quality and energy efficiency, there are searches for the application of hybrid methods in which the FMD technique is applied together with different drying methods. In this study, foam drying methods were used in the production of powder from hawthorn fruit, which is one of the fruits that have been emphasized more in recent years due to its positive effects on health, and the effects of the methods and different drying temperatures on the antioxidant bioactive components of the powders were determined.

The freeze foam mat dried samples showed similar bioactive content to the samples that were convective foam mat driedat 60 °C. According to these results, convective foam mat drying application at the appropriate temperature for the product can be considered a preferable method in terms of bioactive component content. Hawthorn powders produced by foam mat drying methods, whose bioactive content is highly preserved, can be used as a natural functional ingredient or food supplement in the food industry. Dried fruit and vegetable products are valuable foodstuffs as a concentrated source of nutrients and bioactive compounds. Optimizing the process conditions is important to maximize both their quality properties and their bioactive content.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The author declared that for this research article, they have no actual, potential, or perceived conflict of interest.

Author contribution

The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

This research was funded by the Süleyman Demirel University Scientific Research Fund (FAB-2021-8252)

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

The author is thankful to Sevval Bostancı for their assistance at the laboratory.

REFERENCES

Barbosa-Cánovas, G. V., Ortega-Rivas, E., Juliano, P., & Yan, H. (2019). *Food Powders: Physical Properties, Processing and Functionality*. Kluwer Academic/Plenum Publishers.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Cemeroğlu, B. (2003). *Meyve ve Sebze İşleme Teknolojisi*. Nobel Kitap. (in Turkish)

Chang, Q., Zuo, Z., Harrison, F., Sing, M., & Chow, S. (2002). Hawthorn. *Herbal Medicine*, *42*, 605–612.

Choi, Y., Lee, S. M., Chun, J., Lee, H. B., & Lee, J. (2006). Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (Lentinus edodes) mushroom. *Food Chemistry*, *99*(2), 381–387. https://doi.org/10.1016/j.foodchem.2005.08.004

Coklar, H., & Akbulut, M. (2016). The Change in Antioxidant Activity , Total Phenolic Content and Phenolic Profile of Hawthorn (Crataegus orientalis ... *Fruit Science*, *3(2)*

(November 2016), 30–37.

Cuevas-Bernardino, J. C., Lobato-Calleros, C., Román-Guerrero, A., Alvarez-Ramirez, J., & Vernon-Carter, E. J. (2016). Physicochemical characterisation of hawthorn pectins and their performing in stabilising oil-in-water emulsions. *Reactive and Functional Polymers*, *103*, 63–71. https://doi.org/10.1016/j.reactfunctpolym.2016.03.024

Dahmer, S., & Scott, E. (2010). Health effects of hawthorn. *American Family Physician*, *81*(4), 465–468.

Darniadi, S. (2017). *Optimisation of foam-mat freeze-drying conditions for blueberry powder and evaluation of powder properties*. *August*, 1–202. https://etheses.whiterose.ac.uk/18476/

Darniadi, S., Ho, P., & Murray, B. S. (2018). Comparison of blueberry powder produced via foam-mat freeze-drying versus spray-drying: evaluation of foam and powder properties. *Journal of the Science of Food and Agriculture*, *98*(5), 2002–2010. https://doi.org/10.1002/jsfa.8685

Dev, S. R. S., & Raghavan, V. G. S. (2012). Advancements in Drying Techniques for Food, Fiber, and Fuel. *Drying Technology*, *30*(11–12), 1147–1159. https://doi.org/10.1080/07373937.2012.692747

Dewanto, V., Xianzhong, W., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, *50*(10), 3010–3014. https://doi.org/10.1021/jf0115589

González-Jiménez, F. E., Salazar-Montoya, J. A., Calva-Calva, G., & Ramos-Ramírez, E. G. (2018). Phytochemical Characterization, in Vitro Antioxidant Activity, and Quantitative Analysis by Micellar Electrokinetic Chromatography of Hawthorn (Crataegus pubescens) Fruit. *Journal of Food Quality*, *2018*. https://doi.org/10.1155/2018/2154893

He, G., Sui, J., Du, J., & Lin, J. (2013). Characteristics and antioxidant capacities of five hawthorn wines fermented by different wine yeasts. *Journal of the Institute of Brewing*, *119*(4), 321–327. https://doi.org/10.1002/jib.101

Heras-Ramírez, M. E., Quintero-Ramos, A., Camacho-Dávila, A. A., Barnard, J., Talamás-Abbud, R., Torres-Muñoz, J. V., & Salas-Muñoz, E. (2012). Effect of Blanching and Drying Temperature on Polyphenolic Compound Stability and Antioxidant Capacity of Apple Pomace. *Food and Bioprocess Technology*, *5*(6), 2201–2210. https://doi.org/10.1007/s11947-011-0583-x

Jakubczyk, E., Gondek, E., & Tambor, K. (2011). Characteristics of selected functional properties of apple powders obtained by the foam-mat drying method. *Food Process Engineering in a Changing World*, *January 2015*, 1–6.

Jiang, H., Zhang, M., & Adhikari, B. (2013). Fruit and vegetable powders. *Handbook of Food Powders: Processes and Properties*, 532–552. https://doi.org/10.1533/9780857098672.3.532

Kisioglu, B., & Nergiz-Unal, R. (2018). The powerful story against cardiovascular diseases: Dietary factors. *Food Reviews International*, *34*(8), 713–745. https://doi.org/10.1080/87559129.2017.1410172

Koç, M., & Ertekin, F. K. (2016). Türk Tarım -Gıda Bilim ve Teknoloji Dergisi Şeker İçeriği Yüksek Gıdaların Püskürtülerek Kurutulması: Ürün Kazanımı ve Toz Ürün Özelliklerinin Geliştirilmesi. *Türk Tarım – Gıda Bilim ve Teknoloji Dergisi*, *4*(5), 336–344.

Li, M., Chen, X., Deng, J., Ouyang, D., Wang, D., Liang, Y., Chen, Y., & Sun, Y. (2020). Effect of thermal processing on free and bound phenolic compounds and antioxidant activities of hawthorn. *Food Chemistry*, *332*(483), 127429. https://doi.org/10.1016/j.foodchem.2020.127429

Lin, J. Y., & Tang, C. Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chemistry*, *101*(1), 140–147. https://doi.org/10.1016/j.foodchem.2006.01.014

Liu, H., Liu, J., Lv, Z., Yang, W., Zhang, C., Chen, D., & Jiao, Z. (2019). Effect of dehydration techniques on bioactive compounds in hawthorn slices and their correlations with antioxidant properties. *Journal of Food Science and Technology*, *56*(5), 2446–2457. https://doi.org/10.1007/s13197-019-03720-x

Muthukumaran, A., Ratti, C., & Raghavan, V. (2008). Foam-mat freeze drying of egg white-mathematical modeling Part II: Freeze drying and modeling. *Drying Technology*, *26*(4), 513–518. https://doi.org/10.1080/07373930801929615

Nazhand, A., Lucarini, M., Durazzo, A., Zaccardelli, M., Cristarella, S., Souto, S. B., Silva, A. M., Severino, P., Souto, E. B., & Santini, A. (2020). Hawthorn (Crataegus spp.): An updated overview on its beneficial properties. *Forests*, *11*(5), 1–21. https://doi.org/10.3390/F11050564

Nijhuis, H. H., Torringa, H. M., Muresan, S., Yuksel, D., Leguijt, C., & Kloek, W. (1998). Approaches to improving the quality of dried fruit and vegetables. *Trends in Food Science and Technology*, *9*(1), 13–20. https://doi.org/10.1016/S0924-2244(97)00007-1

Perera, C. O. (2005). Selected quality attributes of dried foods. *Drying Technology*, *23*(4), 717–730. https://doi.org/10.1081/DRT-200054180

Qadri, O. S., Srivastava, A. K., & Yousuf, B. (2020). Trends in foam mat drying of foods: Special emphasis on hybrid foam mat drying technology. *Critical Reviews in Food Science and Nutrition*, *60*(10), 1667–1676. https://doi.org/10.1080/10408398.2019.1588221

Raharitsifa, N., & Ratti, C. (2010). Foam-mat freeze-drying of apple juice part 1: Experimental data and ann simulations. *Journal of Food Process Engineering*, *33*(SUPPL. 1), 268–283. https://doi.org/10.1111/j.1745-4530.2009.00400.x

Ratti, C. (2013). Freeze drying for food powder production. In *Handbook of Food Powders: Processes and Properties*. Woodhead Publishing Limited. https://doi.org/10.1533/9780857098672.1.57

Re, R., Nicoletta, P., Anna, P., Ananth, P., Min, Y., & Catherine, R.-E. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*((9-10)), 1231–1237.

Reis, F. R., de Moraes, A. C. S., & Masson, M. L. (2021). Impact of Foam-Mat Drying on Plant-Based Foods Bioactive Compounds: a Review. *Plant Foods for Human Nutrition*,

76(2), 153–160. https://doi.org/10.1007/s11130-021-00899-3

Rigelsky, J. M., & Sweet, B. V. (2002). Hawthorn: Pharmacology and therapeutic uses. *American Journal of Health-System Pharmacy*, *59*(5), 417–422. https://doi.org/10.1093/ajhp/59.5.417

Sangamithra, A., Venkatachalam, S., John, S. G., & Kuppuswamy, K. (2015). Foam Mat Drying of Food Materials: A Review. *Journal of Food Processing and Preservation*, *39*(6), 3165–3174. https://doi.org/10.1111/jfpp.12421

Seerangurayar, T., Manickavasagan, A., Al-Ismaili, A. M., & Al-Mulla, Y. A. (2018). Effect of carrier agents on physicochemical properties of foam-mat freeze-dried date powder. *Drying Technology*, *36*(11), 1292–1303. https://doi.org/10.1080/07373937.2017.1400557

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178. https://www.sciencedirect.com/science/article/abs/pii/S0076687999990171

Suvarnakuta, P., Chaweerungrat, C., & Devahastin, S. (2011). Effects of drying methods on assay and antioxidant activity of xanthones in mangosteen rind. *Food Chemistry*, *125*(1), 240–247. https://doi.org/10.1016/j.foodchem.2010.09.015

Türker, İ., Koç, B., & İşleroğlu, H. (2018). Püskürtmeli-Dondurarak Kurutma İşleminin Maltodekstrinin Fiziksel Özellikleri Üzerine Etkisi. *Gıda*, *43*, 197–210. (in Turkish) https://doi.org/10.153237/gida.GD17101

Volf, I., Ignat, I., Neamtu, M., & Popa, V. I. (2014). Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols. *Chemical Papers*, *68*(1), 121–129. https://doi.org/10.2478/s11696-013-0417-6

Zhang, L. L., Zhang, L. F., & Xu, J. G. (2020). Chemical composition, antibacterial activity and action mechanism of different extracts from hawthorn (Crataegus pinnatifida Bge.). *Scientific Reports*, *10*(1), 1–13. https://doi.org/10.1038/s41598-020-65802-7

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, *64*(4), 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 206-212
DOI: https://doi.org/10.31015/jaefs.2023.1.25

Effects of ultraviolet and ultrasound treatments applied before the storage period on egg quality characteristics

Ozlem Varol Avcilar[1] • **Ebrunur Yilmaz**[2]

[1] Department of Nutrition and Dietetics, Faculty of Health Sciences, Osmaniye Korkut Ata University, Osmaniye, Türkiye
[2] Institute of Graduate Education, Hasan Kalyoncu University, Gaziantep, Türkiye

Citation: Varol Avcılar, O., Yilmaz, E. (2023). Effects of ultraviolet and ultrasound treatments applied before the storage period on egg quality characteristics. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 206-212

Received: 18 January 2023
Revised: 20 February 2023
Accepted: 22 February 2023
Published Online: 22 March 2023

Correspondence: Ozlem Varol Avcilar
E-mail: ovarolavcilar@osmaniye.edu.tr

Abstract

Egg is an important source of animal protein with its nutritional substances. These nutritional substances and the quality characteristics of the egg are affected by the physicochemical changes with the effect of various factors during storage. Different studies have been carried out to preserve the quality values of eggs during storage. In these studies, Ultraviolet applications were mostly used for the sanitation of eggs, while Ultraviolet applications were made to determine the internal quality of the egg without breaking the shell. The aim of this study is to determine the effects of ultraviolet and ultrasound applications applied before storage to the quality characteristics of the eggs collected from laying hens. Eggs collected in the morning were subjected to ultraviolet and ultrasound treatments before storage and stored for 28 days in an environment of 20°C and approximately 60% humidity. The applied treatments made a significant difference between the groups in egg shell thickness ($p < 0.01$), albumen weight ($p < 0.01$) and yolk dry matter value ($p < 0.01$). Storage time, on the other hand, made significant changes between groups on weight loss, shell breaking strength, albumen and yolk quality characteristics. The interaction of applications and storage time was statistically significantly in weight and height of albumen, Haugh unit and dry matter of yolk. As a result, the effects of processes such as ultraviolet and ultrasound applied to eggs, especially on egg shell thickness, reveal the importance of application time and application amount in such studies.

Keywords: Egg, Quality changes, Storage time, Ultrasound, Ultraviolet

INTRODUCTION

The laying hen industry is invaluable for producing the cheapest and best quality protein source. Egg is among the indispensables of low-calorie diets with its antioxidant properties and some essential amino acids. It also strengthens the immune system and protects against diseases (Benede and Molina, 2020; Puglisi and Fernandez, 2022).

When the egg is first taken from the chicken, it cannot carry the characteristics it has until consumption. In particular, the factors (care and feeding conditions, oviposition time, genetic structure, age, temperature, huminity, storage time etc.) that cause changes in internal and external quality can show their effects both during and after the production stage and cause changes until the consumption stage. Extending the shelf life of the egg by maintaining its quality properties increases the value of this animal food (Onbaşılar and Avcılar, 2011; Lou et al., 2020; Tabib et al., 2021; Sarıyel et al., 2022).

During storage, changes in egg internal quality can be observed as a result of

increase in albumin pH, thinning of albumin thickness, liquefaction and evaporation of water. Due to the increase in pH, carbon dioxide loss occurs from the shell (Kumari et al., 2020).

The long storage period of the egg may cause the breakdown of fat and proteins in its structure, and the release of ammonia and sulfur components. This results in the formation of undesirable taste and odor (Brodacki et al., 2019). The less the processes to be applied to the egg change the internal and external quality during the storage period, the better the quality properties for both the consumer and the products to be processed will be preserved, as deterioration will be prevented.

In the researches, various methods were applied after the eggs were collected in order to preserve the quality characteristics of the eggs and to extend the shelf life. Some of these methods are; coating of eggs with various substances (zein (Caner and Yüceer, 2015), soy proteins (Xie et al., 2002), mineral oils (Jirangrad et al., 2010), chitosan (Bhale et al., 2003)) and ultraviolet applications (UV) (Mehdizadeh et al., 2015; Melo et al. 2019; Cassar et al. 2021).

It is mostly used in egg sanitation processes such as ultraviolet applications other sanitation aids (chlorine, hydrogen peroxide, quaternal ammonium cation, etc.) in the egg industry (Al-Ajeeli et al., 2016).

Turtoi and Borda (2014) in their study on the decontamination of eggshell by the application of ultraviolet light, stated that compared to chemical disinfectants, clean and recently contamination eggshells resulted as significant reduction in the bacterial population. In a study in which UV applications used in chicken breeding were compiled, it was reported that ultraviolet-A light had positive effects on reducing fear and stress responses, but in some studies, it significantly increased feather pecking due to age in the production phase (Rana and Campell, 2021).

Ultrasound procedures applied to the egg have been studies on determining and improving the internal quality of the egg. In a study, ultrasonic waves (frequency of 150 kHz) were used to determine the internal quality of the egg without damaging the egg shell, and it was stated that it could be used to provide information about the freshness of eggs stored for 3 weeks (Aboonajmi et al., 2010).

According to Sert et al. (2011) in their studies where they applied 35 kHz ultrasound to eggs at different times (5, 15, 30 min); detected high specific weight, shell strength, albumen height, and Haugh unit in eggs that were subjected to ultrasound. It was stated that egg quality was significantly improved by ultrasonic treatment ($p < 0.01$). Caner and Yüceer (2014) reported that the application of 300 W and 400 W ultrasound improved the internal quality of fresh eggs during storage. It has been stated that it affects the shell strength negatively. Yüceer end Caner (2020) used different methods (ozone, ultrasound and coating with shellac and lysozyme-chitosan) to preserve the freshness of eggs during storage. It has been stated that ultrasound application has a positive effect on the preservation of the internal quality of the egg after storage.

In this study, it was aimed to determine the effects of ultraviolet and ultrasound treatments applied to before storage on egg external and internal quality characteristics at the same temperature and humidity levels during storage.

MATERIALS AND METHODS

Materials

In the research, eggs were obtained from a private enterprise in December 2021. A total of 300 egg were collected from 34 weeks old Nick-Brown layer hybrids. Eggs were collected in the morning (10:00-11:00). While collection process, the poultry house temperature was measured as 22 °C and the humidity is 60%. It was known that animals were given standard layer feed during the laying period. Precaution was taken to ensure that the eggs were not broken, dirty and different size. The eggs were brought to the laboratory where they would be stored for 28 days. Collected eggs were weighed (Pioneer Ohaus, USA) and divided into three groups (control (non-treatment), ultraviolet treated, ultrasound applied). Eggs were kept at 22 °C and 60% humidity until the last day of storage. To determine the quality characteristics of the eggs, 10 eggs from each group were examined on the 1st, 7th, 14th, 21st and 28th days. During storage, egg weight, egg weight loss, shell breaking strength, shell thickness, shell weight, albumen measurements (length, width, height, ph), yolk measurements (diameter, weight, height, ph), albumen and yolk index values, and Haugh unit value has been determined.

Applications

UV-C Irradiation application: For this process, the eggs were placed in a closed system UV device (BLX-254, France) with a metal flat tray of 10 eggs at a time. Eggs were exposed to UV lamps (5 x 8 W, 254 nm tube) for 3 minutes. UV area was 20 x 50 cm^2. Eggs were stored in the same environment with other groups throughout store time.

Ultrasonic Application: Ultrasonic water bath (Ultrasound HD Selecta, Spain) was used for this process. Each time 8 eggs were placed in the basket apparatus of the device and immersed in distilled water at 24°C, and ultrasound (120 W 10 min) was applied (modification Yuceer and Caner, (2020)). Afterwards, the eggs were dried and stored in the same environment as the other groups.

Egg Quality Analysis

Eggshell breaking strength;

10 eggs from each group were used to determine

the breaking strength of the eggshell. The CT3 Texture Analyzer (BrookfieldEngineering Labs Inc., Middleborough, MA, USA) device was used to measure this. For analysis, the eggs were conveniently placed under the piercing apparatus of the device. Force was applied to make hole in the top and bottom eggs parts. Probe model TA39. Test speed was 5mm/s.

The internal and external egg quality;

In order to determine the internal and external quality characteristics of the eggs, firstly, it was broken on a flat surface. Egg shell thickness was measured from 3 different points (top, bottom and equatorial) and averaged. Some properties of albumen (length, width, height) and yolk (diameter, weight and height) were measured with an electronic digital vernier caliper (Insize, 1183-150A, Chine). These values were used to calculate the yolk index [(yolk height / yolk diameter) x100], albumen index [(albumen height / (average of albumen length and albumen width)/2 x 100] and Haugh unit (HU) value (Haugh unit (HU); [100 x log (H + 7.57 - 1.7W0.37), where H is albumen height and W is egg weight] (Yalçın et al. 2014).

In addition, after the albumen and yolk were brought to a homogeneous form, the dry matter values were calculated by using a digital refractometer (A. Krüss Optronic, Germany). pH measurements (Thermo Scientific Orion 2 Star, Singapore) were also made on specimens.

Statistical analysis

Distribution, the homogenity of variance of data, was analysed. Two-way ANOVA determined the effects of applications and storage period on egg properties. Tukey test was used to check the significance of the difference between the groups. The statistical analysis was performed by means of the SPSS Statistics 23.0 package software. $p < 0.05$ was taken into account statistically (Dawson and Trapp, 2001).

RESULTS

In Table 1, egg weight losses and shell quality characteristics of the groups are given. While there was no difference in weight loss among UV, Ultrasound and control groups, it was observed that the weight loss increased as the storage time increased ($p < 0.001$). The highest weight loss was determined as 2.60 g on the 28th day. Although the eggshell breaking strength was determined as the highest value (4.056 kg/cm^3) in the upper part of eggs the ultrasound group, as there is no statistical difference was observed between the groups. There was no difference between the eggshell breaking strength in the upper part of eggs the groups in the storage time. While there was no difference between the treatment groups in the breaking force values applied to the lower pole of the eggs, it was determined that 3.539 km/cm^2 on the 1st day, 3.631 kg/cm^2 on the 21st day and 3.152 kg/cm^2 on the 28th day during the storage period. According to these values it was concluded that this difference was statistically significant ($p < 0.05$).

It was determined that the highest shell thickness was in the control group (0.48 mm) the lowest shell thickness was in the ultraviolet applied group (0.44 mm), and this difference was significant ($p < 0.01$). Although the highest shell thickness was determined on the 21st day, the storage time did not make a significant difference in terms of this value. Shell weight was a value that was not affected by both the application processes and the storage time ($p > 0.05$).

Table 1. Effects of UV, Ultrasond and storage period on egg weight loss and shell quality of eggs.

Treatments	Storage period (day)	Initial egg weight (g)	Weight loss (g)	Breaking strength (top) (kg/cm²)	Breaking strength (bottom) (kg/cm²)	Shell thickness (mm)	Shell weight (g)
UV		61,25	1,41	4,031	3,494	0,44^{A}	7,37
Ultrasond		61,64	1,41	4,056	3,395	0,45^{A}	7,49
Control		59,47	1,41	4,027	3,340	0,48^{B}	7,27
	1		0,15^{a}	3,926	3,539^{bc}	0,44	7,39
	7		0,83^{b}	4,183	3,419^{abc}	0,46	7,58
	14		1,45^{c}	3,953	3,308^{abc}	0,46	7,29
	21		2,04^{d}	4,286	3,631^{c}	0,47	7,37
	28		2,60^{e}	3,844	3,152^{a}	0,45	7,25
					P		
Treatment			NS	NS	NS	**	NS
Storage period			***	NS	*	NS	NS
Interaction			NS	NS	NS	NS	NS

n=10. Values were shown as mean. $^{a, b, c, d, e}$: The difference among means carrying different letters in the same column was statistically significant ($p < 0.05$). $^{A, B}$:With in treatments grups, values with the same superscript do not significantly differ ($p<0.05$).
NS: $p > 0.05$; *: $p<0.05$; **: $p<0,01$; ***: $p<0,001$

Albumen weight was determined as the highest in the ultrasound group (37.22 g) and the lowest in the control group (35.29 g) in the treated eggs ($p < 0.01$). During the storage period, albumen weight decreased gradually ($p < 0.001$) and it was measured as 38.70 g on the 1st day and 34.74 g on the 28th day (Table 2). Albumen pH values were similar between the treated groups. It was observed that this value increased gradually during the storage period and reached the highest value on the 28th day (9.54) ($p < 0.001$). While no difference was observed between the treated groups in terms of albumen height values ($p > 0.05$), it was determined that the highest numerical value was in the group treated with ultraviolet (5.46 mm). Moreover, it was found that as the storage time increased, the albumen height value decreased statistically significantly ($p < 0.001$).

While there was no difference in albumen length and width measurements in the treated egg groups ($p > 0.05$), it was determined that these values increased significantly as the storage time extended ($p < 0.001$). There was no difference in Haugh unit and albumen dry matter values in the treated egg groups. It was determined that the haugh unit value decreased ($p < 0.001$) and albumen dry matter value increased ($p < 0.001$) as the storage time increased.

When the egg albumen quality values were examined, it was determined that there was a statistically significant interaction between the processes applied to the eggs and the storage time ($p < 0.05$), this interaction in the albumen weight, albumen height and Haugh unit respectfully.

In egg yolk quality values, it was determined that the treatments applied to the eggs did not generally differ between the groups (Table 3). It was determined that only the difference in egg dry matter value was significant ($p < 0.01$) and this difference was due to the control group (46.18%). As the storage time increased, the egg yolk; diameter, weight and pH value increased ($p < 0.001$); it was determined that the yolk height ($p < 0.05$), yolk index ($p < 0.001$) and dry matter amount ($p < 0.001$) decreased.

DISCUSSION

Egg, which contains many essential amino acids in its structure and carries valuable elements for the emergence of a living thing, takes its place as an ingredient in the preparation of consumer products and most foods prepared in different ways in many countries without any limitation.

The nutrients and quality characteristics of the egg may change during the storage of the egg. The temperature, humidity and storage time of the environment during storage are among the effective factors in the formation of these changes. In order to reduce the negative effects of these factors, studies on the effects of processes that do not require heat (Sert et al. (2011); Caner and Yüceer (2014); Mehdizadeh et al., 2015; Melo et al. 2019; Cassar et al. 2021).

In the study, it was determined that the ultrasound and ultraviolet applications applied to the eggs did not make any difference between the groups on egg weight loss, shell breaking strength and shell weight. It was determined that only shell thickness was lower in the treated groups compared to the control.

It can be thought that the thickness of the shell will decrease because the processes applied to the egg damage the shell surface (cuticle layer). However, it is

Table 2. Effects of UV, Ultrasond and storage period on albumen quality and Haugh unit of eggs.

Treatments	Storage period (day)	Alb. weight (g)	Alb. pH	Alb. height (mm)	Alb. length (mm)	Alb. width (mm)	Alb. index	Haugh unit	Alb. dry matter (%)
UV		$36,83^{B}$	9,31	5,46	112,42	93,12	5,86	70,65	15,55
Ultrasond		$37,22^{B}$	9,32	5,28	115	92,57	5,5	67,97	15,5
Control		$35,29^{A}$	9,31	5,04	110,64	89,01	5,54	68,78	15,65
	1	$38,70^{c}$	$8,84^{a}$	$7,76^{d}$	$82,86^{a}$	$69,27^{a}$	$9,96^{c}$	$88,55^{d}$	$14,84^{a}$
	7	$37,66^{b}$	$9,42^{b}$	$5,68^{c}$	$109,59^{b}$	$81,08^{b}$	$6,03^{b}$	$73,42^{c}$	$15,61^{b}$
	14	$35,70^{a}$	$9,29^{c}$	$4,86^{b}$	$119,86^{c}$	$95,81^{c}$	$4,57^{a}$	$67,12^{b}$	$15,47^{bc}$
	21	$35,45^{a}$	$9,49^{bd}$	$4,07^{a}$	$124,72^{d}$	$107,70^{d}$	$3,60^{a}$	$58,80^{a}$	$15,80^{b}$
	28	$34,74^{a}$	$9,54^{d}$	-	-	-	-	-	$16,11^{bd}$
					P				
Treatment		**	NS	NS	NS	NS	NS	NS	NS
Storage period		***	***	***	***	***	***	***	***
Interaction		*	NS	*	NS	NS	NS	*	NS

n=10. Values were shown as mean. , $^{a, b, c, d}$: The difference among means carrying different letters in the same column was statistically significant ($p < 0.05$). $^{A, B}$:With in treatments grups, values with the same superscript do not significantly differ ($p < 0.05$). NS: $p > 0.05$; *: $p < 0.05$; **: $p < 0,01$; ***: $p < 0,001$

Table 3. Effects of UV, Ultrasond and storage period on yolk quality of eggs.

Treatments	Storage period (day)	Yolk weight (g)	Yolk pH	Yolk height (mm)	Yolk diameter (mm)	Yolk index	Yolk dry matter (%)
UV		15,44	6,29	14,61	41,35	35,60	$46,78^{B}$
Ultrasond		15,58	6,31	14,49	41,42	35,72	$46,76^{B}$
Control		15,41	6,30	14,24	41,88	34,40	$46,18^{A}$
	1	$14,47^{a}$	$6,12^{a}$	$14,60^{ab}$	$38,47^{a}$	$38,42^{b}$	$48,53^{c}$
	7	$15,29^{a}$	$6,17^{a}$	$15,30^{b}$	$39,62^{a}$	$38,66^{b}$	$46,92^{b}$
	14	$15,54^{b}$	$6,31^{b}$	$14,51^{ab}$	$42,21^{b}$	$34,50^{a}$	$46,43^{b}$
	21	$16,11^{b}$	$6,42^{c}$	$13,95^{a}$	$43,78^{c}$	$32,79^{a}$	$45,41^{a}$
	28	$15,96^{b}$	$6,47^{c}$	$13,88^{a}$	$43,68^{bc}$	$31,82^{a}$	$45,59^{a}$
				P			
Treatment		NS	NS	NS	NS	NS	**
Storage period		***	***	*	***	***	***
Interaction		NS	NS	NS	NS	NS	**

n=10. Values were shown as mean. $^{a, b, c}$: The difference among means carrying different letters in the same column was statistically significant ($p < 0.05$). $^{A, B}$:With in treatments grups, values with the same superscript do not significantly differ ($p<0.05$).
NS: $p > 0.05$; *: $p<0.05$; **: $p<0,01$; ***: $p<0,001$

noteworthy that this thinning is not reflected in the shell breaking strength. Yan et al., (2014) stated that there is a correlation between eggshell thickness and breaking strength, and eggs with thin and homogeneous eggshells are stronger.

In a study done, it was stated that the application of ultrasound to the egg increased egg weight loss, unlike this study (Sert, 2011). In another study, it was stated that the egg shell strength was negatively affected in different levels of ultrasound application (300-400W) (Caner and Yuceer, 2014).

In this study, it was determined that the egg weight loss increased as the egg storage period increased. Weight loss occurs due to the loss of water and carbon dioxide in the albumen during storage (Yimenu et al., 2017). At the same time, it was determined that the breaking strength at the wide pole of the egg decreased due to the prolongation of the storage period. This may be caused by thinning of the shell surface, depending on the prolonged storage period.

Albumen properties are among the important factors in determining the internal quality. Albumen weight was determined to be the highest in the ultrasound group. In contrast, Albumen weight was determined as lowest in the control group. Yüceer end Caner, (2020) used different methods (ozone, ultrasound and coating with shellac and lysozyme–chitosan) to preserve the freshness of eggs during storage, and it was stated that ultrasound application positively affects the preservation of the internal quality of the egg after storage.

In the study, the albumen weight, height, index and Haugh unit values decreased as a result of the physico-chemical changes occurring in the internal structure of the egg as the storage period was prolonged. Increases in albumen length, width, pH and dry matter values were found similar to other studies. These changes may develop due to reasons such as prolongation of the storage period, loss of water and CO_2, and increase in the pH of the albumen (Kopacz and Drazbo 2018; Kumari et al., 2020; Sheng et al., 2020).

Many properties of the yolk, which was a valuable part of the nutritional diversity of the egg, were not affected by the ultrasound and ultraviolet treatments applied in this study, and had similar values with the control. It was determined that only the differences in the amount of yolk dry matter were significant. Depending on the processes applied, changes in the egg internal quality and its effect on the transitions in the vitelline membrane may be effective these results (Kumarin et al., 2020).

It was determined that the storage period was effective on all yolk quality characteristics. The increase in vitelline membrane permeability with time may have caused changes in yolk quality characteristics. Similar results have been reported in other studies depending on the time storage period (Drabik et al., 2021; Tabib et al., 2021).

The interaction between the processing applied to the eggs and the storage period was statistically significant. This interaction was determined in the albumen weight, height, Haugh unit and the yolk dry matter value respectufuly. The albumen weight of the ultraviolet and ultrasound treatments was higher than the control group. Processes can reduce water loss by affecting the pores in the shell, and as a result, less weight loss may occur during storage.

CONCLUSION

As a result, in this study, it was determined that ultrasound and ultraviolet treatments applied to eggs are important in terms of some properties on both albumen and yolk quality values. The application time of the methods and the amount of application may be effective in the formation of these effects. Prolongation of the egg storage period caused changes in the internal egg quality. It is noteworthy that the interaction of both the processes applied to the egg and the storage time is mostly observed in the albumen values. Considering this interactions, future studies may bring up the use of these two methods in different ways.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Study conception, design, data analysis, and writing of the manuscript were performed by Ö. Varol Avcılar. Material preparation and data collection were Ö. Varol Avcılar and E. Yılmaz. All the authors read and approved the final manuscript. All the authors verify that the Text, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

All data generated or analysed during this study are in cluded in this published article.

Consent for publication

Not applicable.

Acknowledgements

Authors are thankful to Prof.Dr. Ayşe Tülin ÖZ.

REFERENCES

Aboonajmi, M., Akram, A., Nishizu, T., Kondo, N., Setarehdan, S. K., Rajabipour, A. (2010). An ultrasound based technique for the determination of poultry egg quality. Research in Agricultural Engineering, 56(1), 26–32.

Al Ajeeli, M. N., Taylor T. M., Alvarado C. Z., Coufal C. D. (2016). Comparison of eggshell surface sanitization technologies and impacts on consumer acceptability. Poultry Science, 95, 1191–1197. https://doi.org/10.3382/ps/pew014

Benede, S., Molina E. (2020). Chicken egg proteins and derived peptides with antioxidant properties. Foods, 9(6), 735. https://doi.org/10.3390/foods9060735

Bhale, S., No, H. K., Prinyawiwatkul, W.,Farr, A. J., Nadarajah, K., Meyers, S. P. (2003). Chitosan Coating Improves Shelf Life of Eggs. Journal of Food Science, 68, 7, 2378-2383. https://doi.org/10.1111/j.1365-2621.2003.tb05776.x

Brodacki, A., Batkowska, J., Drabik, K., Chabroszewska, P., Luczkiewicz, P. (2019). Selected quality traits of table eggs depending on storage time and temperature. British Food Journal 121 (9), 2016-2026. https://doi.org/ 10.1108/BFJ-10-2018-0688

Caner, C., Yuceer, M. (2014). Maintaining functional properties of shell eggs by ultrasound treatment. Journal of The Science of Food and Agriculture 95, 2880–2891. https://doi.org/10.1002/jsfa.7029

Caner, C., Yüceer, M. (2015). Efficacy of various protein-based coating on enhancing the shelf life of fresh eggs during storage. Poultry Science 94(7), 1665–1677. https://doi.org/10.3382/ps/pev102

Cassar, J. R., Bright, L. M., Patterson, P. H., Mills, E. W., Demirci, A. (2021). The efficacy of pulsed ultraviolet light processing for table and hatching eggs. Poultry Science 100,100923. https://doi.org/10.1016/j.psj.2020.12.021

Dawson, B., Trapp, R. G. (2001). Basic and Clinical Biostatistics, 3rd ed. Lange Medical Books. McGraw-Hill Medical Publishing Division, New York.

Drabik K., Próchniak T., Kasperek K., & Batkowska J. (2021). The use of the dynamics of changes in table eggs during storage to predict the age of eggs based on selected quality traits. Animals, 11, 3192. https://doi.org/10.3390/ani11113192

Jirangrat, W., Torrico, D. D., No, J., No, H. K., Prinyawiwatkul, W. (2010). Effects of mineral oil coating on internal quality of chicken eggs under refrigerated storage. International Journal of Food Science and Technology, 45(3), 490–495.

Kopacz, M., Aleksandra, D. 2018. Changes in the quality of table eggs depending on storage method and time. Scientific Annals of Polish Society of Animal Production, 14(3), 37-45. https://doi.org/10.5604/01.3001.0013.5084

Kumari, A., Utkarsh Kumar Tripathi, U. K., Vipin Maurya, V., Kumar, M. (2020). Internal qualıty changes ın eggs durıng storage. International Journal of Science, Environment and Technology, 9(4), 615 – 624.

Luo, W., Xue, H., Xiong, C., Li, J., Tu, Y., Zhao, Y. (2020). Effects of temperature on quality of preserved eggs during storage. Poultry Science, 99, 3144–3157, https://doi.org/10.1016/j.psj.2020.01.020

Melo, E. F., Clımaco, W. L. S., Triginelli, M. V., Vaz, D. P., De Souza, M. R., Baiao, N. C., Pompeu, M. A., Lara, L. J. C. (2019). An evaluation of alternative methods for sanitizing hatching eggs. Poultry Science, 98, 2466–2473. https://doi.org/10.3382/ps/pez022

Mehdizadeh, S. A., Minaei, S., Karimi Torshizi, M. A., Mohajerani, E. (2015). Effect of UV irradiation, sample thickness and storage temperature on storability, bacterial activity and functional properties of liquid egg. Journal of Food Science and Technolohy, 52(7), 4414–4422. https://doi.org/10.1007/s13197-014-1509-1

Onbaşılar, E. E., Avcılar, Ö. V. (2011). Kahverengi yumurtacı tavuklarda yaş ve yumurtlama zamanının yumurta ağırlığı ve kabuk kalitesi üzerine etkileri. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 51(1), 15-19.

Puglisi, M. J., Fernandez, M. L. (2022). The health benefits of egg protein. Nutrients, 14(14), 2904. https://doi.org/10.3390/nu14142904

Rana, M. S., Campbell, D. L. M. (2021). Application of ultraviolet

light for poultry production: a review of ımpacts on behavior, physiology, and production. Frontiers in Animal Science, 2, 699262. https://doi.org/10.3389/fanim.2021.699262

Sarıyel, V., Aygun, A., Coklar, H., Narınc, D., Akbulut, M. (2022). Effects of prestorage application of gum arabic coating on the quality of table eggs during storage. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 28(3), 363-370. https://doi.org/10.9775/kvfd.2022.27077

Sert, D., Aygun, A., Demir, M. K. (2011). Effects of ultrasonic treatment and storage temperature on egg quality. Poultry Science, 90, 869–875. https://doi.org/10.3382/ps.2010-00799

Sheng, X. W., Bing, S. H., Lu, C. Q., Yuan, X, Y., Zang, T., Zhan, Z. W., Shu, D. Q., Li, Y. J., Li, M. T., Wu, B. Q. (2020). A combined approach using slightly acidic electrolyzed water and UV exposure to improve egg internal quality during storage. Poultry Science, 99, 6007–6012. https://doi.org/10.1016/j.psj.2020.07.034

Tabib, I., Onbaşılar, E. E., Yalçın, S. (2021). The effects of cage type, oviposition time and egg storage period on the egg quality characteristics of laying hens. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 68, 329-336. https://doi.org/10.33988/auvfd.779272

Turtoi, M., Borda, D. (2014). Decontamination of egg shells using ultraviolet light treatment. World's Poultry Science Journal, 70(2), 265-278. https://doi.org/10.1017/S0043933914000282

Xie, L., Hettiarachchy, N. S., Ju, Z. Y., Meullenet, J., Wang, H., Slavik, M. F., Janes, M. E. (2002). Edible film coating to minimize eggshell breakage and reduce post-wash bacterial contamination measured by dye penetration in eggs. Journal of Food Scıence, 67, 280-284. https://doi.org/10.1111/j.1365-2621.2002.tb11398.x

Yalçın, S., Yalçın, S., Onbaşılar, İ., Eser, H., Sahin, A. (2014). Effects of dietary yeast cell wall on performance, egg quality and humoral immune response in laying hens. Ankara Universitesi Veteriner Fakültesi Dergisi, 61, 289-294.

Yan, Y. Y., Sun, C. J., Lian, L., Zheng, J. X., Xu, G. Y., Yang, N. (2014). Effect of uniformity of eggshell thickness on eggshell quality in chickens. The Journal of Poultry Science, 51, 338–342. https://doi.org/10.2141/ jpsa.0130032

Yimenu, S. M., Kim, J. Y., Koo, J., Kim, B. S. (2017). Predictive modeling for monitoring egg freshness during variable temperature storage conditions. Poultry Science, 96, 2811–2819. https://doi.org/10.3382/ps/pex038

Yuceer, M., Caner, C. (2020). The effects of ozone, ultrasound and coating with shellac and lysozyme–chitosan on fresh egg during storage at ambient temperature. Part II: microbial quality, eggshell breaking strength and FT-NIR spectral analysis. International Journal of Food Science and Technology, 55, 1629–1636. https://doi.org/10.1111/ijfs.14422

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 213-222
DOI: https://doi.org/10.31015/jaefs.2023.1.26

Precipitation forecast with logistics regression methods for harvest optimization

Mesut Samasti[1] • **Tarik Kucukdeniz**[2]

[1] TUBİTAK Turkish Management Sciences Institute, Türkiye
[2] Department of Industrial Engineering, Istanbul University-Cerrahpasa, Türkiye

Abstract
This paper proposes a model that forecasts the weather and then, based on that forecast, uses an income-oriented linear programming method to optimize the harvesting process. Data representing a total yearly output capacity of 472,878 tons from 214 different field locations were used to test the model for sugar beet production. Prior to optimization, long-term one-year weather rainfall forecasting was done using 10 years of actual weather data for the field locations. Weather precipitation was forecasted using logistic regression with an accuracy of 84.16%. The outcome of the weather precipitation prediction model was a parameter in the optimization model. The weather forecast for precipitation led to the 120-day harvest planning being optimized. Comparative analysis was done on the outcomes of the developed model and the current scenario. Comparing the current situation to the proposed one, revenue would have increased by 16.7%. Given that it incorporates weather forecasts into the harvest optimization process, the methodology presented in this paper is more practical than other harvest optimization models.
Keywords: Precipitation forecast, Machine learning, Logistic regression, Harvest efficiency, Optimization

Citation: Samasti, M. and Kucukdeniz, T. (2023). Precipitation forecast with logistics regression methods for harvest optimization. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 213-222

Received: 08 January 2023
Revised: 05 February 2023
Accepted: 10 February 2023
Published Online: 27 March 2023

Correspondence: Mesut Samasti
E-mail: mesutsamasti@gmail.com

INTRODUCTION

Throughout human history, agricultural pursuits have been of utmost significance. Farmers are now looking for different alternatives to boost their earnings due to the rise in global competition in agricultural activities conducted at the micro and macro levels. Some of these alternatives include switching from low-yielding seeds to high-yielding yet unsustainable seeds, as well as using excessive irrigation, fertilizers, and pesticides. The environmental sustainability of agricultural production is jeopardized by these possibilities, which also drive up the costs of production for farmers daily.

Aside from the current challenges in agricultural activity production, extraordinary occurrences like pandemics and recent wars have disrupted the global supply chain network and made already challenging agricultural activity production processes much more challenging. Cost items including seeds, fertilizers, gasoline, electricity, and equipment have gone up, and agricultural activities that try to produce with poor profit margins have come to a halt as a result of the disruption of the global supply chain and the onset of economic bottlenecks.

Farmers that attempt to carry on their agricultural operations using conventional methods either search for other solutions for the production processes or discontinue their production activities when their profit margins decline, which

is a bad situation. States have created incentive programs to encourage the continuation of manufacturing of essential production products because of this (Şaşmaz and Özel, 2019).

For cost items incurred during production activities, there are alternate chances for improvement and cost savings. Sectoral clustering and collective purchasing can create economies of scale and lower the cost of a unit of production, even though it may not be possible to cut cost items in the import-based procurement for manufacturing at the first stage (TÜSSİDE, 2019). Operational plans created using scientific methods may even be more successful in lowering production costs. the selection of ideal planting sites, the identification of ideal products based on soil and climate analysis, the use of experimental design methods to increase productivity during the production period (Ranka and Sharma, 2012), the selection of irrigation and fertilization techniques that will increase productivity (Hill and Keller, 1980), the real-time continuous monitoring of irrigation needs by controlling humidity with Internet of Things (IoT) sensors for optimal irrigation (Mat et al., 2016), and harvest planning.

Recent or continuing rain increases harvesting costs while the commodity is being harvested. The duration of harvesting time per unit increases when these expenditures are considered because of the muck that is present during and after rainfall. Additionally, the mud creates a lot of sludge in the harvested product, increasing the operating costs of washing and cleaning the products before final consumption while reducing the useable unit product load on the transport vehicles.

This study looks at how sugar beet products are harvested and transported to a processing plant with an annual output of 472,878 tons. The alternate optimum harvesting plan based on weather forecasting and the current harvest plan were compared and contrasted. Ten years' worth of daily weather data were gathered for the study from the Turkish State Meteorological Service. The maximum, lowest, and average temperatures as well as the amount of precipitation per square meter are all included in this weather information. Regression models based on data mining were employed to attempt to forecast precipitation in the relevant area. Python language coding was used to create a solution for the prediction approach. Categorical categorization was employed to identify whether the weather would be rainy. An yearly harvest plan was created following the completion of the long-term weather forecast. The OpenSolver plugin was used to resolve the integer linear programming model created for the harvest plan.

Section 1 is informed in general terms about the study. The Section 2 literature review is also included. This section examines studies on the topic and the article's addition to the body of knowledge. Section 3 provides a definition of the issue and an explanation of its scope. The procedures and model are the same as those in Chapter 4. Real data were used in the study connected to part 5, and the findings were shared with section 6. There was a broad assessment given in the final section.

For the business sectors of manufacturing, agriculture, and tourism to employ resources effectively, weather forecasting is crucial. When businesses have access to weather information in advance, they can make plans more successfully. Numerous research studies have been conducted to forecast meteorological events as a result. A linear link between weather-dependent data and weather conditions has been proposed in recent studies on weather forecasting. Because weather patterns are erratic and nonlinear, artificial neural networks have been employed as an alternative. One study used artificial neural networks to try and estimate the maximum temperature from the minimum and highest temperature data (Abhishek et al., 2012). Another study used machine learning techniques to create minimum and maximum temperature projections for the past two days. This was done since traditional weather forecast models are unreliable in the presence of disruptions (Holmstrom et al., 2016). Other studies used temperature, humidity, dew point, pressure, wind, and rain data to create weather forecast models using deep learning techniques and iterative neural networks (RNN) (Salman et al., 2015), LSTM (Long Short Term Memory) methods (Fente and Singh, 2018), and temperature prediction models using the LSTM method (Karevan and Suykens, 2018).

Forecasting precipitation is a challenging task for weather forecasting techniques. Pressure, temperature, wind speed, and wind direction are only a few of the many factors that affect the amount and spatial distribution of precipitation. Due to the complexity of the atmospheric processes that cause precipitation and a lack of data, forecasting precipitation is frequently impossible (Luk et al., 2001). Alternative approaches to precipitation forecasting are now available because to advancements in artificial intelligence applications. It has been demonstrated that artificial neural networks, which execute a non-linear mapping between input and output, can predict precipitation with reasonable accuracy. The month-based ANN model created for precipitation forecasting has been found to be accurate in determining the risk level of heavy precipitation events (Sulaiman and Wahab, 2017). Artificial neural networks have been used to create new methods that enhance the performance of precipitation forecasting. The relative humidity, air pressure, wet bulb temperature, and cloudiness data from 75 measurement locations over a 4-year period were used in one novel model to produce relevant findings (Hung et al., 2009).

When the parameters of the data set (minimum and maximum temperature, average temperature, average humidity, atmospheric pressure, precipitation amount,

sunshine duration, maximum and average wind speed) are enlarged in precipitation forecasting systems, the forecasts' accuracy rises. While the accuracy rate of processes covering short time periods can be up to 72% with a fuzzy inference system model (Safar et al., 2019), the accuracy rate of precipitation forecasts can be up to 86% with machine learning techniques (Anwar et al., 2020). Utilizing extensive weather data six hours in advance, substantial precipitation forecasts can be made, and effective outcomes can be attained using genetic algorithms (Lee et al., 2014). Similarly, using machine learning techniques, it is feasible to forecast harmful severe rains four days in advance (Choi et al., 2018).

For a variety of industries, including business, tourism, and agriculture, weather and precipitation forecasting is crucial. When precipitation affects the harvest plan, operating costs are higher than anticipated, the harvest plan is delayed, and ongoing activities are disrupted. Planning for harvest is crucial for determining capacity for product transportation and storage activities (Khalilzadeh and Wang, 2021). Real-time techniques have been established about when and how much product will be harvested, stored, and processed in order to preserve the post-harvest product quality for perishable food goods (Lin et al., 2018).

Statistical models have been created to help analyze the impact of changes in characteristics like temperature, fog, humidity, and precipitation on sugarcane production when the research evaluating the weather and crop harvest together in the literature are examined (Priya and Suresh, 2009). Similar to this, a harvest prediction model based on weather characteristics was created in another study. This prediction model utilized the multiple regression model (MLR). A region that produces rice was used to test the appropriate model. The created estimate model can account for the yield variation at a rate of 89% (Dhekale et al., 2014). The estimated harvest date, which is determined by the weather, is determined by the product's level of maturity and productivity. Some products need to be harvested quickly in order to preserve their economic value. So that the product can be collected at a time when it is commercially viable, models that forecast harvest dates based on weather conditions have been established in this scenario. The created model was used to harvest plums with a 30-day maturation period (DeBuse et al., 2010). The operational procedures used for harvesting are also impacted by the weather. Through interviews with wheat harvest specialists, the effects of the harvesting process on operational and logistic services were investigated (Medvediev et al., 2017). There are no studies that optimize the harvesting process using a cost-oriented strategy depending on whether it is raining or not, according to the literature review. This study tries to close this gap in the body of knowledge.

Problem Definition

When harvest time comes, the grown agricultural goods must be rapidly collected, processed, or consumed while being mindful of the perishability of the product. This makes it vital to arrange the labor, equipment, and tools, as well as the logistics activities, before the commodity is harvested. Planning is also necessary for the facility's capacity use if the items are to be transported there for processing or storage. These strategies must serve as the foundation for resource planning. Failure at any point in the supply chain between the harvest and the production facility results in idle planned resources and raises wastage rates as the product's delivery time is prolonged. For this reason, when resource planning has a small margin of error, economic damages to the businesses will be kept to a minimal.

Natural occurrences like rain can make it challenging to employ resources, even while resources like the labor, equipment, vehicles, capacity, and logistics can be controlled in the issues experienced in planning based on resource assignments. Because personnel, equipment and trucks cannot operate in the muddy fields where the harvest will take place during rainfall, this results in a waste of resources.

While resource allocation plans are being formed, natural occurrences like rain cannot be controlled; nevertheless, if such weather conditions are expected and resource distribution plans are made based on these forecasts, the danger of resources becoming idle can be avoided. In order to manage resource distribution more effectively,

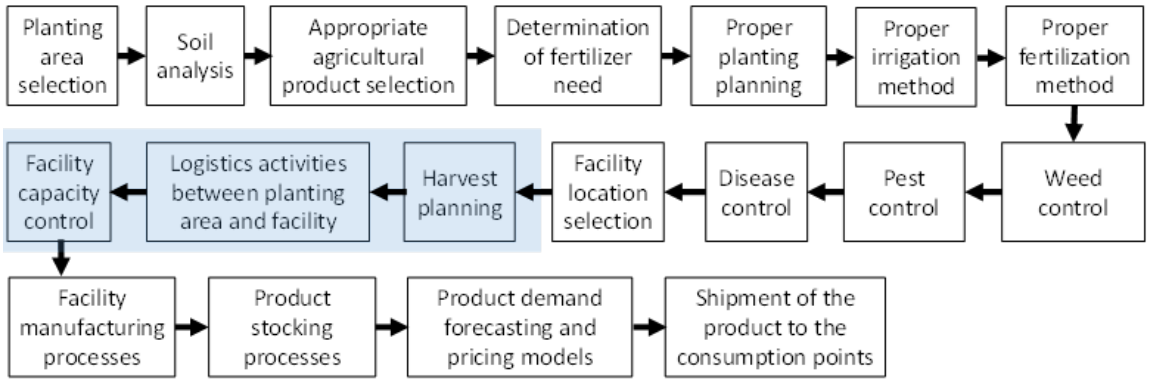


Figure 1. Life cycle of agricultural goods and research area

this study tries to forecast rain.

The problem for which a solution proposal is developed covers the stages of "harvest removal planning," "logistic activities between the planting area and the facility," and "plant capacity control" taking into account the general life cycle of agricultural products from the planting decision to the consumption point. The broad life cycle of agricultural products and the place of the research scope within this life cycle are schematized in the picture below.

Through reductions in cost factors for agricultural activities, this study will support the sustainability of agricultural activities.

METHOD

In order to solve the issue, two approaches were utilized sequentially. The first method involved examining the weather information at the harvesting locations and using a logistic regression algorithm to forecast precipitation. An integer linear programming model was used to optimize the harvest after weather predictions. The methodology listed below provides a general method for solving problems.

machine learning process. Machine learning algorithms work iteratively until the right model is produced after preparing the data for the learning process. The program is run after the proper model has been chosen. The learning process will be improved by having more data. The general machine learning process is depicted in the following diagram.

Categorical data are used in logistic regression algorithms to produce better results. As a result, the weather forecast that will be fed into the model will categorize predictions as either raining or not. machine learning algorithms are classified under 4 groups. Classification, Regression, Clusrting and Deep Learning. Logistic regression is included in the Regression group within these groups (Wang et al., 2020).

The linear relationship between the dependent and independent variables is investigated by linear regression models. With linear regression, variables can be projected (Maulud and Abdulazeez, 2020). When using categorical or continuous data, logistic regression is employed (LaValley, 2008). When handling problems involving categorical classification, the logistic regression method yields trustworthy findings. The data are classed as 1-0

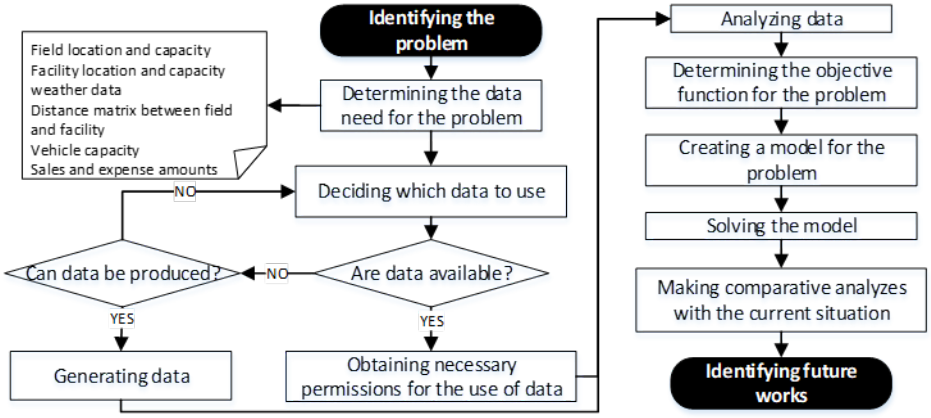


Figure 2. General study methodology

Logistic regression method

Artificial intelligence has a component called machine learning that focuses on learning by mimicking human intellect. Algorithms are used to examine data, and learning that is based on these analyses goes on and gets better (Helm et al., 2020). With data comes the

in logistic regression, and the s-shaped logistic function curve is utilized since it better fits the data (Gianey and Choudhary, 2017).

Determine which category the people belong to using logistic regression (Çokluk, 2010). When some of the case studies created with the logistic regression model

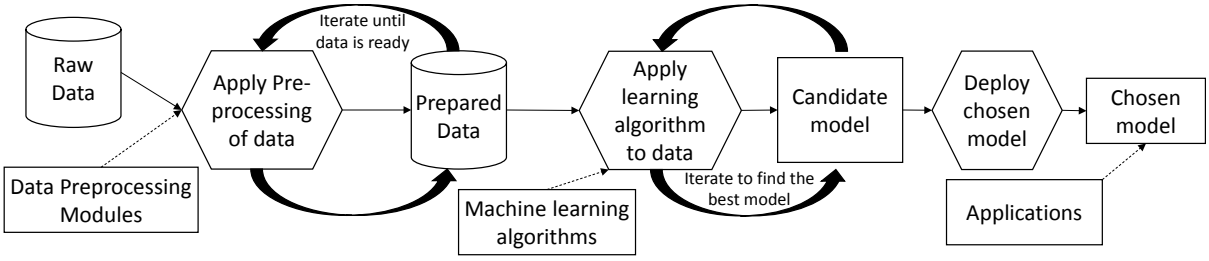


Figure 3. Machine learning process (Chappell, 2015)

are examined, it can be used to classify financial success of enterprises (Kaygın et al., 2016), to evaluate the risk status of bank credit customers (Budak and Erpolat, 2012), to examine the effects of factors that create customer satisfaction on customer adherence (Mutlubaş and Soybalı, 2017), and to model weather conditions like fog (Aktaş and Erkuş, 2009). Compared to linear models, logistic regression models are distinct. The graphic below shows the graph and formula variations between logistic and linear regression.

- The soil at the field point will continue to be wet for three days after the precipitation.
- The car is a unique creation.
- When the relevant field is unable to fill the vehicle tonnage, it is unable to purchase goods from another field to meet the available capacity.
- The amount of daily trips may require vehicle exits from one or more fields.
- The product's unit kilogram value is five times its dry air operating cost.

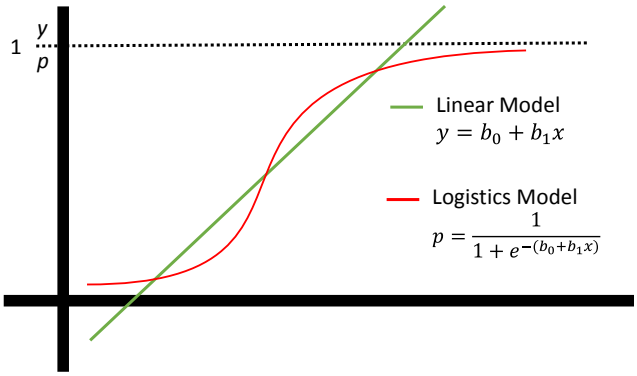


Figure 4. Linear regression and logistics regression (Gianey and Choudhary, 2017)

Weather precipitation forecasting was accomplished using Python and the Scikit-Learn library's logistic regression techniques, one of the libraries frequently utilized in machine learning challenges. Whether there will be rain or not, regardless of how much rain falls per square meter, it is rated as 1-0 in the weather forecast. With the logistic regression method, the first nine years of the pertinent data set will serve as training data and the last year will serve as test data, and daily estimates of whether it will rain or not encompass a one-year period.

Harvest optimization model

After using the logistic regression technique to predict the weather, an integer linear programming model was created to optimize the harvesting process. The system's overall revenue will be maximized by the developed model.

Model Assumptions

To address the issue, several assumptions have been made. Following the model solution, comparative analyses were assessed within the parameters of these hypotheses.

- The product is shipped from a special facility with a finite capacity.
- In the fields, only one kind of product is produced.
- Field locations and plant locations are already known.
- The actual road distances are the ones between the field and the plant.
- The product's economic value fluctuates with time.
- Operating costs for harvesting in dry soil and wet soil are split in half.

Model Sets

The harvests from the fields shall be brought to a single facility within the parameters of the issue.

$i \in I$ To be harvested fields

$i \in I$ Points of facilities (There is one facility within the scope of this problem)

k ∈ K Weather condition

t ∈ T Day

Parameters [unit]

$UC_{i,j,t}$ The cost of transportation on day t from fields i to facility j

$P_{i,t}$ ∈N Time dependent amount of income of the crops in the fields i

TKi Production capacity of the field i

FKj Production capacity of the facility j

NF: Number of active facilities (There is 1 facility for this problem)

Variables

$X_{i,j,t}$ ∈ N Amount transported daily on day t from field i to facility j

$W_{i,t}$ ∈ B Harvesting of crops from field on day {0,1}

K_{it} ∈ N Weather-dependent unit operation cost coefficient from field on day t (obtained by logistic regression)

Y_j ∈ B Facility is active or passive

MODEL

$$\max z = \sum_{i \in I} \sum_{j \in J} \sum_{t \in T} (P_{i,t} - K_{it} UC_{i,j,t}) X_{i,j,t} \qquad (1)$$

Constraints

$$\sum_{i \in I} (X_{i,j,t} - TK_i Y_{i,t}) = 0 \quad \forall i \in I, t \in T \qquad (2)$$

$$\sum_{i \in I} X_{i,j,t} \leq FK_j \qquad \forall j \in J, t \in T \qquad (3)$$

$$\sum_{j \in J} Y_j = NF \qquad (4)$$

214 fields in various locations and with varying output capacity are used to grow sugar beets. Figure 6 shows the distribution of the fields' production capacities, and Figure 7 shows the distribution on a map.

The product is harvested over a 120-day period, and lorries with a 25-ton capacity maximum are used to transport it to the plant. In order to determine how

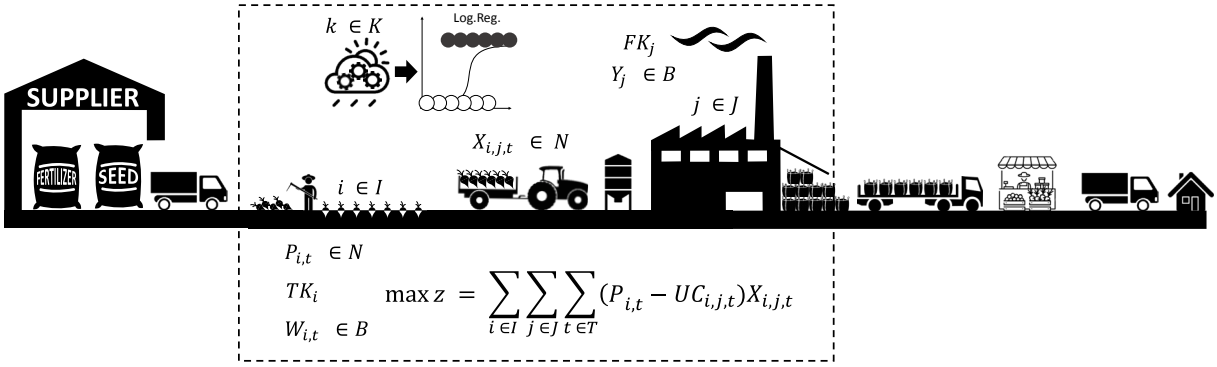


Figure 5. Model scope

(1) Displays the mathematical model's objective function. It seeks to optimize revenue from the arrival of weather-dependent, variable-cost goods at the facility.

(2) Illustrates the restriction that sends the goods to the plant on the day of field harvest.

(3) Illustrates the constraint that the facility's capacity is not exceeded by the products arriving at the facility.

(4) Displays the restriction with the number of open facilities. One facility is present in this instance.

The OpenSolver optimization plugin is used to resolve the weather-dependent harvest optimization model. The ba of the issue is depicted in the figure below.

Case study

The approach created within the parameters of the issue was used as an actual case in a facility with a total yearly capacity of 472,878 tons for processing sugar beet.

many cars would travel from each field to the facility, the field tonnage was divided into 25 and the total number of visits to the fields was spread evenly across the 120-day harvesting plan. These calculations show that there would be 19,007 truck trips overall. Figure 8 shows the distribution of the fields' distances from the facility, which will be taken into account when calculating the expenses associated with logistics.

In terms of the problem, there is only one facility. The total revenue from the products entering the facility is the goal of the integer linear programming model created to solve the challenge. In order to create a solution suggestion for the issue, 10 years' worth of meteorological data from 214 field points were gathered and examined from the Turkish State Meteorological Service. Measurement stations near field locations were found and included in the estimation after 31.9 million lines of data from all

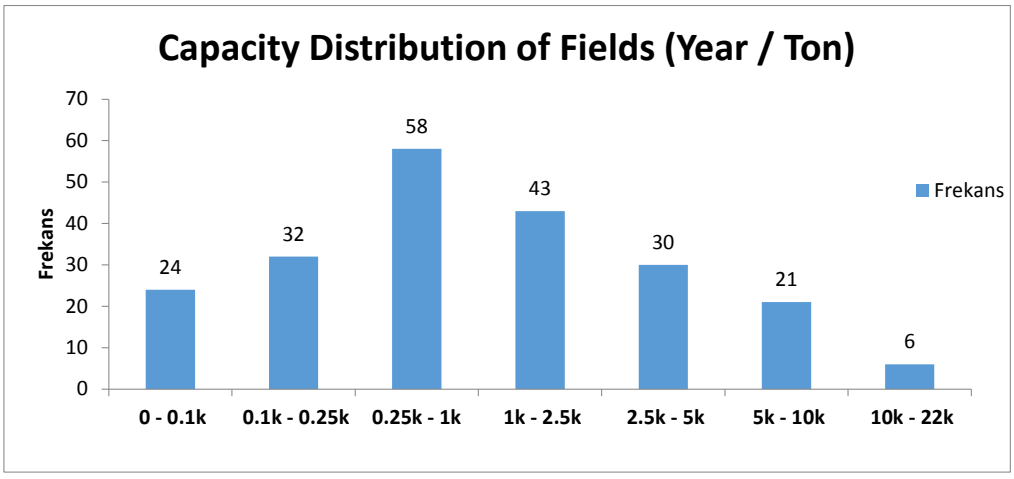


Figure 6. Capacity distribution of fields

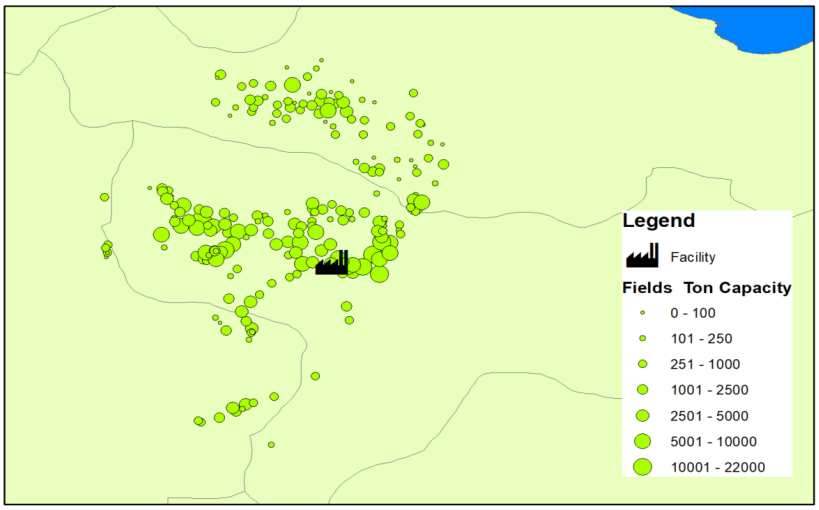


Figure 7. Facility and fields locations

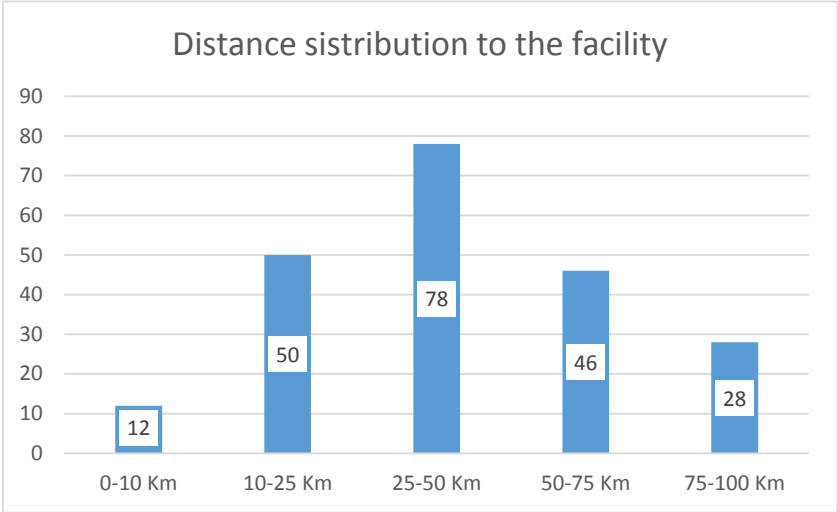


Figure 8. Distance distribution of the facility

around Turkey were evaluated. The maximum, lowest, average temperature, and precipitation are broken down into 4 columns in the pertinent field locations. The daily 365 days x 10 years for each field location are detailed in the 10-year data.

The literature review demonstrates how challenging it is to handle complicated, multidimensional problems like weather forecasting technologies. Data from lowest and maximum temperatures, average temperatures, average humidity, atmospheric pressure, amount of precipitation, length of sunshine, and maximum and average wind speeds are used to forecast weather and precipitation when previous studies are taken into consideration. The study's use of weather data might have less scope than earlier research. The estimating model, however, includes a 1-year time frame, in contrast to prior studies. Additionally, because the study's primary objective is income maximization, its scope includes an examination of the benefits of harvest planning through weather forecasting.

RESULTS

In the annual precipitation forecast within the scope of the study, the 120-day period covering September-December dates was determined as the harvest time. When the 10-year weather data of the harvest period is examined, it is seen that the number of rainy days in the harvest period tends to decrease and the average temperature values tend to increase.

In the weather predictions made using the logistic regression method, it was seen that precipitation was favorably associated to the minimum temperature and date and negatively related to the maximum temperature. Whether the length is long or short, the accuracy rate in prediction models varies. Although short-term forecast models have a high accuracy rate, long-term projections may have a lower accuracy rate. In this work, logistic regression was used to forecast long-term precipitation. It was attempted to estimate the precipitation of the weather in 2020 by using the meteorological data from the field locations as training data for the years 2010

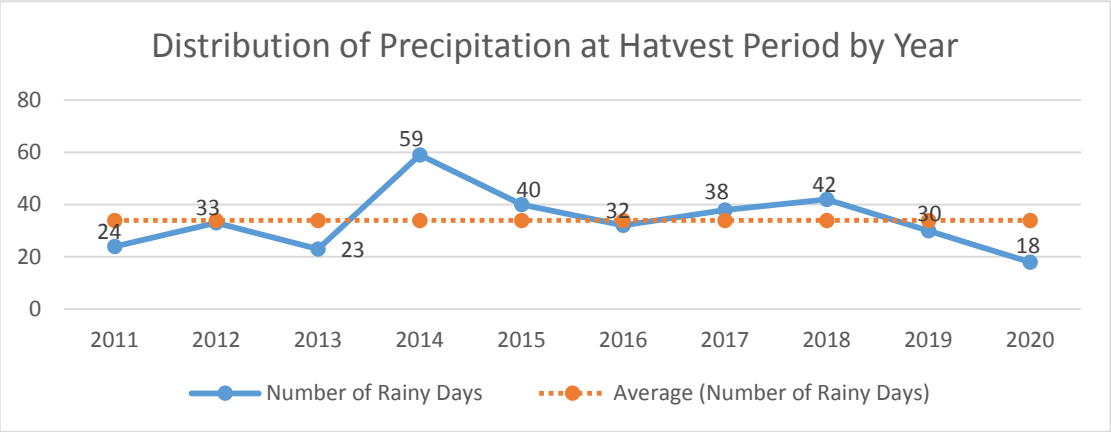


Figure 9. Distribution of precipitation at harvest time by years

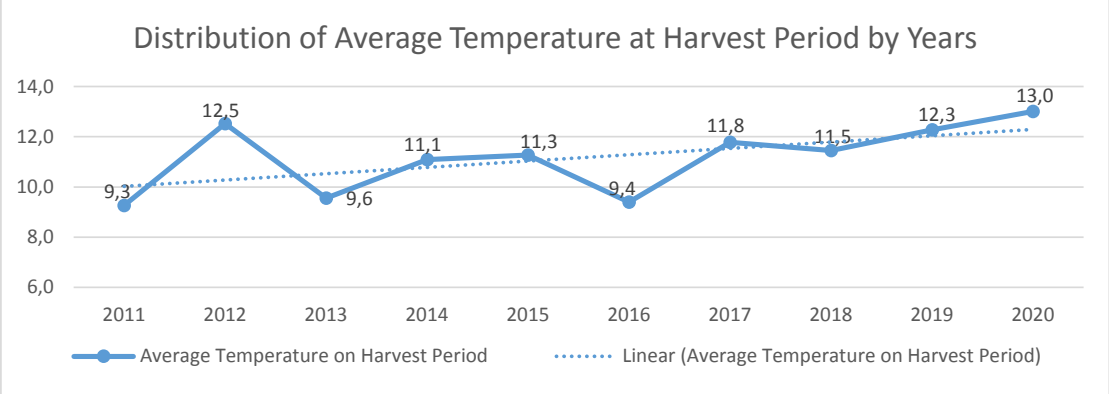


Figure 10. Distribution of average temperature at harvest by years

through 2019. The acquired results were compared to the data from 2020. The estimation using the logistic regression model had an accuracy rate of 84.16%. The outcomes support effective resource allocation in the current harvest plan, which is created well in advance of the harvest.

The economic return of the product's maturity due to sugar polarization depends on time at 4 distinct levels in the problem of harvesting after the weather forecast, whether it is raining or not at 2 different levels, and the weather itself at 1 level. The optimal choice was determined using opensolver using 214 fields, 120 harvesting days, and 205.440 account combinations. 159 vehicles per day were transported to the factory during the 119 days of harvesting, with 86 vehicles being transported on the final day. The calculated average tonnage load for each vehicle was 24.88 tons.

Depending on whether it is raining or not, the expenses in the example studied within the parameters of the problem differ. Rainy days result in higher operational costs because regular harvest planning is weather-independent. In the integer linear programming model solution implemented via OpenSolver utilizing the weather forecast data generated by logistic regression, it is evident that the operational profit of the business will increase by 16.7% compared to the usual harvesting plan. (Present harvest plan revenue is 16.566.398, alternative harvest plan revenue dependent on weather forecast is 19.329.205). Making short-term weather forecasts when necessary can help an organization boost its operational profit when harvest season, which is prepared in accordance with the long-term strategy, starts.

CONCLUSION

Results from supplementing the planning process for the sugar beet harvest with weather forecasting techniques were compared to those from the existing harvest plan in this study. Within the parameters of the study, 10 years of weather data from the field sites where the harvest will be made were examined, and a logistic regression model was used to anticipate precipitation. A harvest schedule for the crop was created using integer linear programming and included the results of the precipitation forecast as a parameter. When compared to the present harvest plan, it was determined that the alternative harvest plan may potentially boost operating profit by 16.7%.

If the forecast model is created in two stages in a way that they complement one another in the long and near term, further research may try to go into greater depth about the weather data sets and look at the overall possible benefit. Alternative model approaches that could improve the planning of tools, machines, and labor in harvesting operations may be possible when weather forecasting systems for harvest planning are established.

The examination of the weather data shows that there is a risk for the productions to be achieved in the upcoming years because to the rise in yearly average temperature values and the decline in precipitation data. Future research can create models that take into account weather trends to get outcomes that are more accurate.

Over 6.5 million tons of sugar beets are produced in Turkey overall. Averaging 2200 vehicles per day are required during the 120-day harvest period. A short-term vehicle demand constraint in the logistics industry may result from poor supply chain management choices for these products. For this reason, it's critical to effectively and efficiently arrange the logistical procedures for sugar beet.

This investigation was conducted in order to produce sugar beets. Fresh perishable products such as fresh olives, strawberries, mushrooms, tomatoes, lettuce, etc. degrades more quickly than sugar beet. Because of this, it is essential to design the harvest strategy for these products in a way that minimizes waste. In the future, fertilization activities of the products and plans that will spread the ripening process of the product throughout time based on the demand by estimating the demand in harvest planning for perishable agricultural products may be new research areas.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Abhishek, K., Singh, M.P., Ghosh, S., Anand, A. (2012). Weather forecasting model using Artificial Neural Network, Pocedia Technology, 311-318

Aktaş, C., Erkuş, O. (2009). Investigation of fog forecastıng of eskişehir using logistic regression analysis, İstanbul Commerce University Journal of Science, 47-59

Anwar, M.T., Nugrohadi, S., Tantriyati, V., Windarni, V.A. (2020). Rain prediction using rule-based machine learning approach, advance sustainable science, Engineering and Technology, 200104

Budak, B., Erpolat S. (2012). Comparison of artificial neural networks and logistic regression analysis in the credit risk prediction, Online Academic Journal of Information Technology, 3(9), 23

Chappell, D. (2015). Introducing azure machine learning, DavidChappell & Associates,

Choi, C., Kim, J., Kim, J., Kim, D., Bae, Y., Kim, H.S. (2018). Development of heavy rain damage prediction model using machine learning based on big data, Hindawi Advances in Meteorology, 11

Çokluk, Ö. (2010). Lojistik regresyon analizi: kavram ve uygulama, In: Educational Sciences: heory & Practice, 1357-1407, (in Turkish)

DeBuse, C., Lopez, G., DeJong, T. (2010). Using spring weather data to predict harvest date for 'Improved French' prune, In: Proc. 9th IS on Plum & Prune Genetics, 107-112

Dhekale, B.S., Sawant, P.k., Upadhye, T.P. (2014). Weather based pre-harvest forecasting of rice at Kolhapur (Maharashtra), Trends in Biosciences, 925-927

Fente, D.N., Singh, D.K. (2018). Weather forecasting using artificial neural network, In: Proceedings of the 2nd International Conference on Inventive Communication and Computational Technologies, 1757-1761

Gianey, H.K., Choudhary, R. (2017). Comprehensive review on supervised machine learning algorithms, In: , 37-43

Helm, J.M., Swiergosz, A.M., Haeberle, H.S., Karnuta, J.M., Schaffer, J.L., Krebs, V.E., Spitzer A.I., Ramkumar, P.N. (2020). Machine learning and artificial ıntelligence: definitions, applications, and future directions, Current Reviews in Musculoskeletal Medicine, 69-76

Hill, R.W., Keller, J. (1980). Irrigation system selection for maximum crop profit, American Society of Agricultural Engineers, 366-372

Holmstrom, M., Liu, D., Vo, C. (2016). Machine learning applied to weather forecasting, Stanford University, 1-5

Hung, N.Q., Babel, M.S., Weesakul, S., Tripathi, N. K. (2009). An artificial neural network model for rainfall forecasting in Bangkok, Thailand, Hydrol. Earth Syst. Sci, 1413-1425

Karevan, Z., Suykens, J.A.K. (2018). Spatio-temporal stacked LSTM for temperature prediction in weather forecasting, 1-5

Kaygın, C.Y., Tazegül, A., Yazarkan H. (2016). Estimation capability of financial failures and successes of enterprises using data mining and logistic regression analysis, Ege Academic Review, 147-159

Khalilzadeh, Z., Wang, L. (2021). An MILP model for corn planting and harvest scheduling considering storage capacity and growing degree units, Biorxiv, 2021-02

LaValley, M.P. (2008). Logistic Regression, Circulation, 2395–2399

Lee, J., Hong, S., Lee, J.H. (2014). An efficient prediction for heavy rain from big weather data using genetic

algorithm, In: IMCOM (ICUIMC)'14, 1-7
Lin, X., Negenborn, R.R., Lodewijks, G. (2018). Predictive quality-aware control for scheduling of potato starch production, Computers and Electronics in Agriculture, 266-278
Luk, K.C., Ball, J.E., Sharma, A. (2001). An application of artificial neural networks for rainfall forecasting, Mathematical and Computer Modelling, 683-693
Mat, İ., Kassim, M.R.M., Harun, A.N., (2016). IoT in precision agriculture applications using wireless moisture sensor network, In: IEEE Conference on Open Systems, 24-29
Maulud, D.H., Abdulazeez, A.M. (2020). A review on linear regression comprehensive in machine learning, Journal of Applied Science and Technology Trends, 140-147
Medvediev, I., Lebid, I., Bragin, M. (2017). Assessment of the weather and climate conditions impact on the organization and planning of transport support for wheat harvesting, TEKA. Commission of the motorization and energetics in agriculture, 45-54
Mutlubaş, C., Soybalı, H.H. (2017). The ivestigation of effect of customer satisfaction factors on customer loyalty with logistics regression analysis, Journal of Turkissh Tourism Research, 1-15
Priya, S.R.K., Suresh, K.K. (2009). A study on pre-harvest forecast of sugarcane yield using climatic variables, Statistics and Applications, 1-8
Ranka, N.M., Sharma, L. (2012). Design of experiments: a powerful tool for agriculture analysis, Statistics, 11356-11358
Safar, N.Z.M., Ramli, A.A., Mahdin, H., Ndzi, D., Khalif, M.N.K. (2019). Rain prediction using fuzzy rule based system in North-West Malaysia, Indonesian Journal of Electrical Engineering and Computer Science, 1572-1581
Salman, M.G., Kanigoro, B., Heryadi, Y. (2015). Weather forecasting using deep learning techniques, In: ICACSIS, 281-285
Sulaiman, J., Wahab, S.H. (2017). Heavy rainfall forecasting model using artificial neural network for flood prone area, IT Convergence and Security, 69-76
Şaşmaz, M.Ü., Özel, Ö. (2019). Effect of agricultural ıncentives on the development of agricultural sector: example of Turkey, Dumlupinar University Journal of Social Sciences, 61, 50-65
TÜSSİDE (2019). National suger beet logistics optimization in Turkey, In: Turkish Management Sciences Institutes' Research,
Wang, G., Pu, P., Shen, T. (2020). An efficient gene bigdata analysis using machine learning algorithms, Multimedia Tools and Applications, 9847-9870

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 223-233
DOI: https://doi.org/10.31015/jaefs.2023.1.27

Evaluation of database and some soil characteristic of Kumkale Agricultural Enterprise soils in GIS

Yavuz Sahin Turgut[1] • **Yakup Kenan Koca**[1]

[1] Soil Science and Plant Nutrition, Agriculture Faculty, Çukurova University, Adana, Türkiye

Abstract

General Directorate of Agricultural Enterprises (GDAE), which has been operating since 1994, is a state institution whose aim is to protect the gene resources as well as the production of seeds, breeding and raw materials for agriculture and agriculture-based industry. There are more than 30 agricultural enterprises established for similar purposes in Türkiye. One of these enterprises is Kumkale Agricultural Enterprise (KAE) located within the borders of Çanakkale province. The aim of this study is to reveal the current potentials of KAE lands, for which detailed studies have been made before, to create a database in Geographic Information System (GIS) and to produce various thematic maps. In this study, important soil characteristics such as slope, soil depth, surface stones, drainage, as well as soil series and physiographic units included in the survey report were mapped and the areas covered were calculated in GIS. With the digitization has determined that the agricultural enterprises lands in question are spread over an area of 7309 decares in total. In terms of soil series, the highest distribution area belongs to Karabatak series (948.2 da), while Alluvial soils (3094.8 da) have the highest distribution area in terms of physiographic units. In addition, most of the lands consist of 0-2% slope (4478.9 da), deeper than 120 cm (5936.8 da), stone-free (5677.2 da) and without drainage problems. As a result, it will be useful to examine both the temporal and spatial changes of the lands of GDAE, which has a significant production capacity in Türkiye, to make new field studies and compare them with the old survey studies in terms of guiding future studies.

Keywords: Kumkale agricultural enterprise, GIS, Soil survey, Soil mapping, GDAE

Citation: Turgut, Y.S., Koca, Y.K. (2023). Evaluation of database and some soil characteristic of Kumkale Agricultural Enterprise soils in GIS. International Journal of Agriculture, Environment and Food Sciences, 7 (1), 223-233

Received: 24 November 2022
Revised: 01 January 2023
Accepted: 05 January 2023
Published Online: 27 March 2023

Correspondence: Yavuz Sahin Turgut
E-mail: ysturgut@cu.edu.tr

INTRODUCTION

The General Directorate of Agricultural Enterprises (GDAE), whose purpose is to produce seeds, breeding stock and raw materials for agriculture and agriculture-based industry, and to protect gene resources, is an Economic State Entity. GDAE was established as a Public Economic Organization (PEO) with the merging of Stud Farms and State Farms under one roof in 1984, and was transformed into Economic State Enterprise (ESE) in 1994. The central organization of GDAE is the General Directorate, and the provincial organization is the business directorates. (Anonymous, 2019). GDAE had 37 business directorates spread over all geographical regions of Türkiye. Twenty of these enterprises have been rented for a long period of time with the decision taken. Seventeen of them continue to be operated within GDAE. One of GDAE's long-term rented enterprises is Kumkale Agricultural Enterprise (KAE). Detailed soil surveys of all GDAE enterprises in Türkiye, including the aforementioned enterprise soils, were

completed within the scope of a study conducted by Çukurova University Faculty of Agriculture, Department of Soil Science and Plant Nutrition (Anonymous, 1995). However, these study reports could not be transferred to the GIS environment due to the technological limitations of that period. Soil survey and mapping studies are the whole of evaluation and mapping studies of lands such as defining the characteristics of soils in an area, classifying soils according to a standard classification system, showing the boundaries of soil types on a map, making predictions about the behavior of soils (Dinç and Şenol, 2009) and determining the important physical and chemical properties of soils. Soil maps, including various office, field and laboratory studies, were produced in the traditional method, mostly in printed form. In the following years, the developments in computer technology and Geographic Information Systems (GIS) related to this technology have made it necessary to keep the printed maps in digital form. Recently, inferences regarding soil property information or land suitability made by transferring heritage soil data to digital environment (Tuğaç, 2021; Kılıç et al., 2021, Kaya et al., 2022, Koca and Turgut, 2022). The literature review showed that there were no studies conducted in previous years on the production potentials and soil properties of the lands in the study area. However, some researchers have conducted few studies that affect production preferences and economic profitability in Kumkale Plain (Alp, 2018; Uçan, 2018; Kocaköse and Aktürk, 2019; Demirel, 2020). Additionaly, in the study conducted by Özcan and Uygun (2004), salt and pH analyzes were made in soil samples taken from 13 points determined in the Kumkale Plain at 5 different depths and 7 different months, and distribution maps were created in GIS. Özcan and Akbulak (2006), hydraulic conductivity and texture data in the surface and subsurface layers of soils in the Kumkale plain were analyzed in GIS. Maps were created showing the spatial distribution of the texture and hydraulic conductivity of the surface and subsurface layers. In order to evaluate the suitability of Kumkale Plain for paddy cultivation, Everest and Özcan (2016) determined that 38.89% of the paddy lands are S1 (very suitable), 26.16% are S3 (marginally suitable) and 34.45% are N1 (temporarily unsuitable). Camoglu et al. (2018), 20 randomly selected businesses in the Kumkale Plain were handled and these businesses were evaluated in terms of design. In the study, it was concluded that in drip irrigation systems installed in the plain, uniform water distribution could not be achieved in general, that is, the systems were installed incorrectly. Everest and Everest (2020) analyzed the processes that decide the land use types of farmers in the Kumkale plain by paired comparison. In the study, a survey was conducted with 114 farmers who produce in the plain.

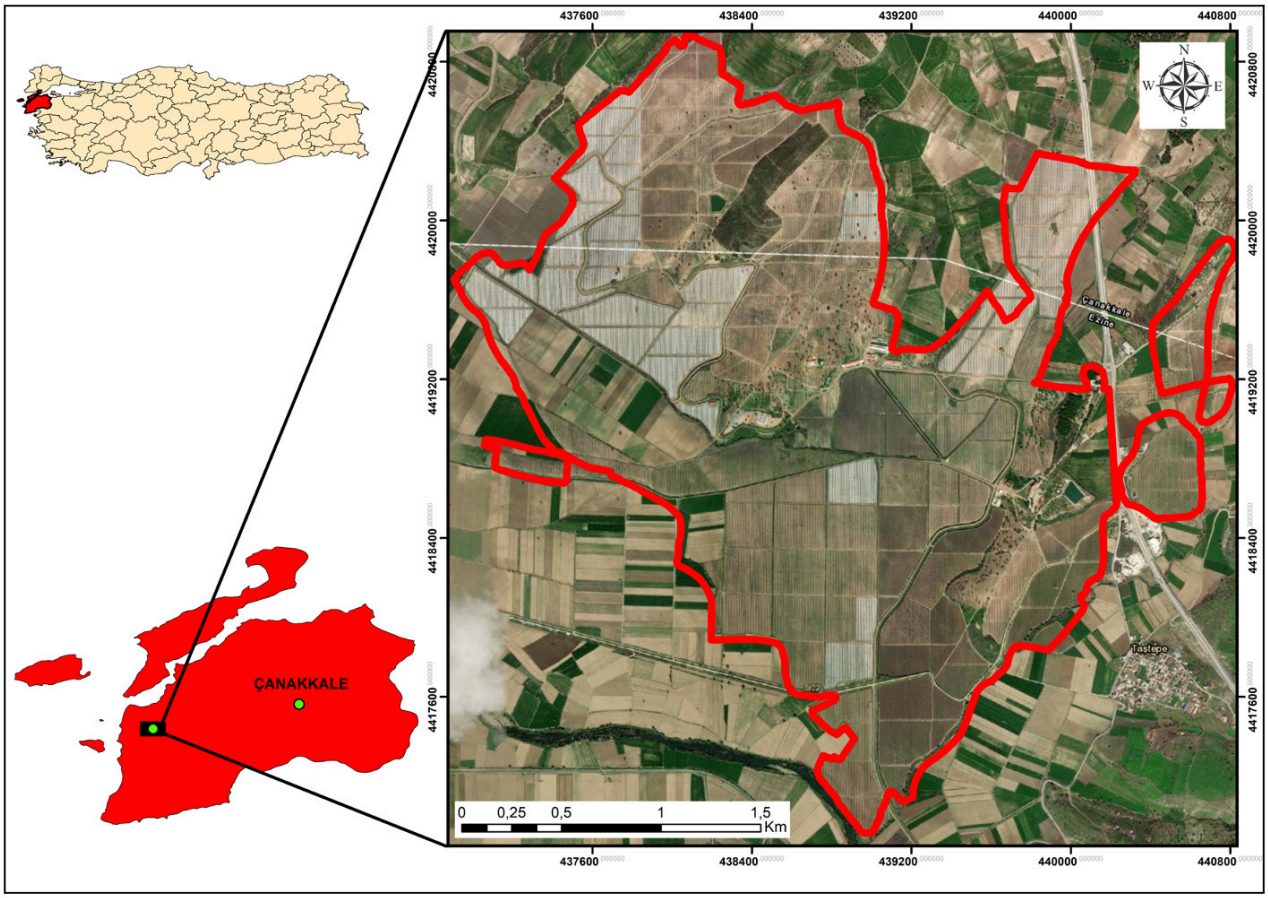


Figure 1. Location map of study area

It has been determined that the most effective factor in the farmers' decision on land use types is the cost of production. Considering the technological possibilities and conditions of the period in which this study was conducted, the lack of current coordinate information, scale shifts and deviations in the printed maps, and a number of numerical evaluation deficiencies for the area necessitated this study. Thus, the aim of this study is to create a database and produce various thematic maps of the soils of KAE (Anonymous, 1995), for which detailed soil surveys by means of the GIS.

MATERIALS AND METHODS

Material

KAE within the provincial borders of Çanakkale is located approximately 2 km west of Akçapınar town of Çanakkale central district (436886.00 E, 4419675.00 N; 440844.00 E, 4419110.00 N: WGS84 UTM Zone 35N). Çanakkale-Ezine highway in the east of the enterprise and the Ezine district border begins in the south of the enterprise and some of the lands in the south of the enterprise are located in the district of Ezine (Fig.1). Karamenderes Stream, located to the west of the enterprise, is located in the south of the enterprise and passes through it. In addition, Gökçalı village is located in the north of the enterprise. In a significant part of the lands, which cover an area of approximately 7.200 da, horticultural agriculture is carried out. Stone fruits such as peach, nectarine and apricot produces in almost all of the study area (6200 da).

According to the climate data of the study area, the annual average precipitation is 625.5 mm (between 1929 and 2021 years). Precipitation is generally in the form of rain and very little snow in December and January (Table 1). Although it is similar to the Mediterranean climate type, the winter months are colder and in summer, evaporation is less in summer. For many years, average temperature values rise above 25 °C in summer and fall below 10 °C in winter. The annual average temperature is 15.2 °C. The study area, which is semi-humid according to the Aydeniz and Erinç climate classification, has a C1: semi-arid-less humid structure according to the Thornthwaite climate classification (Anonymous, 2022). Soil moisture regime in the study area is xeric and temperature regime is mesic.

A large part of the lands consists of unconsolidated alluvial material stored in the Holocene. These deposits are usually calcareous and very calcareous materials, depending on the geological characteristics of the materials in the places where the rivers pass. Apart from the alluvial formations, in the area between the northwest and east of the enterprise, there are marine-derived marl, calcareous clay deposits and Paleocene aged caliches. In the study area, 14 different series were defined, spreading over 4 different physiographic units. Among these series, Menderes, Hanay, Fidanlık, Karabatak, Köprübaşı, Kumkale, Boğaz, Çıplaktepe, Kemerdere and Karabağlar series are included to Entisol. Gökçalı, Akçeşme, Maltepe and Akçapınar series are included to Inceptisol (Anonymous, 1995).

Method

In this study, which was carried out in an office environment, the maps of the enterprise land, for which detailed soil surveys were made, were transferred to the GIS, and various thematic maps were produced. Therefore, the basic soil map, which is in the form of a printed map, was scanned with a scanner and then geographically corrected with the help of Google Earth in the GIS. Soil boundaries and other mapping units were digitized manually, database (attribute) was created and maps were produced. ArcGIS 10 software was used throughout this phase.

Table 1. Climate Data of the Study Area (1929-2021)

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Annual
AT	6.3	6.7	8.4	12.6	17.6	22.2	25.1	25.1	21.1	16.3	12.1	8.4	15.2
AHT	9.6	10.2	12.5	17.2	22.7	27.7	30.7	30.7	26.4	20.8	16.0	11.7	19.7
ALT	3.2	3.4	4.7	8.3	12.7	16.6	19.3	19.6	16.1	12.2	8.6	5.4	10.8
ANRD	12.8	11.3	10.7	7.5	6.2	5.6	1.8	1.0	4.2	7.3	7.7	11.5	87.6
MTP	92.4	72.3	66.0	45.0	30.1	25.7	14.3	9.2	25.0	55.6	84.3	105.6	625.5

AT: Average temperature (°C); AHT: Average highest temperature (°C); ALT: Average lowest temperature (°C); ANRD: Average number of rainy days; MTP: Monthly total precipitation (mm)

RESULTS AND DISCUSSION

Soil Series

Soil series (n:14) were defined in the agricultural enterprise. These series are named as Akçapınar, Akçeşme, Boğaz, Çıplaktepe, Fidanlık, Gökçalı, Hanay, Karabağlar, Karabatak, Kemerdere, Köprübaşı, Kumkale, Maltepe and Menderes. Among these series, the series with the highest distribution area is Karabatak series with 948.2 da. This is followed by the Karabağlar series

with 796.4 da. Series with the least distribution area is Fidanlık series with 54 da (Table 2). Series map prepared in GIS is given in the Fig.2.

Karabatak series soils, which are most widespread in the study area, are soils formed on alluvial terraces. $CaCO_3$ content is moderate and generally dominant cations are Ca^{+2} and Mg^{+2}. Increasing ESP and salt amounts towards the lower parts of the profile may cause serious salinity and alkalinity problems in the future if precautions are not taken. In these soils, where the texture is mostly clay, horizons below the Ap horizon are completely massive. Their color is mostly pale yellowish brown. Intense

Table 2. Soil Series in the study area

Series (n:14)	Area (da)	Percentage (%)
Akçapınar	788.4	11
Akçeşme	291.8	4
Boğaz	227.5	3
Çıplaktepe	774.6	11
Fidanlık	54.0	1
Gökçalı	566.3	7
Hanay	769.6	11
Karabağlar	796.4	11
Karabatak	948.2	13
Kemerdere	268.8	4
Köprübaşı	346.3	5
Kumkale	313.5	4
Maltepe	592.8	8
Menderes	394.2	5
Others	176.5	2
Total Area	7309.0	100

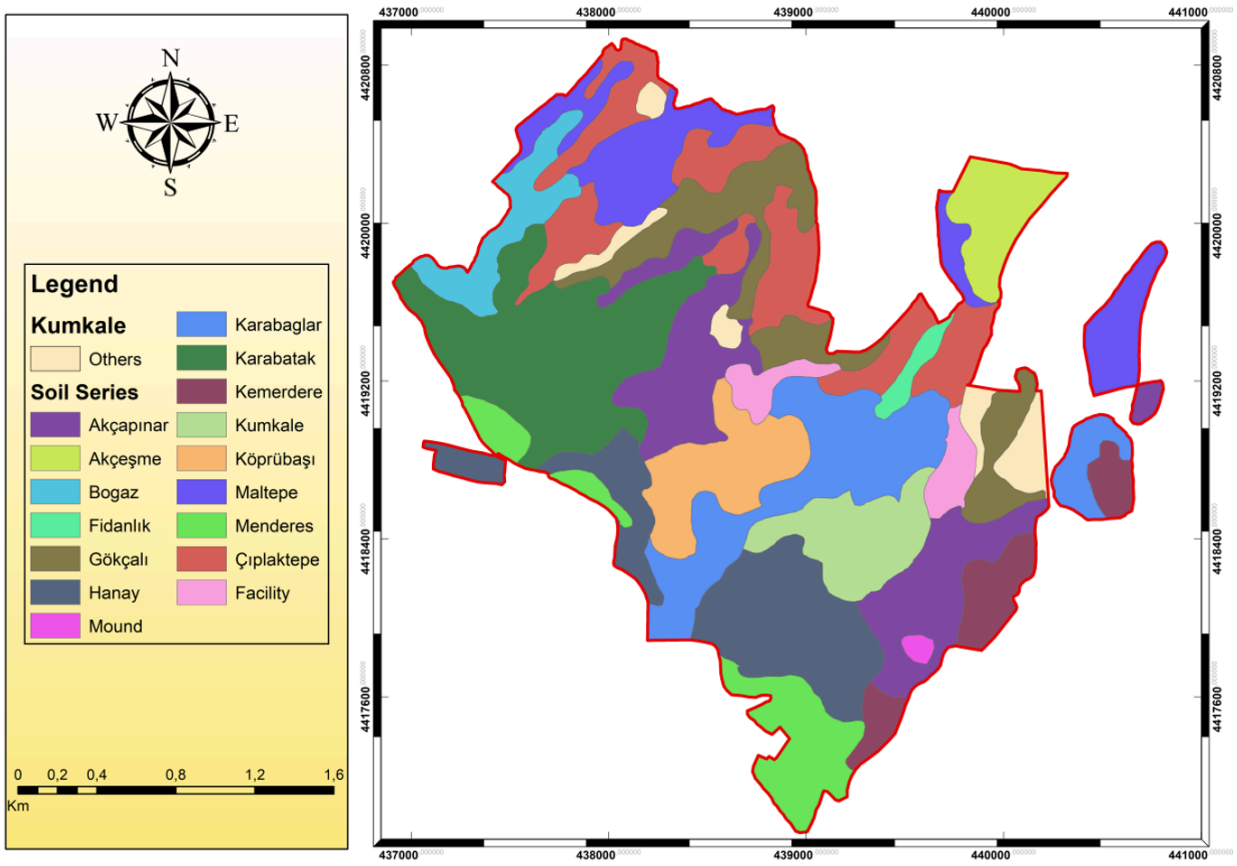


Figure 2. Distribution map of the soil series

yellowish red rust spots are seen in all profiles below the Ap horizon and intense black colored Mn concretions in addition to rust spots in lower layers. The Karabağlar series, which has the highest distribution area after Karabatak series, are deep soils with AC horizons, formed on the Alluvial parent material, in the base land formed at the intersection of Alluvial-Coluvial lands, and located in slightly wavy topographies. As in the Karabatak series, yellowish-reddish rust spots and rare Mn concretions are observed in the soils of this series as a result of the seasonal rise and fall of the ground-water.

Physiographic Units

Within the boundaries of the enterprise, 4 different physiographic units were defined. These are Alluvials, Lowlands, Highlands (Colluvial) and Bajadas. Alluvials occupy the most space among these units. This physiography, which spreads in 42% of the agriculture enterprise, is defined in the field of 3094.8 da. Lands that covered the least area are the Bajadas. This physiographic unit, which spreads over 227.5 da of land, constitutes only 3% of the enterprise (Table 3). Physiographic units map prepared in GIS environment is shown in the Fig.3.

Most of the series defined in the enterprise were developed on the Alluvial parent material. The high lands between the northeast, north and west of the enterprises center are generally Mesozoic and Paleozoic in age. Maltepe, Gökçalı, Akçapınar, Akçeşme and Çıplaktepe series were defined on these lands. Kumkale, Köprübaşı, Hanay and Karabatak series were defined on the river terraces that occupy the most space in the enterprise. Among these four series, apart from the Hanay series, the soils belonging to the other three series were covered horizons, which are indicative of the important periodic floods of the Kemer and Karamenderes streams, which are especially effective in the southern part of the enterprise.

Table 3. Physiographic Units in the study area

Physiographic Units	Area (da)	Percentage (%)
Alluvials	3094.8	42
Lowlands	796.4	12
Bajadas	227.5	3
Highlands(Colluvial)	3013.9	41
Others	176.5	2
Total Area	7309.0	100

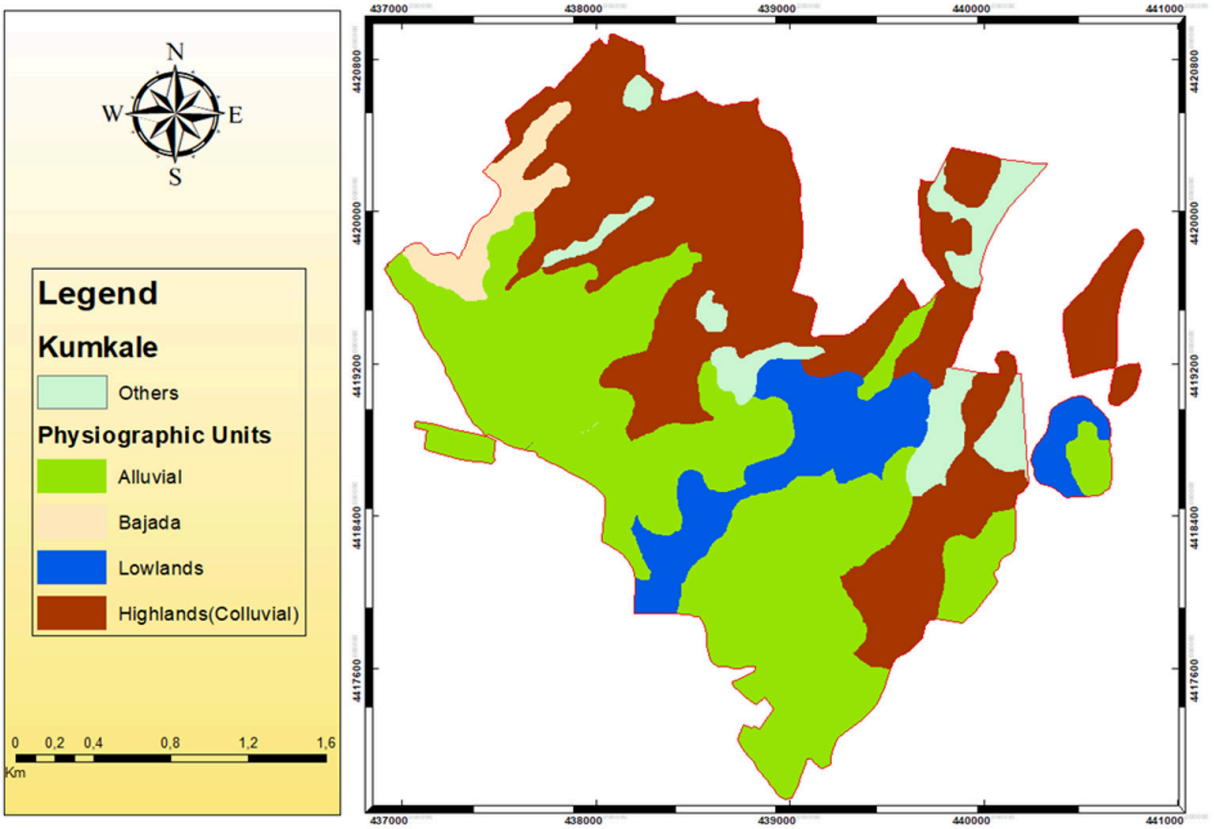


Figure 3. Map of the physiographic units created in GIS

Slope

Significant part of the enterprise consists of flat or almost flat alluvial lands. Therefore, a significant part of the enterprise lands consists of lands with 0-2% slope, which is almost flat (Table 4). According to the maps produced in the GIS environment (Fig. 4) and the database inquiries, 61% of the enterprise area (4478.9 da) consists of nearly flat lands with 0-2% slope class. Lands with a slope of 2-6% within the boundaries of the enterprise were determined as 32% (2292 da), while the lands with a slope of 6-12% were determined as 4% (289.60 da). Also, the proportion of areas with a slope of more than 12% is 1% (72 da). The sloping lands are located in the northeast of the enterprise and are generally defined as colluvials.

In addition, there is an area with a very steep (+12%) slope at the intersection of Alluvial lands and Highlands (shown in red on the slope map) in the northwest part of the enterprises center. It is necessary to keep under control the surface flow in such lands where slope is high and soil depth is shallow. Therefore, these areas should be constantly kept covered with vegetation. In this way, surface runoff can be prevented as well as the preservation of soil held by plant roots without soil erosion.

Soil Depths

Soil water-storage capacity and effective rooting depth are mainly related to the soil depth. Soil degradation due to soil erosion is a serious threat to the soil quality and productivity in hilly areas. The effects of soil erosion on productivity depend largely on the thickness and quality of the topsoil and on the nature of the subsoil. Productivity of deep soils with thick topsoil and excellent subsoil properties may be virtually unaffected by erosion.

Table 4. Slope Levels in the study area

Slope (%)	Area (da)	Percentage (%)
0-2	4478.9	61
2-6	2292.0	32
6-12	289.6	4
12+	72.0	1
Others	176.5	2
Total Area	7309.0	100

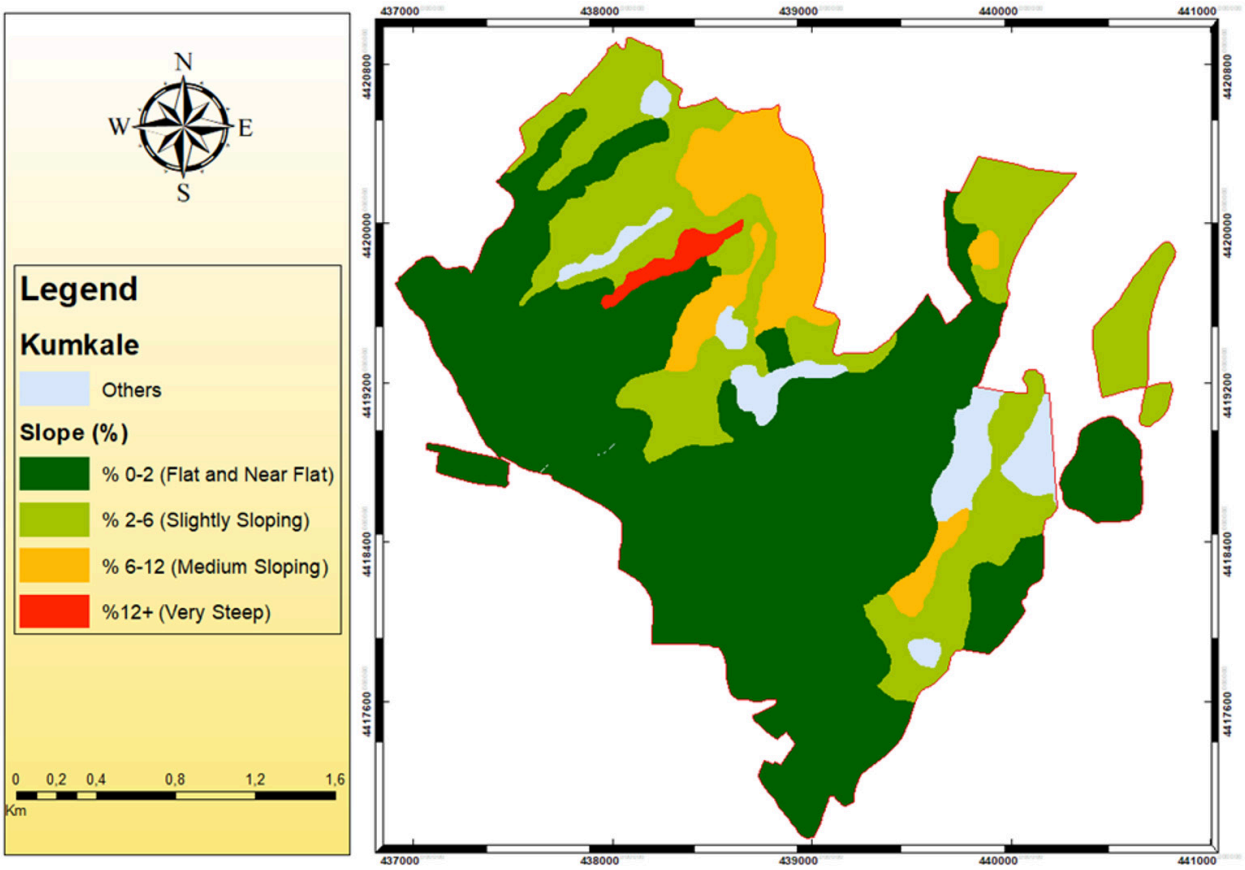


Figure 4. Map of the slope classes created in GIS

However, most hilly soils are shallow or have some undesirable properties in the subsoil such as petrocalcic horizon, or bedrock that adversely affects yields. In either cases, productivity will decrease as the topsoil gets thinner and undesirable subsoil is mixed into the topsoil by tillage. Soil depth largely affects plant productivity and therefore farm income. Soil depth defines the root space and the volume of soil from where the plants fulfil their water and nutrient demands (Kosmas et al., 1999). As a result of the inquiries made in the map and GIS of the enterprise, it was determined that soils consisted of very shallow (30-60 cm), shallow (60-90 cm), deep (90-120 cm) and very deep (120 cm+) soils (Table 5). The depth map produced as a result of digitization is shown in Fig 5.

In KAE, the areas that have problems in terms of soil protection and need to be taken precautions are generally located in the north, northwest and northeast of the enterprises center. In these regions, the slope is "medium (6-12%)" (Fig 4.) and the soil depth varies between "very shallow (30-60 cm) and shallow (60-90 cm)". Apart from these areas, most of the study area consists of deep soils. Generally, relevant lands where slope and soil depth limit agricultural productivity are class IV lands. Crop selection and soil tillage methods to be grown on the lands included in this class are important. In addition, the low water holding capacity of the soils in the aforementioned lands and the continuous flooding that may damage plant cultivation, surface stones, water-wind erosion and shallow soil depth are other important issues that may affect agricultural production. The lands opened to cultivated agriculture without the necessary precautions will result in the loss of existing lands.

Table 5. Soil Depths in the study area

Depths (cm)	Area (da)	Percentage (%)
30-60	174.7	2
60-90	338.0	5
90-120	683.0	9
120+	5936.8	82
Others	176.5	2
Total Area	7309.0	100

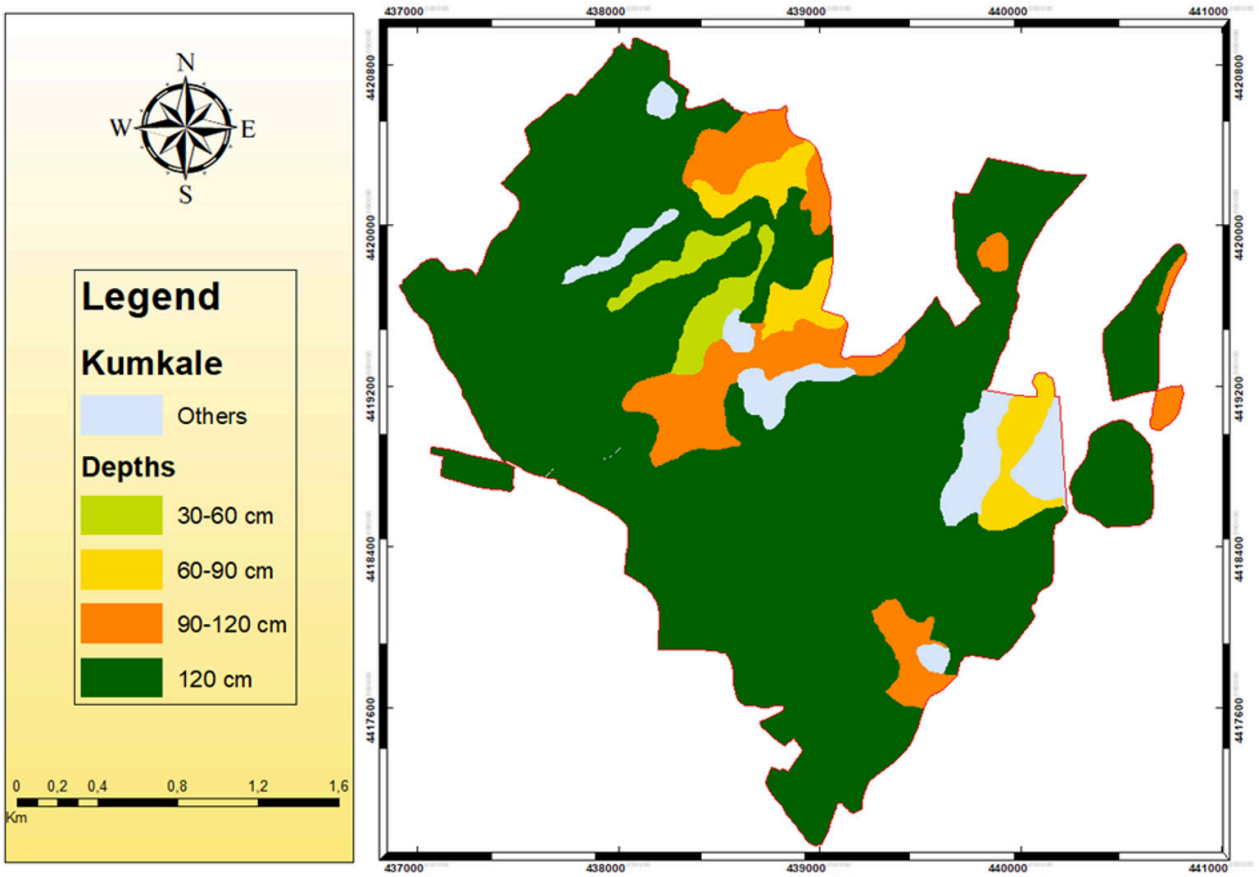


Figure 5. Map of soil depth classes created in GIS

Surface Stones

Stone content is particularly important when planning the agricultural productivity of an area, as high stone content can cause difficulties for both soil preparation and planting (Saksa et al. 2018). Knowledge of soil stones can also be helpful when developing tree growth, weathering, and hydrological models (Panagos et al. 2014, Melander,2019). Almost all of the enterprise lands consist of Alluvial and Colluvial lands with flat or almost flat slopes, without or with less stones. Accordingly, only 5% of the lands (stony or very stony) have stony problems. 78% (5677.2 da) of the study area consists of stone-free and 15% (1058.6 da) less stony soils (Table 6). The surface stones map obtained in GIS is given in Fig 6.

As the surface stones map is carefully examined, it is resulted that there is no stoniness problem in the alluvial and lowlands shown on the physiographic map (Fig.3). On the other hand, especially in the slope (Fig.4) and depth maps (Fig.5), it is seen that the stones problem is high in lands where slope is high and soil depth is weak.

Drainage

The drainage problem seen in the flat and nearly flat, young and alluvial lands of the enterprise and the bottom lands in the middle of the coluvial and alluvial lands, limiting factor that reduces productivity of agricultural areas and even causes bigger problems such as salinity and alkalinity, if timely measures are not taken. Especially the most serious problem limiting the agricultural production of Kumkale agricultural enterprise is the drainage problem, which exists in 28%

Table 6. Surface Stones in the study area

Surface Stones	Area (da)	Percentage (%)
Stone-free	5677.2	78
Less Stony	1058.6	15
Medium Stony	174.7	2
Very Stony	222.1	3
Others	176.5	2
Total Area	7309.0	100

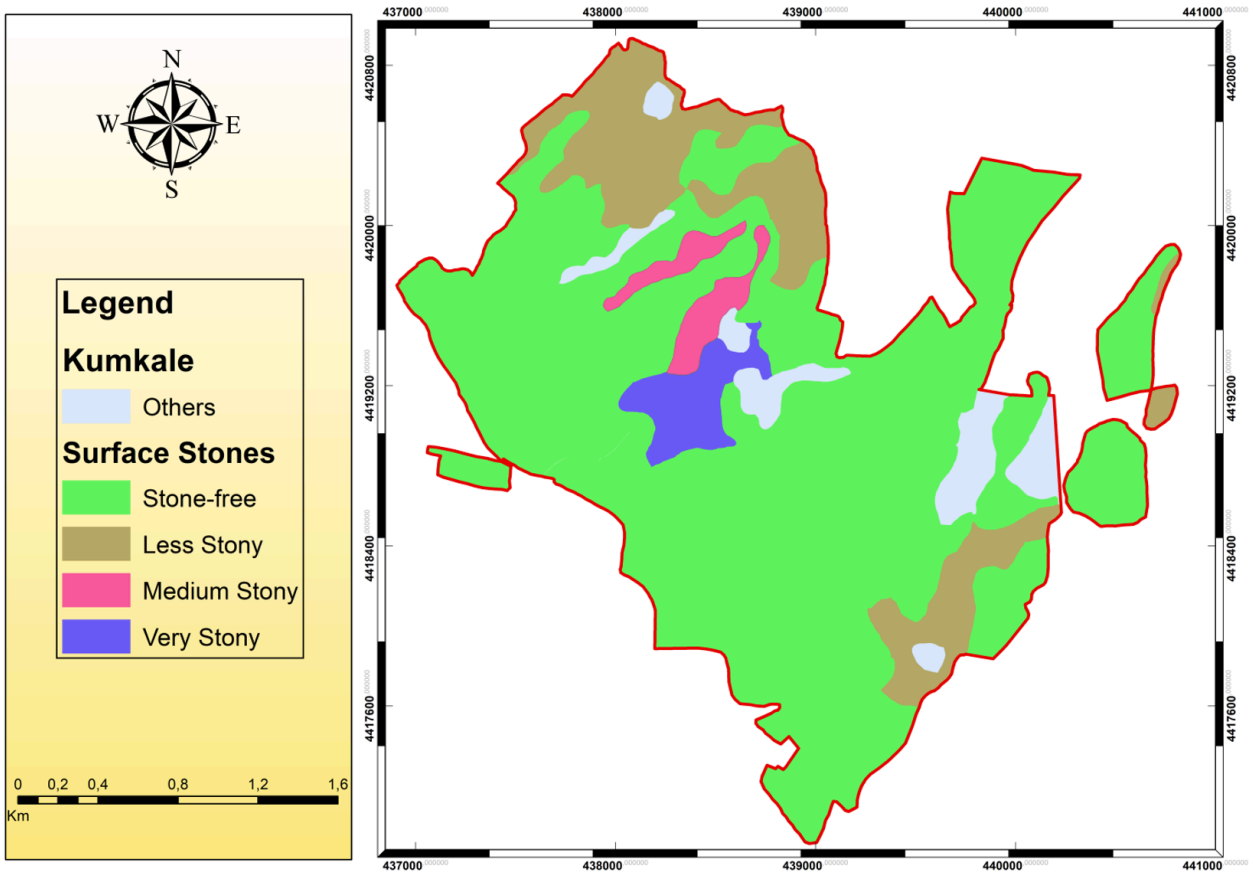


Figure 6. Map of surface stoniness classes created in GIS

of the total area (Table 7). According to this, drainage is well drained in 69% (5052.1 da) of the area, moderately drained in 16% (1132.5 da), somewhat poorly drained in 6% (406.4 da), 6% (465.4 da) poorly drained and 1% (76.2 da) very poorly drained. Fig. 7 shows drainage map of the enterprise lands.

In the drainage map prepared in the GIS, it is seen that there is a significant drainage problem in the center of the enterprise lands and especially in the regions where the base lands are common. There are drainage problems ranging from insufficient to bad in the soils of the Boğaz, Karabağlar, Menderes, Kemerdere, Kumkale, Hanay, Köprübaşı and Karabatak series. Especially the insufficiency of unleveling and surface drainage systems of a significant part of the soils belonging to some series (Boğaz, Köprübaşı, Karabatak and Fidanlık) cause drainage problems and surface ponding. In the northwest of the enterprise lands at the intersection of high lands and alluvial lands are in a more pitted position compared to their surroundings, so rain water and irrigation waters and waters coming from the environment cause the ground water to rise. Open surface drainage channels should be opened and proper soil leveling should be done in order to prevent water accumulation on the surface and to ensure a healthy drainage of water.

Table 7. Drainage Classes in the study area

Drainage	Area (da)	Percentage (%)
Well Drained	5052.1	69
Moderately Drained	1132.5	16
Somewhat Moderately Drained	406.4	6
Poorly Drained	465.4	6
Very Poorly Drained	76.2	1
Others	176.5	2
Total Area	7309.0	100

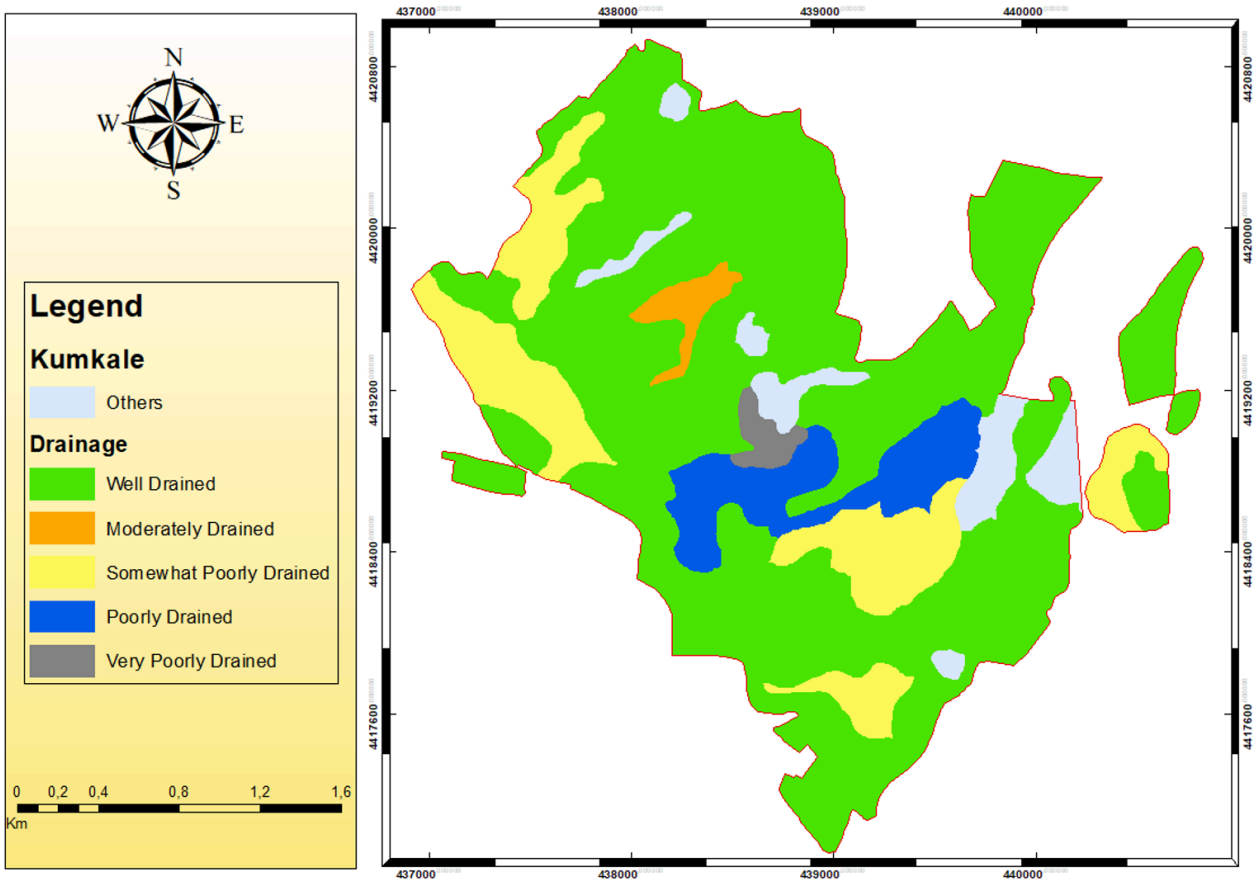


Figure 7. Map of drainage classes created in GIS

CONCLUSION

In this study, which deals with the important soil/land characteristics of KAE, the agricultural potential of the enterprise has been tried to be revealed. 14 soil series were defined in the agricultural enterprise. These series are named as Akçapınar, Akçeşme, Boğaz, Çıplaktepe, Fidanlık, Gökçalı, Hanay, Karabağlar, Karabatak, Kemerdere, Köprübaşı, Kumkale, Maltepe and Menderes. Among these series, the series with the highest distribution area is Karabatak series with 948.2 da. This is followed by the Karabağlar series with 796.4 da. Series with the least distribution area is Fidanlık series with 54 da. Most of the series defined in the enterprise were developed on the Alluvial parent material. The high lands between the northeast, north and west of the enterprises center are generally Mesozoic and Paleozoic in age. Kumkale, Köprübaşı, Hanay and Karabatak series were defined on the river terraces that occupy the most space in the enterprise.

In KAE, the areas that are problematic in terms of soil protection and need to be taken precautions are generally located in the north, northwest and northeast of the enterprises center. In these regions, the slope is "medium (6-12%)" and the soil depth varies between "very shallow (30-60 cm) and shallow (60-90 cm)". It is necessary to keep under control the surface flow in such lands where slope is high and soil depth is shallow. In this way, surface runoff can be prevented as well as the preservation of soil held by plant roots without soil erosion.

Another important trouble encountered in the enterprise is the drainage problem of the soils. The drainage problem seen in the flat and nearly flat, young and alluvial lands of the enterprise and the bottom lands in the middle of the colluvial and alluvial lands, limiting factor that reduces productivity of agricultural areas and even causes bigger problems such as salinity and alkalinity. In particular, the most serious problem limiting the agricultural production of KAE is the drainage problem, which exists in 28% of the total area. As a result, some soil characteristics and areal distributions were obtained by detailed soil surveys of KAE lands and digitizing the maps obtained in GIS. The survey report and detailed soil map of study area, which was carried out in the past, were digitized with this study. All relevant soil properties and classes are given in detail in the findings section of this study. With this and similar studies, it will be useful to examine both the temporal and spatial changes of the lands connected to GDAE, which has a significant production capacity in Türkiye, and to compare them with the old survey studies by making new field studies. In addition, the creation of a new database to be digitized in the GIS and the application of precision agriculture techniques can help the evaluation of the enterprise soils according to their suitable agricultural potentials.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Alp, Ç. (2017). Risk analysis in agricultural enterprises engaged in tomato cultivation: The example of Canakkale province Kumkale plain. COMU Institute of Science and Technology, Department of Agricultural Economics, Master Thesis.118 p.

Anonymous, (2022). General Directorate of Meteorology, Official Statistics. Internet access: https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=A&m=CANAKKALE

Anonymous, (1995). Detailed Soil Survey and Mapping of Kumkale Agricultural Enterprise Soils. General Directorate of Agricultural Enterprises, Ankara.

Anonymous. (2019). General Directorate of Agricultural Enterprises 2019 Administrative Activity Report. http://www.sp.gov.tr/upload/xSPRapor/files/bh8cq+TIGEM_2019_Yili_Faal_Rap_.pdf

Çamoğlu, G., Demirel, K., Genç, L., Eroğlu, İ., Boran, A. (2018). Evaluation of Drip Irrigation Systems with a CAD Based Program: The Example of Kumkale, Çanakkale. Journal of the COMU Agr.Fac.. 6(1):61-70.

Demirel, G., (2020). Classification and Analysis of Wheat Growing Agricultural Enterprises According to Farm Accounting Data Network (CMVA) system: The Example of Canakkale Province Kumkale Plain. COMU Institute of Science and Technology, Department of Agricultural Economics, Master Thesis.82 p.

Dinç, U., Şenol, S. (2009). Soil Survey and Mapping. (4th Edition). C.U. Faculty of Agriculture General Publication No:161; Textbooks Publication No: A-50. Adana: Çukurova University Faculty of Agriculture Offset Workshop.

Everest, B., Everest, T. (2020). Analysis of Factors Influencing Farmers' Decision on Land Use Types: The Example of Kumkale Plain. Anadolu, Journal of AARI, 30(2): 221-228.

Everest, T., Özcan H. (2016). Land Evaluation of the Suitability of Canakkale Province Karamenderes Sub-Basin Flood Plain for Rice Cultivation. Soil Water Journal, 5(2): 18-24.

Kaya F., Başayiğit L., Keshavarzi A., Francaviglia R. (2022) Digital mapping for soil texture class prediction in northwestern Türkiye by different machine learning algorithms, Geoderma Regional, Volume 31, e00584, ISSN 2352-0094,

https://doi.org/10.1016/j.geodrs.2022.e00584

Kılıç, M., Gundogan, R., Günal, H. (2021). Development and Applications of Digital Soil Mapping. In K. Bellitürk, M.F. Baran, A. Çelik (Eds.), Food for Sustainability, Environment, Agro-Forestry and New Research in Agriculture (pp. 53–87). IKSAD Publishing House.

Koca, Y.K., Turgut, Y.Ş. (2022). Overview of the lands of Dalaman agricultural enterprises using geographic information systems. International Journal of Agriculture Environment and Food Sciences, 6(1), 41-49. DOI: 10.31015/jaefs.2022.1.7

Kocaköse, B., Aktürk, D. (2019). Economic Analysis of Enterprises Operating Agricultural Activities in Çanakkale. Turkish Agriculture, Journal of the Food Science and Technology, 7(11), 2001-2011. Doi: 10.24925/turjaf.v7i11.2001-2011.2994.

Kosmas, C., Kirkby, M., Geeson, N. (1999). Manual on: Key indicators of desertification and mapping environmentally sensitive areas to desertification. European Commission, Energy, Environment and Sustainable Development, EUR 18882, 87 p.

Melander, L., Ritala, R., Strandström, M. (2019). Classifying soil stoniness based on the excavator boom vibration data in mounding operations. Silva Fennica vol. 53 no. 2 article id 10068. https://doi.org/10.14214/sf.10068

Özcan, H., Uygun, R. (2004). Monitoring and Evaluation of Spatial and Temporal Changes of Salt Movement in Soil Profile in Çanakkale-Kumkale Plain. Journal of Harran University Faculty of Agriculture, 8(2):33-41.

Özcan, H., Akbulak C., (2006). Examination of soil texture-hydraulic conductivity relationship with GIS: Example of Kumkale Plain. I. National UZAL-GIS Congress, Proceedings.

Panagos P., Meusburger K., Ballabio C., Borrelli P., Alewell C. (2014). Soil erodibility in Europe: a high-resolution dataset based on LUCAS. Science of the Total Environment 479–480(1): 189–200. https://doi.org/10.1016/j.scitotenv.2014.02.010

Saksa T., Miina J., Haatainen H., Kärkkäinen K. (2018). Quality of spot mounding performed by continuously advancing mounders. Silva Fennica 52(2) article 9933. https://doi.org/10.14214/sf.9933

Tuğaç, M.G. (2021). GIS-Based Land Suitability Classification for Wheat Cultivation Using Fuzzy Set Model. International Journal of Agriculture Environment and Food Sciences, 5 (4) , 524-536 . https://doi.org/10.31015/jaefs.2021.4.12

Uçan, M. (1994). Mixture Structures of Mixture of Vetch + Oat Mixtures at Different Nitrogen Doses in Kumkale Arid Conditions. Trakya University Institute of Science and Technology, Department of Field Crops, Master Thesis.109s.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(1): 234-243
DOI: https://doi.org/10.31015/jaefs.2023.1.28

Larvicidal and pupicidal effects of some essential oils against *Musca domestica* Linnaeus, 1758 (Diptera:Muscidae)

Meltem Kökdener[1]

[1] Department of Social Services, Faculty of Health Science, Ondokuz Mayıs University, Samsun, Türkiye

Abstract

Essential oils (EOs) from plants can provide an eco-friendly alternatives to traditional synthetic insecticides. This study aimed to evaluate the effects of six different essential oil (*Foeniculum vulgare, Cinnamomum verum, Allium sativum*, *Capsicum annum, Mentha piperita, Urtica dioica*) against *Musca domestica*. Larvicidal and pupicidal, efficacy of six EOs were evaluated by contact toxicity method at four different doses (1%; 2.5%, 5%, and 10%) in 5 replications. The research was performed at 60 ± 1.5% humidity and 27 ± 0.5 ^{0}C temperature at the Animal Physiology Laboratory of Ondokuz Mayıs University. The LC_{50} and LC_{90} values were determined by probit analysis. The effects of treatment and concentrations on different exposure periods, larval and pupal survival and development time were analyzed by a two-way analysis of variance. The larval toxicity of six EOs increased significantly with increased exposed periods. The larval and pupal mortality percent was also increased as the concentration increased for testing all essential oil. The highest larval mortality percent (100.0%) was observed at the (10%) concentration of six essential oils, and all concentrations of *Cinnamomum verum* oil. Besides, the lowest larval mortality percent (46%) was caused by the 1% concentration of *Foeniculum vulgare*. The highest pupicidal effect was shown by 10, 5, 2.5% *Cinnamomum verum* EOs (100% mortality) and 1% *Cinnamomum verum* EOs (97.36%). The present study revealed that tested essential oil had significant potential for affecting biological parameters of *M. domestica*. The *Cinnamomum verum* EOs can be used as an eco- friendly product for the control of housefly larvae and pupae.

Keywords: Essential oil, Control, *Musca domestica,* Toxic effect

Citation: Kökdener, M. (2023). Larvicidal and pupicidal effects of some essential oils against *Musca domestica* Linnaeus, 1758 (Diptera:Muscidae). International Journal of Agriculture, Environment and Food Sciences, 7 (1), 234-243

Received: 28 November 2022
Revised: 23 January 2023
Accepted: 26 January 2023
Published Online: 27 March 2023

Correspondence: Meltem Kökdener
E-mail: kokdener@omu.edu.tr

INTRODUCTION

The housefly, *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae), is an opportunistic and cosmopolitan species (Gupta et al., 2012) and found in close association with the activities of humans or humans and domestic animals throughout the world (Schou et al., 2013). More than 100 pathogens can cause diseases such as diarrhea, cholera, typhoid, tuberculosis bacillary, dysentery, paratyphoid shigellosis, and myiasis in animals and humans by houseflies (Ogbalu et al., 2014; Förster et al., 2007).

Houseflies develop on various ephemeral food substrates, such as kitchen waste, swine, goat, bovine, sheep and horse dung, wet hay, fermenting organic matter, human excrement cut grass, urine, (Meyer and Petersen, 1983). Dairy farms are ideal places for housefly breeding and, they pose a serious threat to dairy operations all over the world (Neupane et al., 2019). In addition to

transmitting diseases, *Musca domestica L.* causes spoiled food, stress, and irritations to livestock and poultry that lead to reduced efficiency in their production (Kumar et al., 2011a). Besides, it causes heavy economic losses, unnecessary staff annoyance, and a significant decrease in animal management quality (Pavela et al., 2008; Chantawee and Soonwera, 2018a). Therefore, considering the stress and disease caused by houseflies in domestic animals, it is paramount to control them (Kumar et al., 2011a). *M. domestica* population is greatly managed using insecticides. Indiscriminate use of conventional insecticides may develop insecticide resistance and also it leads to environmental and toxicological problems for animals and humans (Khan et al., 2013).

The essential oils (EOs) obtained from plants (Chantawee and Soonwera, 2018a) are considered a good alternative to conventional chemical pesticides and produce environment-friendly effects on living organisms (Scott et al., 2000). There are several researches regarding the toxicity of some essential oils against houseflies such as galangal (*Alpinia galanga*), star anise (*lillicium verum*), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), ylang-ylang (*Canaga odorata*), clove (*Eugenia caryophyllata*), cinnamon (*Cinnamomum verum*), eucalyptus (*Eucalyptus globulus*), peppermint (*Mentha piperita*), sweet orange (*Citrus sinensis*), lavender (*Lavandula angustifolia*), ginger (*Zigiber officinale*) phlai (*Zigiber cassumunar*), and calamondin (*Citrus madurensis*). Several studies have shown the adulticidal, larvicidal, ovicidal, repellence and deterrence effects of essential oils on house fly which may be used for its management (Chantawee and Soonwera, 2018a; Malik et al., 2007; Pavela, 2008; Kumar et al., 2014).The larval and pupal stages might constitute the majority of the development period of the housefly population (Kumar et al., 2014) and pupiciding and larviciding approaches are effective methods for decreasing the fly population (Chantawee and Soonwera, 2018a).

This present study was conducted to evaluate the efficacy and toxicity of six EOs from *Foeniculum vulgare* Mill (Umbelliferae), *Cinnamomum verum* (Lauraceae), *Allium sativum* L. (Amaryllidaceae), *Capsicum annum* (Solanaceae), *Mentha piperita* (Lamiaceae), and *Urtica dioica* (Urticaceae) against the larvae, and pupae of *M. domestica*

MATERIALS AND METHODS

Collection and rearing of Musca domestica L.

This study was conducted in the Animal Physiology Laboratory, Ondokuz Mayıs University, Samsun, Turkey. Adult houseflies were caught from dairy farms on the campus of Ondokuz Mayıs University using a sweep net and immediately transferred to the laboratory. Adults were kept at a temperature of 27 ± 0.5 °C, 60 ± 1.5% relative humidity (RH) and 12L : 12D light cycle in the cage (40 × 30 × 40 cm), enclosed with cheesecloth. Water and sugar were provided to the housefly adults. A cotton soaked in milk was offered as an oviposition substrate. The newly hatched larvae were transferred to jars containing mixture of wheat and milk. Pupae were kept in another glass jar (1000 ml) for adult emergence. All experimental studies were done under the same conditions (60 ± 1.5% relative humidity = RH and 27 ± 0.5 °C). Pupae and larvae acquired though experimets procedure were used in the pupicidal and larvicidal bioassays.

Larvicidal bioassays

All essential oils (Sigma–Aldrich) used in the study were 98 - 99% pure. From the stock solution, concentrations of 1, 2.5, 5, 10% were prepared. Larvicidal bioassays were performed with 10 larvae (third instar) in plastic cups (200 ml) which had different concentrations of esential oil along with ten grams of larval rearing diet on a filter paper. Control filter paper was sprayed with distile water. Five replicate for each concentration of different essential oil were conducted. Larvae were monitored for any change in mobility and appearance for 1, 5, 10, 15, 20 days, post treatment. Larval mortality was determined by the formation of a brownish appearance and wilting (Kumar et al., 2011b). The two lethal (LC_{50} and LC_{90}) concentrations were determined.

Pupicidal bioassays

Pupicidal bioassays were carried out by following the protocol described by Kumar et al., 2011a; Kumar et al., 2013), with a few modifications. For each pupicidal bioassay, different concentrations of each essential oil were sprayed onto filter paper, using a micropipette in different treatments. Initially, the treated petri dishes with filter paper were air-dried for 6 minutes to allow solvent evaporation, before the introduction of pupae. 10 pupae (aged 2-3 days) were placed in a Petri plate (dia., 90 mm). The Control Petri plate was treated with distilled water alone. Five replicates per treatment were performed. The percentage of pupicidal activity, was determined by the inhibition rate %. Inhibition rate (%IR or PIR) = Cn – Tn / Cn × 100 where Cn depicts the number of newly emerged insects in the control and Tn represents the number of insects in the treated Petri plates. All the treated pupae were recorded for adult emergence for 6 days.

Statistical analysis

Lethal concentrations (LC_{50}, LC_{90}) were computed using Probit analysis. Pupicidal effectivity was calculated in terms of percentage inhibition rate. The effect of treatment and concentrations on different exposure periods, larval and pupal survival and development time were analyzed using a two-way Analysis of Variance (ANOVA using SPSS 20.0). Hence A one-way analysis of variance (ANOVA) was performed to analyze the impacts of exposure period devepment time for each tested dose. In the event of a significant *F*-test ($P < 0.05$), the Tukey's HSD test was used to compare means.

RESULTS

Larvacidal bioassays

All essential oils caused prolongation of larval and pupal durations at all concentrations as compared to the control group (Table 1). The longest larval development time (16.2 days) was recorded at 5% concentration of *U. diocia* essential oil, while the shortest larval development time was found at 1% concentrations of *A. sativum* (7 days). Larval development time differed significantly among essential oils ($F = 1465.631$, $p < 0.001$) and concentrations ($F = 321.103$, $p < 0.0001$). There was significant interaction ($F = 72.242$, $p < 0.001$) among the effects of essential oil and concentraions on larval development time. The longest pupal development time (14.2 days) was recorded at 2.5% and 1% concentrations of *F. vulgare*, and *A. sativum* oil, while the shortest pupal development time (10 days) was recorded at 1% concentrations of *C. annum*, *M. piperita*, and *U. dioica* essential oil (Table 1). The results of the larvicidal activity of essential oil against the larvae of *M. domestica* are presented in Table 1. Pupal development time differed significantly among essential oils ($F = 897.990$; $p < 0.001$) and concentrations ($F = 66.982$, $p < 0.0001$). There was a significant interaction ($F = 42.338$, $p < 0.001$) between the effects of essential oil and concentraions on pupal development time. In the present study the essential oils observed a larvicidal effect which was time-and concentration-dependent. The larval and pupal mortality rates increased as the concentration increased for each of the tested essential oil (Table 1). The highest larval mortality (100.0%) was caused by all concentration of *C. verum* and the lowest mortality rate was caused by all concentration of *F.vulgare* (Table 1).The larval mortality of *M. domestica* differed significantly between essential oil ($F = 102.9$, $p < 0.001$), and concentrations ($F = 823.5$, $p < 0.001$). There was a significant interaction ($F = 39.007$, $p < 0.001$) between concentrations and essential oils. The larval and pupal survival of *M. domestica* (L) at various concentrations of various essential oil are presented in Figure 1. The highest pupal mortality (100.0%) was observed at the highest concentration (10%) of six essential oil, and 5% concentration of six essential oil (except of *M. piperita*) (Figure 1). The percent pupal mortality of *M. domestica* differed significantly between essential oil ($F = 47049.600$, $p < 0.001$), and concentrations ($F = 29382.800$, $p < 0.000$). There was a significant interaction ($F = 29683.860$, $p < 0.001$) between concentrations and essential oils. The larval toxicity of EOs increased significantly with increased exposure periods and concentrations. C. *verum* was the most effective at all concentration (100 %, 100 %, 76%, 46%) after 24 h. of exposure. After 24 hours of exposure, no mortality was observed in *Mentha piperita*, and *Capsicum annum* at 5, 2.5, and 1% concentrations and *Urtica dioica* at 1% concentrations (Table 2). The $LC_{50,}$ LC_{90} values of the essential oils against the house fly are presented in Table 3. In the current study, the LC_{50} and LC_{90} values showed that *Cinnamomum verum* oil was the most toxic ($LC_{50} = 0.021$; LC_{90} 1.688) to house fly larvae. The LC_{50} and LC_{90} values indicated that *Foeniculum vulgare* oil was the least toxic ($LC_{50} = 1.327$; $LC_{90} = 3.312$) to house fly larvae (Table 3).

Pupicidal bioassays

Pupicidal bioassays of *M. domestica* with six essential oils at different concentrations observed diverse efficacy (Table 4).The percentage inhibition rate (PIR), calculated after 6 days, at different concentrations of six essential oils, varied between 52.63 and 100% and the increase in the concentration of all essential oil caused an increased PIR. All EOs at 10% concentration impacted high PIR value, ranging from 86.84% to 100.0%. In pupicidal bioassays at the highest concentration (10%) of *Allium sativum, Cinnamomum verum, Urtica dioica* suppressed the emergence of adult flies by 100%. Cinnamomum verum EOs was the highest performer, with a PIR value of between 100 and 97.36%, for different concentrations of oil tested (10, 5, 2.5, and 1% concentrations) but *Foeniculum vulgare* oil was the poorest performer, with a PIR value of between 52.63 and 86.84%, for different concentrations of tested oil.

DISCUSSION

The control of *Musca domestica* (L.), relies on the use of chemical pesticides. Indiscriminate use of insecticides, has led to the development of insecticide resistance and tolerance, which have adverse effects on consumers and the environment (Vasantha - Srinivasan et al., 2016). Plant-derived products (essential oils) contain bioactive compounds that may be used for the management of pests of animals, crops, and human beings (Pavela, 2011; Khater and Geden, 2018). Essential oils provide an alternative to synthetic insecticides (Khan et al., 2019). This study tested the activity of six essential oils against the pupae and larvae of *M. domestica*. Our findings suggest that the six EOs exhibited higher mortality rates than the controls against the larvae of house fly. The insecticidal potential of essential oils that are derived from plants has been investigated by different researchers for the control of houseflies (Malik et al., 2007; Kumar et al., 2011a; 2012a,b, 2013, 2014; Zahoor et al., 2020; Pavela, 2008). The present results demonstrated that the mortality percent of *Musca domestica* larvae increased by increasing the exposure period and oil concentration for all botanical extracts tested (Fig. 1). In the current study, the effects of all tested essential oils at the 10% concentration had more toxicity against the larvae of housefly compared to other tested concentrations (5, 2.5, and 1%). Similar results have been observed for the extracts of *Origanum onites* L. (Lamiales: Lamiaceae), *Satureja thymbra* L. (Lamiales: Lamiaceae), and *Myrtus communis* L. (Rosales: Myrtaceae) to the adults of three stored-product insects (Ayvaz et al., 2010). The *Cinnamomum verum* oil had the highest larvicidal activity. The least larvicidal activity

Table 1. Growth and development impact of essential oil on larvae of *M. domestica* (L.).

Essential oil	Concentrations (%)	Mortality (%)	Larval development (day)	Pupal development (day)
Capsicum annum	10	100.0 ± 0.0c*	-	-
	5	80.0 ± 5.4a	14.2 ± 0.5b	-
	2.5	76.0 ± 4.0a	13.2 ± 0.4b	10.2 ± 0.4b
	1	74.0 ± 5.1a	13.2 ± 0.4b	10.0 ± 0.4b
	Control	6.0 ± 0.0b	5.2 ± 0.4a	4.2 ± 0.4a
		F = 9.40 ± 0.0, p < 0.000	F = 1231.500, p < 0.000	F= 1090.500, p < 0.000
Mentha piperita	10	100.0 ± 0.0c	-	-
	5	96.0 ± 2.4bc	14.2 ± 0.5c	10.2 ± 0.4b
	2.5	86.0 ± 2.4b	12.2 ± 0.4b	10.2 ± 0.4b
	1	70.0 ± 2.4a	12.2 ± 0.4b	10.0 ± 0.4b
	Control	6.0 ± 0.0c	5.2 ± 0.4a	4.2 ± 0.4a
		F=18.157, p <0.000	F = 1114.625,p < 0.000	F= 684.000, p < 0.000
Urtica dioica	10	100.0 ± 0.0d	-	-
	5	94.0 ± 5.4c	16.2 ± 0.2d	-
	2.5	82.0 ± 2.4ab	14.2 ± 0.2c	10.2 ± 0.4b
	1	74.0 ± 5.4a	12.2 ± 0.4b	10.0 ± 0.0b
	Control	6.0 ± 0.0c	5.2 ± 0.4a	4.2 ± 0.4a
		F=21.610, p < 0.000	F=1429.625, p <0.000	F = 1090.500, p < 0.000
Foeniculum vulgare	10	100.0 ± 0.0d	-	-
	5	80.0 ± 1.4d	7.4 ± 0.2b	-
	2.5	64.2.0 ± 1.1bc	7.2 ± 0.2b	14.2 ± 0.5b
	1	46.0 ± 1.1a	7.1 ± 0.2b	14.2 ± 0.5b
	Control	6.0 ± 0.0c	5.2 ± 0.4a	4.2 ± 0.4a
		F = 2451.34, p < 0.000	F = 561.33, p < 0.000	F = 2170.50, p < 0.000
		F = 469.72, df = 20;4, p < 0.000	F = 561.33, df = 20;4, p < 0.000	F = 302.35, p < 0.000
Cinnamomum verum	10	100.0 ± 0.0b	-	-
	5	100.0 ± 0.0b	-	-
	2.5	100.0 ± 0.0b	-	-
	1	100.0 ± 0.0b	-	-
	Control	6.0 ± 0.0a	5.2 ± 0.4a	4.2 ± 0.4a
		F = 560.485 df = 20;4, p < 0.000	F = 532.667, df = 20;4, p < 0.000	F = 522.667, p < 0.000
Allium sativum	10	100.0 ± 0.0d	-	-
	5	84.0 ± 0.0c	7.4 ± 0.2b	-
	2.5	74.4 ± 0.9b	7.2 ± 0.2b	14.2 ± 0.5b
	1	57.8 ± 0.8a	7.0 ± 0.0b	14.2 ± 0.5b
	Control	6.0 ± 0.0cd	5.2 ± 0.4a	4.2 ± 0.4a
		F = 947.0, df = 20;4, p < 0.000	F = 398.0, df = 20;4, p < 0.000	F = 3208.250, p < 0.000

*Means in the same column followed by different letters are different by Tukey test at 5% significance.

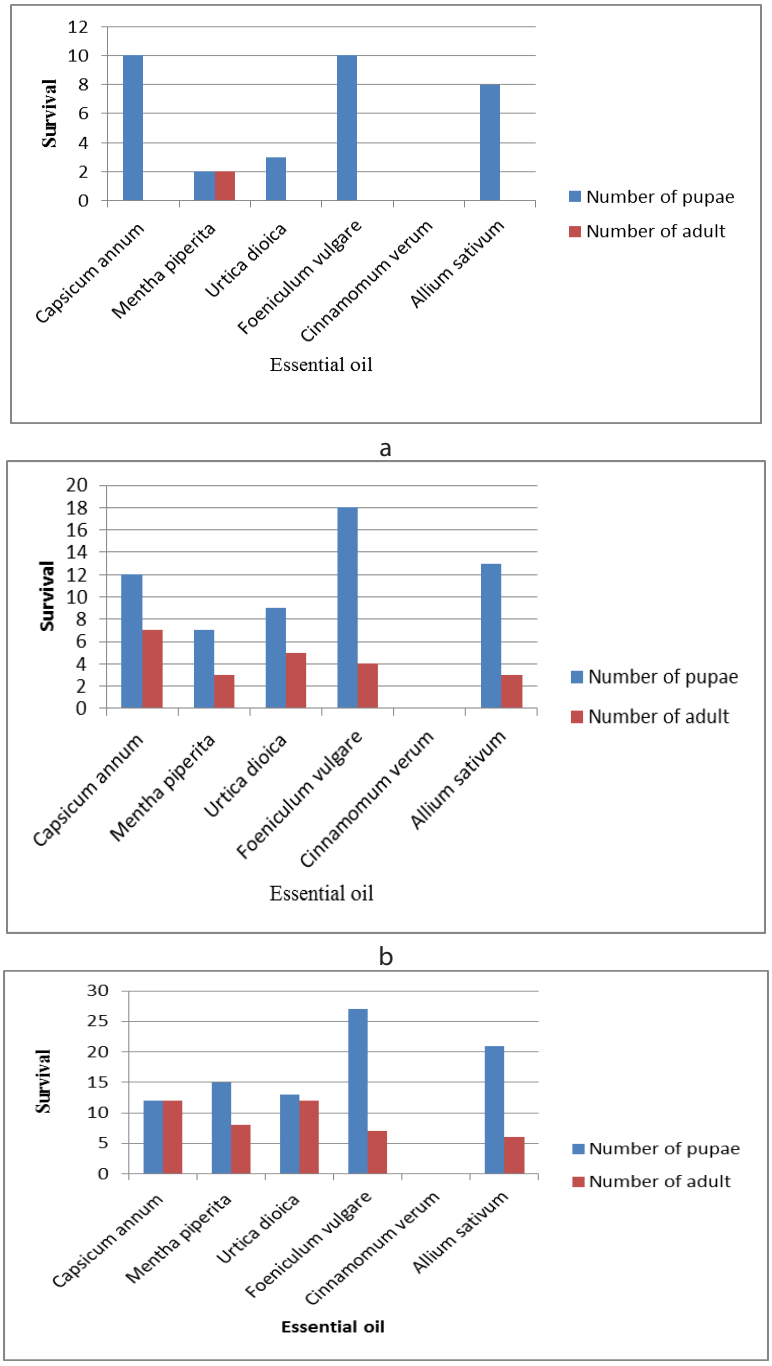


Figure 1. Mean mortality of 3rd instar larvae of *M. domestica* (L) at various concentrations of various essential oil (a: 5%, b: 2.5%, c: 1% concentration)

Table 2. Mortality rate of essential oil against larvae of *M. domestica* (L.) after 24 hour of exposure

Concentration of essential oil	10	5	2.5	1	F value
	Mortality percent after 24 h exposure				
Capsicum annum	72.00b	0.00a	0.00a	0.00a*	F=152.4, p <0.000
Mentha piperita	96.00b	0.00a	0.00a	0.00a	F=1536.0, p <0.000
Urtica dioica	94.00c	64.00b	6.00a	0.00a	F=77.0, p < 0.000
Foeniculum vulgare	89.60c	42.00c	26.00 b	16.80a	F = 3496.17, p < 0.000
Cinnamomum verum	100.00d	100.00c	76.00 b	46.00a	F = 120.923, df = 3,16, p < 0.000
Allium sativum	74.80d	43.00c	28.20 b	20.40a	F = 947.104, p < 0.000

*Means in the same column followed by different letters are different by Tukey test at 5% significance.

Table 3. Lethal concentrations of six essential oils against housefly larvae in larvicidal assay with their LC_{50} and LC_{90} values.

Essential oil	Lethal Concentration	
	LC_{50}	LC_{90}
Capsicum annum	0.311	3.249
Mentha piperita	0.565	2.358
Urtica dioica	0.239	2.298
Foeniculum vulgare	1.327	3.312
Cinnamomum verum	0.021	1.688
Allium sativum	0.831	2.772

Table 4. Percentage inhibition rate (PIR) against pupae of *M. domestica* with different concentrations of six essential oils in contact toxicity assay.

Esential oil	Concentration of oil (µ/L)	PIR
Capsicum annum	10	92.10
	5	78.94
	2.5	73.68
	1	71.05
Mentha piperita	10	92.10
	5	76.31
	2.5	71.05
	1	63.15
Urtica dioica	10	100
	5	92.10
	2.5	81.57
	1	71.05
Foeniculum vulgare	10	86.84
	5	76.31
	2.5	68.42
	1	52.63
Cinnamomum verum	10	100
	5	100
	2.5	100
	1	97.36
Allium sativum	10	100
	5	94.73
	2.5	92.10
	1	89.47

was achieved by *Foeniculum vulgare*. In a present study, the *Cinnamomum verum* EOs at a 5,10% concentration caused 100% mortality in larvae of *M. domestica* after 24 h of exposure. The mortality effect of the *Cinnamomum verum* EOs was 100% after 5 days of exposure at a concentration of 2.5% and after 10 days of exposure at a concentration of 1%. At all four concentrations tested for *Cinnamomum verum* EOs (1, 2.5, 5, 10%), the mortality rate was 100%. Similarly, Khater and Geden (2019) reported the essential oil of cinnamon exhibited a 100% larvicidal bioassay at a 5% concentration against *M. domestica*. Khater et al. (2018) reported that cinnamon oils showed the highest mortalities against larvae of *Lucilia sericata* (Meigen 1826) (Diptera: Calliphoridae) in contact assays. These results are in line with our results. Contrary to our study, Morey and Khandagle (2012) reported that *C.verum* EOs observed strong larvicidal activity against houseflies but that *M. piperita* EOs was more effective than *Cinnamomum verum* EOs in a larvicidal bioassay.

Cinnamomum verum EOs had a strong larvicidal activity, with an LC_{50} value of 0.021 µl/cm^2, and LC_{90} value (1.688) (Table 3). Morey and Khandagle (2012) tested the larvicidal activity (LC_{50}) of *C. verum* oil against *M. domestica*. *C. verum* oil exhibited moderate larvicidal activity (LC_{50} 159 ppm).

Among the six oils, *U. dioica* and *M. piperita* were highly effective against larvae of *M. domestica*. *U. dioica* EOs at 10%, 5%, 2.5%, and 1% concentrations exhibited larvicidal activity with a mortality rate of 100, 94, 82, and 74% respectively. *M. piperita* EOs at 10%, 5%, 2.5% and 1% concentrations exhibited larvicidal activity with a mortality rate of 100, 96, 86, and 70% respectively. The LC_{50} value was 0.565, while the LC_{90} value 2.358 was Kumar et al. (2011a and 2012a) reported potential larvicidal activity of *M. citrata* and *M. piperita* against the larvae of houseflies. Similarly, *Mentha spicata* EO_s showed high larvicidal activity against *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae), *Culex quinquefasciatus* (Say, 1823) (Diptera: Culicidae), and *Aedes aegypti* (Linnaeus 1762) (Diptera: Culicidae) with LC_{50} of, 49.71, 56.08, and 62.62ppm, respectively (Govindarajan et al., 2012). EOs are also known to decrease levels of lipids, glycogen, proteins, and enzyme activity which leads to retarded growth, and poor nutrient utilization. The reduction in enzyme activity due to treatment with EOs causes the cytotoxic effect (disrupting cell membrane) on epithelial cells of the gut (Chintalchere et al., 2013). Meanwhile, the essential oil disrupts normal neurotransmission and the neuroendocrine system, resulting in developmental abnormalities (Mansour et al., 2011; Mohamed et al., 2016; Khater and Khater, 2009; Nasiruddin and Mordue, 1993). The results of the present study indicated that *A. sativum*

EOs at 10%, 5%, 2.5%, and 1% concentrations produced a mortality rate at 100, 84, 74.4, 57.8% , respectively. The LC_{50} value was 0.831, while the LC_{90} value was 2.772. Previous studies have reported that garlic juice and oils have insecticidal effectiveness against pest invertebrates (Inyang and Emosairue, 2005; Chiam et al., 1999; Karci and Isikber, 2007; Isikber et al., 2009). In the same vein, Meriga et al. (2012) observed that the *A. sativum* EOs (methanol extracts) against *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) larvae exhibited high mortality (81%) at 1% concentration. These results are in line with Prowse et al. (2006) who observed *A. sativum* juice achieved high mortality of *M. domestica* (about 90%) at 5% concentration. Similarly, Shahriari et al. (2018) demonstrated the insecticidal effect of *A. sativum* against *M. domestica* was dependent upon exposure time, and concentrations. Levchenko et al. (2021) showed A. *sativum* EOs against *M. domestica* exhibited strong mortality (99%) at a 0.066% concentration. In the present study, at 10% concentration, all essential oils tested exhibited the highest mortality against *M. domestica* larvae with 100% mortality. *F. vulgare* EOs were found to be less effective against housefly larvae compared to other tested essential oil in our study. The treatment of *F. vulgare* EOs at 10%, 5%, 2.5%, and 1 % concentrations was exhibited as 100%, 80%, 64.2%, and 46% for larval stage. LC_{50} and LC_{90} for *F. vulgare* EOs were 1.327 and 3.312 respectively. Similarly, Chantawee and Soonwera (2018a) reported that *F. vulgare* EOs at 1%, 5%, and 10% concentrations exhibited–larvicidal activity against housefly with a mortality rate of 16.3%, 45.7%, and 89.3%, respectively. El Zayyat et al. (2017) reported that *F. vulgare* EOs observed the highest toxicity against *Lasioderma serricorne* F. (Coleoptera: Anobiidae), *Callosobruchus chinensis* Linnaeus 1758 (Coleoptera: Bruchidae), and *Sitophilus oryzae* (L) (Coleoptera: Curculionidae). Contrary to our results, Chantawee and Soonwera (2018b) indicated that 10% and 5% *F. vulgare* EOs exhibited a strong larvicidal activity (100%) against *A. aegypti* at 24 h after exposure. Sedaghat et al. (2011) reported that *F. vulgare* EOs were the most effective against larvae of *An. stephensi* with an LC_{50} value of 20.10 ppm and LC_{90} value of 44.51 ppm, respectively. Levchenko et al., 2021 indicated *E. caryophyllata*, *F. vulgare*, and *A. sativum* EOs against the *M. domestica* had the highest insecticidal effectiveness at the dilution of 1:500.

The results indicated that the larval and pupal developmental time were significantly extended in EOs treatments. Similarly, Roel et al. (2010) indicated that the duration of the development period of *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), was significantly prolonged at different concentrations of *Azadirachta indica* (Meliaceae) essential oil. *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) was found to be prolonged in the larval and pupal stages by *Mentha piperita* by Yousef et al. (2018).

The essential oil may impact insect development and metabolism (Moutassem et al., 2021). However, EOs causes reduced food ingestion (Roel et al., 2010), inhibits digestive enzymes (Yousef et al., 2018) and can result in prolongation of the development duration. Previously research showed that essential oil caused impaired feeding performance and inactivation of digestive (Bezzar-Bendjazia et al., 2017). The pupicidal assay performed by contact toxicity against the pupae of *M. domestica* showed important variation in growth and development inhibition with different concentrations of the tested EOs. Further, it was observed that with an increase in the concentration of six essential oil % mortality also increased in the pupae of *M. domestica*. The failure of pupation and adult emergence can appear as a result of several factors: unsatur-ated fatty acids, which accelerate the melanization process; hardening of the opercular suture and insufficient pressure in the ptilinum (Mohamed et al., 2016; Khater and Khater, 2009). *C. verum* essential oils were the most toxic to exposed pupae, followed by *A. sativum*, and *U. dioica* essential oils, in that order. In present study, *M. piperita* oil was relatively moderately performer with PIR value between 63.15 and 92.10%, for different concentrations among tested essential oil. The pupicidal potential of M. *piperita* has been reported by different researchers. Kumar et al. (2012b) reported PIR of *M.piperita* oil varied between 54 and 100% against house fly pupae. In this investigation, *F. vulgare* recorded the lowest pupicidal activity compared to tested essential oil and pupicidal bioassays of *M. domestica* with *F. vulgare* essential oil exhibited the percentage inhibition rate of 52.63-86.84% at different concentrations. These investigations were in accordance with that reported by Chantawee and Soonwera (2018a) as they observed that ten percent of *F. vulgare* EOs exhibited the highest toxicity against the pupae with 76% mortality rate at 10 days. These findings were contradicted by the studies of Abdel-Bakı et al. (2021) as they reported that the concentration of 10% of essential oil from *F. vulgare* caused complete inhibition (100% mortality) in the pupae of *M. domestica*.

CONCLUSION

This experiment was conducted to evaluate the insecticidal activities of *F. vulgare, A.sativum, C. verum, C. annum, M. piperita* and *U. dioica* against the pupae and larvae of *M. domestica*. The present results demonstrated that the percent of larval and pupal mortality was increased by increasing concentration and exposure time for all tested essential oils. The essential oils were reported to increase pupal and larval mortality, and developmental time. The current results suggest that the *C. verum* essential oil was found to be most effective against larvae and pupae of *M. domestica* among tested essential oils.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Abdel-Baki, A.A.S., Aboelhadıd, S.M., Sokmen, A., Al-Quraishy, S., Hassan, A.O., Kamel, A.A. (2021). Larvicidal and pupicidal activities of *Foeniculum vulgare* essential oil, trans-anethole and fenchone against house fly *Musca domestica* and their inhibitory effect onacetylcholinestrase. Entomological Research, 51, 568–577.

Ayvaz, A., Sagdic, O., Karaborklu, S., Ozturk, I. (2010). Insecticidal activity of the essential oils from different plants against three stored-product insects. Journal of Insect Science, 10, 21. https://doi.org/10.1673/031.010.2101

Bezzar-Bendjazia, R., Kilani-Morakchi, S., Maroua, F., Aribi, N. (2017). Azadirachtin induced larval avoidance and antifeeding by disruption of food intake and digestive enzymes in *Drosophila melanogaster* (Diptera: Drosophilidae). Pesticide Biochemistry and Physiology, 143, 135–140.

Chantawee, A., Soonwera, M. (2018a). Larvicidal, pupicidal and oviposition deterrent activities of essential oils from Umbelliferae plants against house fly *Musca domestica*. Asian Pacific Journal of Tropical Medicine, 11(10), 621-629.

Chantawee, A., Soonwera, M. (2018b). Efficacies of four plant essential oils as larvicide, pupicide and oviposition deterrent agents against dengue fever mosquito, *Aedes aegypti* Linn. (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine, 8(4), 217-225.

Chintalchere JM, Lakare S, Pandit RS (2013). Bioefficacy of essential oils of *Thymus vulgaris* and *Dianthus caryophyllus* against housefly, *Musca domestica* L. The Bioscan 8 (3): 1029-1034.

Chiam, W.Y., Huang, Y., Chen, S.X., Ho, S.H. (1999). Toxic and antifeedant effects of allyl disulfide on *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae). Journal of Economic Entomology, 92(1), 239–245. https://doi.org/10.1093/jee/92.1.239

El Zayyat, E.A., Soliman, M.I., Elleboudy, N.A., Ofaa, S.E. (2017). Bioefficacy of some Egyptian aromatic plants on *Culex pipiens* (Diptera: Culicidae) adults and larvae. Journal of Arthropod-Borne Disease, 11(1), 147-155.

Förster, M., Klimpel, S., Mehlhorn, H., Sievert, K., Messler, S., Pfeffer, K. (2007). Pilot study on synanthropic flies (e.g. Musca, Sarcophaga, Calliphora, Fannia, Lucilia, Stomoxys) as vectors of pathogenic microorganisms. Parasitology Research, 101, 243–246.

Govindarajan, M., Sivakumar, R., Rajeswari, M., Yogalakshmi, K. (2012). Chemical composition and larvicidal activity of essential oil from *Mentha spicata* (Linn.) against three mosquito species. Parasitology Research, 110, 2023–2032. https://doi.org/10.1007/s00436-011-2731-7

Gupta, A.K., Nayduch, D., Verma, P., Shah, B., Ghate, H.V., Patole, M.S., Shouche, Y.S. (2011). Phylogenetic characterization of bacteria in the gut of house flies (*Musca domestica* L.). FEMS Microbiology Ecology, 79 (3), 581-93.

Inyang, U.E., Emosairue, S.O. (2005). Laboratory assessment of the repellent and antifeedant properties of aqueous extracts of 13 plants against the banana weevil *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae). Tropical and Subtropical Agroecosystems,·5, 33-44.

Isikber, A.A., Ozder, N., Saglam, O. (2009). Susceptibility of eggs of *Tribolium confusum, Ephestia kuehniella and Plodia interpunctella* to four essential oil vapors. Phytoparasitica, 37, 231-239.

Karci, A., Isikber, A.A. (2007). Ovicidal activity of various essential oils against confused flour beetle, *Tribolium confusum* Jacquelin duVal (Coleoptera: Tenebrionidae). Integrated Protection *of* Stored Products, 30, 51-258.

Khan, H.A., Akram, W., Shad, S.A. (2013). Resistance to conventional insecticides in Pakistani populations of *Musca domestica* L. (Diptera: Muscidae): a potential ectoparasite of dairy animals. Ecotoxicology, 22, 522–527.

Khan, H.A.A,. Akram, W., Lee, S, Ahmad, T., Maqsood, K., et al. (2019). Toxic potential of some indigenous plant oils against the rice weevil, *Sitophilus oryzae* (Linnaeus). Entomology Research, 49, 136-140.

Khater, H.F., Khater, D.F. (2009). The insecticidal activity of four medicinal plants against the blowfly *Lucilia sericata* (Diptera: Calliphoridae). International Journal of Dermatology, 48, 492-497.

Khater, H.F., Ali, A.M., Abouelella, G.A., Marawan, M.A., Govindarajan M, et al. (2018) Toxicity and growth inhibition potential of vetiver, cinnamon, and lavender essential oils and their blends against larvae of the sheep blowfly, *Lucilia sericata*. International Journal of Dermatology, 57, 449–457.

Khater, H., Geden, C.J. (2018). Potential of essential oils to prevent fly strike and their effects on the longevity of adult *Lucilia sericata*. Journal of Vector Ecology, 43 (2), 261-270.

Khater, H., Geden, C.J. (2019). Efficacy and repellency of some essential oils and their blends against larval and adult house flies, *Musca domestica* L. (Diptera: Muscidae). Journal of Vector Ecology, 44 (2), 256-263.

Kumar, P., Mishra, S., Malik, A., Satya, S. (2011a). Repellent, larvicidal and pupicidal properties of essential oils and their formulations against the housefly, *Musca domestica*.

Medical and Veterinary Entomology, 25 (3), 302-310.

Kumar, P., Mishra, S., Malik, A., Satya, S. (2011b). Insecticidal properties of *Mentha species*: a review. Industrial Crops and Products, 34, 802–817. https://doi.org/10.1016/j.indcrop.2011.02.019

Kumar, P., Mishra, S., Malik, A., Satya, S. (2012a). Insecticidalevaluationofessential oils of *Citrus sinensis* L. (Myrtales:Myrtaceae) against housefly, *Musca domestica* L. (Diptera: Muscidae). Parasitology Research, 110, 1929–1936.

Kumar, P., Mishra, S., Malik, A., Satya, S. (2012b). Efficacy of Mentha x piperita and Mentha citrata essential oilsagainst housefly, *Musca domestica* L. Industrial Crops and Products, 39, 106–112.

Kumar, A., Dubeya, K.N., Srivastava, S. (2013). Antifungal evaluation of *Ocimum sanctum* essential oil against fungal deterioration of raw materials of *Rauvolfia serpentina* during storage. Industrial Crops and Products, 45, 30-35.

Kumar, P., Mishra, S., Malik, A., Satya, S. (2014). Biocontrol potential of essential oil monoterpenes against housefly, *Musca domestica* (Diptera: Muscidae) Ecotoxicology and Environmental Safety, 100 (1), 1–6. https://doi.org/10.1016/j.ecoenv.2013.11.013

Levchenko, M.A., Silivanova, E.A., Khodakov, P.E., Gholizadeh, S. (2021). Insecticidal efficacy of some essential oils against adults of *Musca domestica* L. (Diptera: Muscidae). International Journal of Tropical Insect Science, 41(4), 2669-2677. https://doi.org/10.1007/s42690-021-00448-0

Malik, A, Singh N, Satya S (2007). Housefly (*Musca domestica*): A review of control strategies for a challenging pest. Journal of Environmental Science and Health B 42: 453–469.

Mansour, L.S.A., Bakr, R.F.A., Mohamed, R.I., Hasaneen. N.M. (2011). Larvicidal Activity of Some Botanical Extracts, Commercial Insecticides and their Binary Mixtures Against the Housefly, *Musca domestica*. The Open Toxicology Journal, 4, 1-13.

Meriga, B., Mopuri, R. MuraliKrishna, T. (2011). Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum*. Asian Pacific Journal of Tropical Medicine, 5(5), 391-395.

Meyer, J.A., Petersen, J.J. (1983). Characterization and seasonal distribution of breeding sites of stable flies and house flies on eastern Nebraska feedlots and dairies. Journal of Economic Entomology, 76, 103-108.

Mohamed, H.S., Fahmy, M.M., Attia, M.M., Rabab, M., El Khateeb, M., Shalaby, H.A., Massoud, A.M. (2016). The insecticidal activity of two medicinal plants (*Commiphora molmol*) and (*Balanites aegyptiaca*) against the Blowfly *Lucilia sericata* (Diptera: Calliphoridae). International Journal of Advanced Research in Biological Sciences, 3(3), 144-158.

Mordue, A.J., Nisbet, A. (2000). Azadirachtin from the Neem Tree *Azadirachta indica*: its Action Against Insects. Anais da Sociedade Entomológica do Brasil, 29, 615–632

Morey, R.A., Khandagle, A.J. (2012). Bioefficacy of essential oils of medicinal plants against housefly, *Musca domestica* L. Parasitology Research, 111", 1799-1805. https://doi.org/10.1007/s00436-012-3027-2

Moutassem, D., Bellik, Y., Sennef, M.E.H. (2021). Toxicity and repellent activities of *Thymus pallescens* and *Cymbopogon citratus* essential oils against *Sitophylus granarius.* Plant Protection Science 57, 2021 (4), 297–309

Nasiruddin, M., Mordue, A.J. (1993). The effect of azadirachtin on the midgut histology of the locusts, *Schistocerca gregaria* and *Locusta migratoria*. Tissue Cell, 25, 875–884.

Neupane, S., Nayduch, D., Zurek, L. (2019). House Flies (*Musca domestica*) Pose a Risk of Carriage and Transmission of Bacterial Pathogens Associated with Bovine Respiratory Disease (BRD). Insects, 10, 358. https://doi.org/10.3390/insects10100358

Ogbalu, O.K., Bobmanuel, R.B., Membere, O. (2014). Larvicidal Effect of Aqueous Leaf Extract of Tobacco (Nicotianatabacum) On the Third Instar Larvae of *Musca domestica* L. IOSR Journal of Agriculture and Veterinary Science, 7 (12), 35-40.

Pavela, R. (2008). Insecticidal Properties of Several Essential Oils on the House Fly (*Musca domestica* L.). Phytotherapy Research, 22, 274–278.

Pavela, R, (2011). Insecticidal and repellent activity of selected essential oils against of the pollen beetle, *Meligathes aeneus* (Fabricius) adults. Industrial Crops and Products, 34, 888–892.

Prowse, G.M., Galloway, T.S., Foggo, A. (2006). Insecticidal activity of garlic juice in two dipteran pests. Agricultural and Forest Entomology, 8, 1-6.

Roel, AR, Dourado DM, Matias R, Porto KRA, Bednaski AV, da Costa, R.B. (2010). The effect of sub-lethal doses of *Azadirachta indica* (Meliaceae) oil on the midgut of *Spodoptera frugiperda* (Lepidoptera, Noctuidae). Revista Brasileira de Entomologia, 54(3), 505-510. https://doi.org/10.1590/S0085-56262010000300024

Sedaghat, M., Dehkordi, A.S., Abai, M., Khanavi, M., Mohtarami, F., Abadi, YS., Rafi, F., Vatandoost, H. (2011). Larvicidal activity of essential oils of Apiaceae plants against malaria vector, *Anopheles stephensi*. Journal of Arthropod-Borne Disease, 5(2), 51-59.

Scott, J.G., Alefantis, T.G., Kaufman, P.E., Rutz, D.A. (2000). Insecticide resistance in house flies from caged-layer poultry facilities. Pest Management Science, 56, 147-153.

Schou, TM, Faurby S, Kjærsgaard A, Pertoldi C, Loeschcke V, Hald, B., Bahrndorffet, S.(2013) Temperature and population density effects on locomotor activity of *Musca domestica* (Diptera: Muscidae). Environment Entomoogy, 42(6), 1322-8. https://doi.org/10.1603/EN13039

Shahriari, M., Zibaee, A., Sahebzadeh, N., Shamakhi, L. (2018). Effects of α-pinene. trans-anethole. and thymol as the essential oil constituents on antioxidant system and acetylcholine esterase of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Pesticide Biochemical Physiology, 150, 40-47.

Vasantha-Srinivasan P, Senthil-Nathan S, Thanigaivel A, Edwin E.S., Ponsankar A, Selin-Rani, S., Pradeepa, V., Sakthi-Bhagavathy, M., Kalaivani, K., Hunter, W.B., Duraipandiyan, V., Al-Dhabi. N. A. (2016). Developmental response of *Spodoptera litura* Fab. to treatments of crude volatile oil

from *Piper betle* L. and evaluation of toxicity to earthworm, *Eudrilus eugeniae* Kinb. Chemosphere, 155, 336-347.

Yousef, D.A., Bayoumi, A.E., Dimetry, N.Z., Amin, A.H., Hoballah, E.M. (2018). Evaluatıng effect of pepper mınt oıl *(Mentha pipreta*) and ıts nano-formulatıons on some enzymatıc actıvıtıes and bıonomıcs of cotton leaf worm *Spodoptera littoralis.* Universities Journal of Agricultural Sciences, 26(2), 1977-1991.

Zahoor, A.M.K., Zahoor, M.A., Mubarik, M.S., Rizvi, H., Majeed, H.N, Zulhussnain, M., Ranian,K., Sultana, K., Imran, M., Qamer, S. (2020). Insecticidal, biological and biochemical response of *Musca domestica* (Diptera: Muscidae) to some indigenous weed plant extracts. Saudi Journal of Biological Sciences, 27, 106–116.