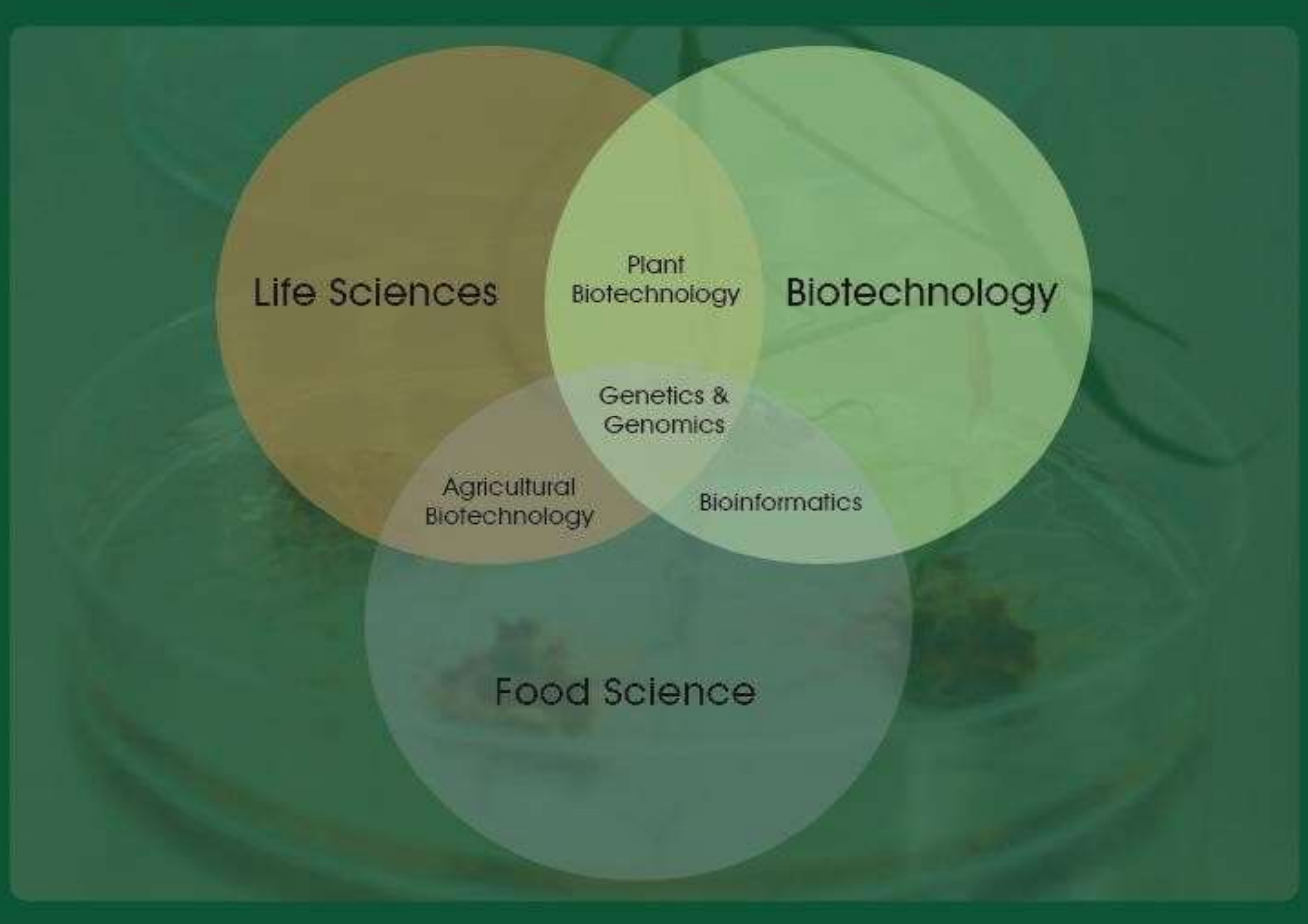
International Journal of Life Sciences and Biotechnology

e-ISSN:2651-4621



Volume/Cilt:4	2021	Issue / Sayı:1

Editor in Chief / Baş Editör
Asst. Prof. Dr. Yilmaz Kaya
Ondokuz Mayis University, Turkey

Section Editors / Bölüm Editörleri*

* sıralama akademik unvan içinde alfabetik sıralamaya göredir. * The ranking is arranged alphabetically within the academic title.

Prof. Dr. Ali ASLAN, PhD, Van Yuzuncuyıl University
Prof. Dr. Ercan BURSAL, PhD, Muş Alparslan University
Prof. Dr. Hasan AKAN, PhD, Harran University
Prof. Dr. Nermin GOZUKİRMIZİ, PhD Istinye University
Prof. Dr. Tengku Haziyamin TENGKU ABDUL HAMİD, PhD, International Islamic University Malaysia
Assoc. Prof. Dr. Ayhan HORUZ, PhD Ondokuz Mayıs University
Assoc. Prof. Dr. Hasan Murat AKSOY, PhD, Ondokuz Mayıs University

Editorial Board / Editör Kurulu
Prof. Dr. İsmail KOCAÇALIŞKAN, PhD, Yildiz Technical University
Prof. Dr. Kasim BAJROVİC, University of Sarajevo
Prof. Dr. Muhammet KURULAY, PhD, Yildiz Technical University
Assoc. Prof. Dr. Gulbubu KURMANBEKOVA, Kyrgyz-Turkish Manas University
Assoc. Prof. Dr. İsmail ERPER, PhD, Ondokuz Mayıs University
Assoc. Prof. Dr. Muhammad Arshad JAVED, PhD, Universiti Teknologi Malaysia
Assoc. Prof. Dr. Roswanira AB. WAHAB, PhD, Universiti Teknologi Malaysia
Assoc. Prof. Dr. Sevgi MARAKLI, PhD, Amasya University
Asst. Prof. Dr. Abdussamat GÜZEL, PhD, Inonu University
Asst. Prof. Dr. Ali Yuksek, PhD, Ondokuz Mayıs University
Asst. Prof. Dr. Cihan İNAN, PhD, Karadeniz Technical University
Asst. Prof. Dr. Ertan ERMİŞ, PhD, Istanbul Sabahattin Zaim University
Asst. Prof. Dr. Feyza TUFAN, PhD, Halic University
Asst. Prof. Dr. Harun ÖZER, PhD, Ondokuz Mayıs University
Asst. Prof. Dr. Kasım TAKIM, PhD, Harran University
Asst. Prof. Dr. Mohamed EDBEİB, PhD, Baniwalid University, Libya
Asst. Prof. Dr. Muhammed YÜCEER, PhD, Canakkale Onsekiz Mart University,
Dr. Aliyu ADAMU, PhD, Kaduna State University
Dr. Abdulwali ABLAT, University of Malaya
Dr. Lect. Abdulgani DEVLET, PhD, Bilecik Seyh Edebali University
Dr. Nedim UZUN, PhD, Taksim Education and Research Hospital, Turkey
Dr. Res. Asst. Kiran NAWAZ, PhD, University of Arizona

Advisory Board / Danışma Kurulu
Prof. Dr. Ahmet OKUMUŞ, PhD, Aydın Adnan Menderes University
Prof. Dr. Didem ÖZÇİMEN, PhD, Yildiz Technical University
Prof. Dr. Fahrul Zaman HUYOP, PhD, Universiti Teknologi Malaysia
Prof. Dr. İbrahim İlker ÖZYİĞİT, PhD, Marmara University
Assoc. Prof. Dr. Funda ARSLANOĞLU, PhD, Ondokuz Mayıs University
Assoc. Prof. Dr. Kadyrbay CHEKİROV, Kyrgyz - Turkısh Manas Unıversıty, Kyrgyzstan
Assoc. Prof. Dr. Sibel YILMAZ, PhD, Yeni Yuzyil University
Assoc. Prof. Dr. Zarina Bt ZAİNUDDİN, PhD, International Islamic University Malaysia

Language Editors/ Dil Editörleri
Assoc. Dr. Sevgi Maraklı, Amasya Unıversıty, Turkey
Res. Assist. Bermet Kıdıralıyeva, Kyrgyz - Turkısh Manas Unıversıty, Kyrgyzstan
Specialist Nurjamal Omurzakova, Kyrgyz - Turkısh Manas Unıversıty, Kyrgyzstan

Managing Editor / Yönetici Editör
Yunus Emre ARVAS, PhD Cand., Yildiz Technical University

Yönetim Ofisi/ Management Office
Yildiz Technical University, Faculty of Chemical and Metallurgical Engineering.
ESENLER/ISTANBUL

Yasal Sorumluluk
Yazıların yasal ve hukuki sorumluluğu yazarlara aittir.
Tüm hakları saklıdır. Derginin hiçbir bölümü, yazılı ön izin olmaksızın ve dergi adına referans gösterilmeden herhangi bir formatta çoğaltılamaz veya kullanılamaz.

Legal Responsibility
The legal responsibility of the articles belongs to the authors. All rights reserved. No part of this journal may be reproduced or used in any form without the prior writen permission and a reference to name of the journal.

Editörden;

Değerli okurlar ve yazarlar,

“International Journal of Life Sciences and Biotechnology” olarak dergimizin dokuzuncu sayısını yayın hayatına sunmaktan mutluluk ve onur duyuyoruz. “International Journal of Life Sciences and Biotechnology” dergisi araştırma- geliştirme ve uygulama ilkeleri baz alınarak yayınlanan uluslararası hakemli açık erişimli akademik bir elektronik dergidir.

“International Journal of Life Sciences and Biotechnology” dergisi Yaşam Bilimleri, Biyoloji, Biyoteknoloji, Biyomühendislik, Ziraat Bilimleri, Gıda Biyoteknolojisi ve Genetik alanlarındaki ilgili araştırmacılara, kurum ve kuruluşlara teorik ve pratik uygulamalarda katkı sağlamayı, tarafsızlık ve bilim etiği ilkelerine bağlı kalarak çözüm temelli, yenilikçi ve katma değeri olan çalışmalara odaklanan, günceli ve geleceği tartışan çalışmaların yayınlanmasını hedeflemektedir.

Bu düşüncelerle 2021 yılı birinci sayısını yayınladığımız “International Journal of Life Sciences and Biotechnology” dergisini, makaleleri ile onurlandıran akademisyenlere, Fikir / Görüş / Öneri / Katkı ve Eleştirileri ile değerlendirme süreçlerine katkılarından dolayı hakem ve yayın kurullarında yer alan kıymetli bilim insanlarına yürekten teşekkür ediyoruz. Bir sonraki sayıda görüşmek ümidiyle…

15.04. 2021
Editör
Dr. Öğrt. Üyesi Yilmaz KAYA

From The Editor;

Dear Readers and Authors,

As “International Journal of Life Sciences and Biotechnology”, we are pleased and honored to present the ninth issue of the journal. "International Journal of Life Sciences and Biotechnology" is an international double peer-reviewed open access academic journal published on the basis of research-development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, We are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in the first issue of 2021 "International Journal of Life Sciences and Biotechnology". Hope to see you in the next issue…

15. 04. 2021

Editor in Chief

Assist. Prof. Dr. Yilmaz KAYA

Sayının Hakemleri / Reviewers of the Issue*

* sıralama akademik unvan içinde alfabetik sıralamaya göredir. * The ranking is arranged alphabetically within the academic title.

Prof. Dr. Burhan KARA, PhD, Isparta University of Applied Sciences
Prof. Dr. Cigdem KUCUK, PhD, Harran University, Turkey
Prof. Dr. Kasim BAJROVİC, PhD, University of Sarajevo
Prof. Dr. Mehmet DEMİRKAYA, PhD, Eskisehir Osmangazi University
Prof. Dr. Satı UZUN, PhD, Erciyes University

Assoc. Dr. Esin SAKALLI ÇETİN, PhD, Muğla University, Turkey
Assoc. Prof. Dr. Funda ARSLANOĞLU, PhD, Ondokuz Mayıs University
Assoc. Prof. Dr. Ismaila Yada SUDI, PhD, Adamawa State University, Nigeria
Assoc. Prof. Dr. İsmail ERPER, PhD, Ondokuz Mayıs University
Assoc. Prof. Dr. İsmail SEZER, PhD, Ondokuz Mayıs University, Turkey

Assist. Prof. Dr. Hüseyin UYSAL, PhD, Aydin Adnan Menderes University
Assist. Prof. Dr. Mehtap USTA, PhD, Trabzon University, Turkey
Assist. Prof. Dr. Parham TASLİMİ, PhD, Bartin University
Assist. Prof. Dr. Samet KOCABAY, PhD, Inonu University, Turkey
Assist. Prof. Dr. Wafaa Hassan MUSLEM, PhD,Mustansiriyah University
Assist. Prof. Dr. Yilmaz KAYA, PhD, Ondokuz Mayis University, Turkey

Dr. Aida BAHARUDDİN, PhD, Uitm Jengka Pahang, Malaysia
Dr. Aliyu ADAMU, PhD, Kaduna State University
Dr. Asli Hocaoglu-OZYİGİT, PhD, Marmara University
Dr. Mohd Firdaus ABDUL-WAHAB, PhD, Universiti Teknologi Malaysia, Malaysia

Dr. Res. Assist. Mehmet TÜTÜNCÜ, PhD, Ondokuz Mayıs University, Turkey
Dr. Res. Assist. Muhammed M. ABED, PhD, University of Anbar, Iraq
Dr. Res. Assist. Serap ÇETİNKAYA, PhD, Sivas Cumhuriyet University, Sivas Turkey
Dr. Res. Assist. Zafer SEÇGİN, PhD, Ondokuz Mayis University, Turkey

İçindekiler/ Contents

Research Articles/ Araştırma Makaleleri

How did the Addition of Indaziflam Affect on Carbon and Nitrogen Mineralizations in a Vineyard Soil?
Burak KOÇAK, Şahin CENKSEVEN, Nacide KIZILDAĞ, Hüsniye AKA SAĞLIKER, Cengiz DARICI....................**1-12**

Lactic Acid Bacterium With Antimicrobial Properties From Selected Malay Traditional Fermented Foods
Tengku Haziyamin TENGKU ABDUL HAMİD, Nur FATİN AMYSYA **13-24**

Delphinidin, Luteolin and Halogenated Boroxine Modulate CAT Gene Expression in Cultured Lymphocytes
Nikolina TOMİC, Maida HADZİC, Naida LOJO-KADRİC, Jasmin RAMİC, Lejla POJSKİC **25-32**

Growth Analysis of Lactobacillus acidophilus Using Different Non-Digestible Carbohydrates
Haia Abobakr AL-KAF, Fahrul Zaman HUYOP, Noor Azwani ZAİNOL... **33-45**

2,4-Dichlorophenoxyacetic Acid Loaded Polymeric Nanoparticle Synthesis and Its Effect on Biomass in Medicago sativa Cell Suspension Cultures
Fatma Şayan POYRAZ, Edibe ABACI, Caner ERTÜRK, Tayfun ACAR, Serap DERMAN, Semiha ERİŞEN, Banu MANSUROĞLU **46-60**

Yield and Quality Stabilities of Waxy Maize Genotypes using Biplot Analysis
Erkan ÖZATA **61-89**

Microbial Population of Soil and Water around Petroleum Depot Suleja, Nigeria, and their Hydrocarbon Utilisation
Chukwunonso ACHİFE, Udeme JOSHUA, Jeremiah BALA, Solomon OYELEKE **90-113**

Review Articles / Derleme Makaleler

Salvia fruticosa'nın (Anadolu Adaçayı) Terapötik Etkileri
Sinem ELMAS, Onur ELMAS **114-137**

Bitkisel Üretimde Yeni Bir Trend: Kenevir
Merve GÖRE, Orhan KURT **137-157**

Antarktika: Yaşam Bilimleri ve Biyoteknoloji Araştırmalarının Gözden Geçirilmesi
Suleyman Faruk KIRKINCI, Sevgi MARAKLI, Hasan Murat AKSOY, Didem ÖZÇİMEN, Yilmaz KAYA **158-177**

Research article

Kocak., B., et al., How did the Addition of Indaziflam Affect on Carbon and Nitrogen Mineralizations in a Vineyard Soil? International Journal of Life Sciences and Biotechnology, 2021.
DOI: 10.38001/ijlsb.751731

How did the Addition of Indaziflam Affect on Carbon and Nitrogen Mineralizations in a Vineyard Soil?

Burak Koçak[1*], Şahin Cenkseven[2], Nacide Kızıldağ[3], Hüsniye Aka Sağlıker[4], Cengiz Darıcı[1]

ABSTRACT
Indaziflam is a herbicide used for weed control in vineyards, apple, peach and orange orchards that inhibit cellulose biosynthesis in plants. The objective of this study was to evaluate the effects of recommended field dose of herbicide Indaziflam (10 ml/ da, RD) and its 2 (RD x2), 4 (RD x4), 8 (RD x8) and 16 (RD x16) folds of RD on carbon and nitrogen mineralizations in a vineyard soil. Herbicide+soil mixtures were humidified at 80% of soil field capacity and then incubated for 42 days at 28ºC . Effects of RD and RD x2 doses on soil carbon mineralization were similar to control and no significant difference was found between them. Higher doses of indaziflam (RD x4, RD x8 and RD x16) stimulated mineralization of soil carbon and there were found significant differences between control and these doses ($P<0.05$). All application doses of herbicide showed variability in ammonium (NH_4-N) and nitrate (NO_3-N) contents while there were generally found no significant differences between control and RD. In general, contents of soil NH_4-N and NO_3-N were increased in all applications as time passed and there were significant differences between days that were measured of these contents ($P<0.05$). Results of soil nitrogen mineralization rate were as following: 1) It was significantly decreased by only RD x2 on 11th day ($P<0.05$) 2) Higher doses of Indaziflam (RD x4, RD x8 and RD x16) significantly stimulated it on 26th day ($P<0.05$) 3) All doses of this herbicide significantly decreased it on 42nd day ($P<0.05$). In conclusion, the recommended field dose of Indaziflam had no negative effect on microorganisms that play an active role in soil carbon and nitrogen mineralization.

ARTICLE HISTORY
Received
11 June 2020
Accepted
15 September 2020

KEY WORDS
Indaziflam,
mineralization,
carbon,
nitrogen,
herbicide

Introduction

The global area under vines and total production of grapevine were reported as 7.400.000 ha and 77.800.000 tonnes in world in 2018 [1]. Turkey takes place as fifth in world countries in the production area of vineyard (4.170.410 da) and total grape

[1] Cukurova University, Faculty of Arts and Sciences, Department of Biology, Adana / Turkey
[2] Cukurova University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Adana / Turkey
[3] Cukurova University, Central Research Laboratory, Adana / Turkey
[4] Osmaniye Korkut Ata University, Faculty of Science and Arts, Department of Biology, Adana / Turkey
*Corresponding Author: Burak Koçak, e-mail: bkocak@cu.edu.tr

(3.933.000 tonnes) [1, 2]. It was noted that grape export is an important income source for the economy of a country [3].

Weed control and elimination in and between grapevine rows by chemicals were more widespread applied as the intensification of viticulture was increased [4]. Vines were competed by weeds for water and nutrients in soils and many pathogens causing diseases in weeds by hosting can cause loss in productivity in grapes at 10.1 % [3]. Prevention of trunk damage by mechanical weeding machines and decrease of working time spent in the vineyard were provided by herbicides [5]. Herbicides have become the most important control method for weed while these chemicals were more considered than other methods due to their easy application and being effective and reliable in weed control [6]. Amount of herbicides that was applied in agricultural fields was reported as 12.644 tonnes and this was 24.6% of total pesticides that were applied in Turkey in 2019 [7]. While the impacts of fungicides and insecticides on soil organisms that were applied in vineyards, it was reported that there is a little knowledge about the effects of herbicides on these organisms [8]. In addition to that, various non-target effects of herbicide on soil microorganisms were found in laboratory and pot studies [9, 10].

Indaziflam is a pre-emergent herbicide that inhibits cellulose biosynthesis and used for perennial grasses and broadleaf weeds [11]. This herbicide has been mainly applied in perennial plants (orange, grape, apple and drupe trees), settlements and areas that were not used for plant production, public domains and forests [12]. Indaziflam can remain as a residue for a long time even in low doses as well as it is resistant to decompose for much longer (150 days) [13]. Researches in four soil samples in Europe and two soil samples in the United States of America (U.S.A) showed that this duration could be in between 22 and 176 days [12].

It was reported that indaziflam showed weak acid ability over soil pH 5.4 [11, 13]. In addition, it was highlighted that indaziflam was non-volatile and could be fade away through decomposition and leaching [11]. It was found that mobility of indaziflam in soil was low and mid-level and sorption of this herbicide in six Brazil oxisol and in three U.S.A. mollisol soils showed positive correlation with organic carbon content [11]. It was indicated that the phytotoxic effects of indaziflam increased in soils containing low organic carbon [14]. Furthermore, it was pointed out that damage of indaziflam to hybrid bermuda grass grown in sandy soil was higher than in silt loam soil

[15]. Finally, it was suggested that the effects of increasing doses of Indaziflam on soil microbial activity should be determined [12].

It was hypothesized that higher doses of Indaziflam would decrease soil microbial activity. The objective of this study was to evaluate the effects of recommended field dose (RD) and its 2 (RD x2), 4 (RD x4), 8 (RD x8) and 16 (RD x16) folds of indaziflam on carbon and nitrogen mineralizations in a vineyard soil.

Material and Methods

Material and study area

Soils used in this study were sampled at 0-10 cm depth from Cukurova University Faculty of Agriculture Vineyard (Adana, Turkey) in May 2018. Indaziflam containing herbicide ($C_{16}H_{20}FN_5$, molecular weight: 301.369 g/mol, 500 g/ 1000 ml active ingredient) was bought commercially and its recommended field dose was 10 ml/da.

Some soil physical and chemical properties

Soils were mixed homogenized and considered as a composite and representative sample and then sieved a 2 mm mesh sieve, plant debris was removed. Soil texture was determined by Bouyoucos hydrometer, field capacity (%, FC) by 1/3 atmospheric pressure with a vacuum pump, pH by a 1:2.5 soil-water suspension with pH-meter (inoLab pH/Cond 720, WTW GmbH, Weilheim, Germany) and $CaCO_3$ content (%) by a Scheibler calcimeter [16]. Organic carbon and total nitrogen (TN) contents of soils (%) were determined by the modified Walkley and Black method and Kjeldahl method, respectively [16]. The determination of soil organic carbon is based on the Walkley & Black chromic acid wet oxidation method. Oxidizable organic carbon in the soil is oxidised by potassium dichromate ($K_2Cr_2O_7$) solution in concentrated sulfuric acid. The determination of total nitrogen in soil is based on digestion of the dried and homogenised soil in a suitable Kjeldahl tube with sulfuric acid. To rise the temperature, potassium sulfate is added and copper sulfate is used as a catalyst. After adding sodium hydroxide to the digestion solution the produced ammonium from all nitrogen species is evaporated by distillation as ammonia. This is condensed in a conical flask with boric acid solution. The amount is titrated against Tashiro's indicator with sulfuric acid [16]. Three replicates were used for each analysis.

Soil carbon and nitrogen mineralizations

Based on the calculation of soil volume weight (1.28 g/cm^3) and introduction of herbicide to the soil at 1 mm depth, recommended field dose of herbicide containing Indaziflam (RD, 10 ml/da) and 2 (RD x2), 4 (RD x4), 8 (RD x8) and 16 (RD x16) folds of RD were mixed with soil. Soils untreated with Indaziflam were used as control.

Soil mixtures (100 g soil+herbicide) were placed in 750 ml incubation vessels and the final moisture content of soils was adjusted to 80% of their own field capacity before incubation at 28 °C over 42 days for carbon mineralization. CO_2 produced from the microbial activity was absorbed periodically in 10 ml saturated 1 M NaOH solution in beakers, which were placed on the top of the soil in incubation vessels. Microbial respiration was measured by titration with 1 M HCl in these closed vessels in the following days of incubation: 1, 3, 7, 14, 28 and 42 [17]. Three replicates were used for each treatment and control. Cumulative carbon mineralization (mg CO_2-C/100 g soil) was calculated by summing up all measured days CO_2 until the end of incubation period while their rates at 42nd day were calculated by dividing cumulative mineralized carbon by its soil organic carbon [18].

Soil samples (100 g) mixed with herbicide were humidified at 80% of soil field capacity, placed in 750 ml incubation vessels and incubated for 42 days at 28°C for nitrogen mineralization. Three replicates were used for each treatment and control. These vessels covered with gauze for aeration were weighed three times every week to determine any weight loss. Distilled water was added when necessary to maintain soil moisture for 42 days. Ammonium and nitrate contents (NH_4-N + NO_3-N) were measured in soils to calculate nitrogen mineralization rate at 11th, 26th and 42nd days of incubation. All soil samples were mixed separately with 200 ml 1 N $CaCl_2$ solution and shaken for 1 h strained samples were distilled to measure mineral nitrogen by the Parnas-Wagner method [19, 20]. Nitrogen mineralization rate was calculated by dividing the total amount of mineral nitrogen by total nitrogen of soil [21].

Statistical analysis

Statistical analyses were performed by the software SPSS v.20. The data were submitted to ANOVA to assess the differences among treatments and incubation days. The separation of means was made according to the Tukey honestly significant difference (HSD). Differences between the data were declared as significant at $P < 0.05$.

Results

Soil analysis

Some physical and chemical properties of soil sampled from the vineyard were summarized in Table 1 below. The soil was loamy and slightly alkaline while field capacity and CaCO3 contents of soil were 24.48% and 43.38%, respectively. Soil organic carbon and nitrogen contents were 1.97% and 0.15% respectively while C/N was determined 12.86.

Table 1 Some physical and chemical properties of soil samples

Clay (%)	25.10 ± 0.06
Silt (%)	30.83 ± 0.09
Sand (%)	44.07 ± 0.03
Texture	Loam
FC (%)	24.48 ± 0.16
pH	7.98 ± 0.03
$CaCO_3$ (%)	43.38 ± 0.24
C (%)	1.97 ± 0.02
N (%)	0.153 ± 0.001
C/N	12.86 ± 0.11

Results are presented as means ± standard error of triplicate analysis (FC: field capacity)

Soil CO_2 evolution

Cumulative carbon mineralization of soils (mg $C(CO_2)$/100 g soil) for 42 days were as following: 55.57 for control, 55.16 for RD, 54.91 for RD x2, 58.02 for RD x4, 58.85 for RD x8 and 66.85 for RD x16 (Figure 1). There were found no significant differences between control, RD and RD x2. RD x4, RD x8 and RD x16 significantly increased soil microbial respiration compared to control ($P<0.05$).

Carbon mineralization rates (%) were determined as at the end of incubation period as following: 2.82 for control, 2.80 for RD, 2.79 for RD x2, 2.95 for RD x4, 2.99 for RD x8 and 3.40 for RD x16 (Figure 2). No significant differences were found between control and both RD and RD x2. There were only found significant differences between control and higher doses of herbicide (RD x4, RD x8 and RD x16) in rate of carbon mineralization.

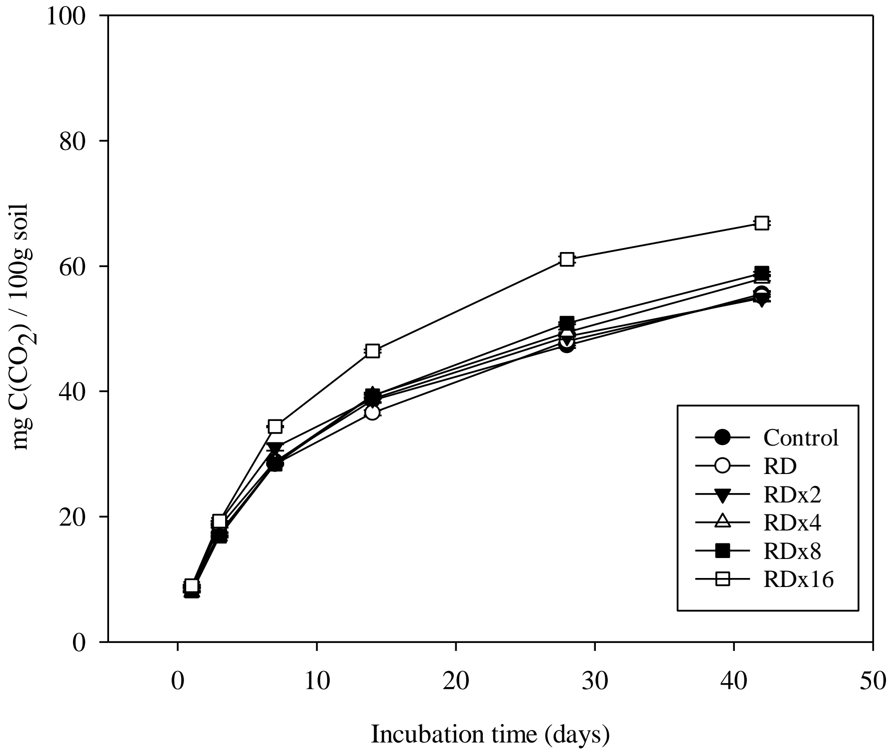


Fig 1 Cumulative carbon mineralization of soils during 42 days at 28ºC (mean ± standard error, mg $C(CO_2)$ /100 g soil, n=3)

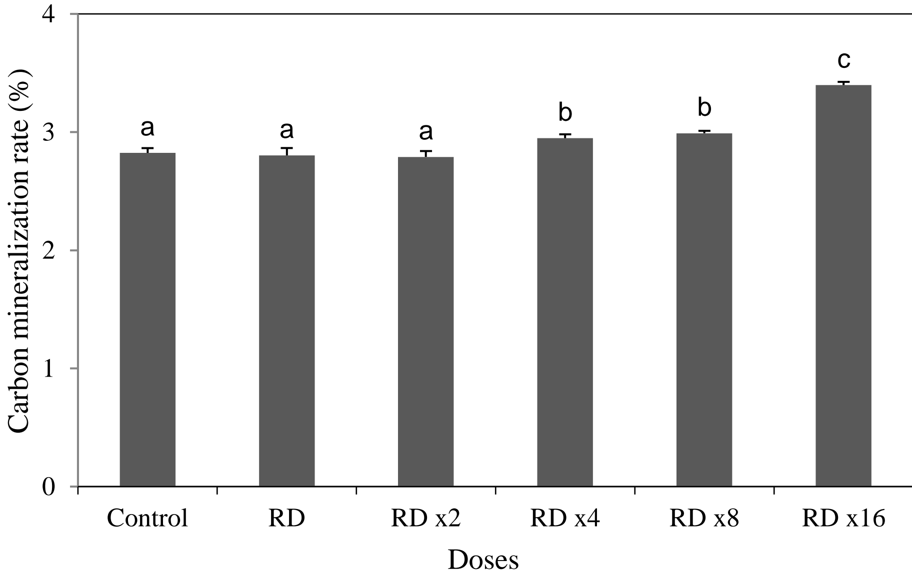


Fig 2 Soil carbon mineralization rates (mean ± standard error, %, n=3, a, b and c indicate significant differences between means)

Soil NH_4-N and NO_3-N contents and nitrogen mineralization rates

Soil NH_4-N and NO_3-N contents (mg/kg) were summarized in Table 2 and were as following: NH_4-N contents were in between 7.92 (RD x16) and 23.97 (Control) while NO_3-N contents were in between 3.92 (Control) and 15.07 (Control) in all measured days.

NH_4-N content results were on 11th day as following: Control was significantly higher than higher doses of herbicide except RD ($P<0.05$). The highest decrease was determined in RD x16 dose and this dose decreased NH_4-N content for 38.1% compared to control. In contrast, NO_3-N contents on 11th day in all herbicide doses were higher than control and there were found only significant differences between control and higher doses (RD x4, RD x8 and RD x16, $P<0.05$). The highest increases in NO_3-N contents were obtained at RD x4 and RD x16 for 106.2% and 97.2% compared to control, respectively (Table 2).

Herbicide doses generally increased mineral nitrogen contents (NH_4-N and NO_3-N) on 26th day as following: there was an only significant difference between control and RD x8 in NH_4-N contents while this increase was 33.3% compared to control ($P<0.05$). On the other hand, RD x4, RD x8 and RD x16 significantly increased NO_3-N contents ($P<0.05$) while these contents were highest increased by RD x16 for 165.5% compared to control (Table 2).

All doses of herbicide decreased soil NH_4-N and NO_3-N contents at the end of incubation (42nd day) as following: Significant differences were found between control and all doses except RD in NH_4-N contents while there were between control and RD and RD x4, separately ($P<0.05$). The highest decrease rates were obtained at RD x16 for 26.4% in NH_4-N contents and at RD for 61.1% in NO_3-N contents compared to control on 42nd day.

Soil NH_4-N and NO_3-N were generally increased in all treatments as time progressed as following: 11th day<26th day<42nd day. There were significant differences in NH_4-N contents of all treatments between 11th and 26th days ($P<0.05$). However, only significant differences were found between 26th and 42nd day in NH_4-N contents of control, RD x4 and RD x8. In contrast, differences between 11th and 26th days in nitrate contents of all treatments except RD x16 were non-significant while differences between 26th and 42nd days in nitrate contents of all treatments except RD x4 were found

significant (P<0.05). There were significant differences between 11th and 42nd days in ammonium contents of all treatments and nitrate contents of all treatments except RD x4 (P<0.05).

Table 2 Soil NH_4-N and NO_3-N contents (mg/kg) on 11th, 26th and 42nd days

	NH_4-N (mg/kg)						NO_3-N (mg/kg)					
	11. Day		26. Day		42. Day		11. Day		26. Day		42. Day	
Control	12.79	abz	17.72	by	23.97	ax	3.92	cy	4.05	dy	15.07	ax
	(0.71)		(1.07)		(0.18)		(0.03)		(0.37)		(0.33)	
RD	14.97	ay	18.15	bxy	23.96	ax	4.20	cy	5.03	dy	9.21	bx
	(0.97)		(0.07)		(1.48)		(0.01)		(0.33)		(0.30)	
RD x2	8.53	cy	16.99	bx	19.51	bcx	4.54	cy	6.01	cdy	13.71	ax
	(0.40)		(0.23)		(1.19)		(0.12)		(0.80)		(0.83)	
RD x4	9.75	bcz	18.01	by	19.44	bcx	8.09	ax	8.60	bcx	9.22	bx
	(0.25)		(0.07)		(0.22)		(0.22)		(0.35)		(0.02)	
RD x8	9.54	cz	23.62	ay	19.07	bcx	6.09	by	7.99	aby	14.16	ax
	(0.04)		(0.33)		(0.05)		(0.09)		(0.47)		(0.49)	
RD x16	7.92	cy	18.75	bx	17.65	cx	7.74	az	10.74	ay	13.12	ax
	(0.44)		(0.23)		(0.07)		(0.42)		(0.26)		(0.43)	

Results are presented as means ± standard error of triplicate analysis. Significant differences between measurement days were indicated in the same column as x, y and z and in the same line as a, b, c and d between treatments (P<0.05)

Rates of nitrogen mineralization in all treatments were given in Figure 3. These rates were between 0.85 % (RD x2 on 11th day) and 2.55% (Control on 42nd day). Only RD x2 significantly decreased this rate on 11th day compared to control (P<0.05). All treatments except RD and RD x2 doses significantly increased this rate on 26th day (P<0.05). At the end of incubation period, all herbicide doses significantly reduced nitrogen mineralization rate (P<0.05).

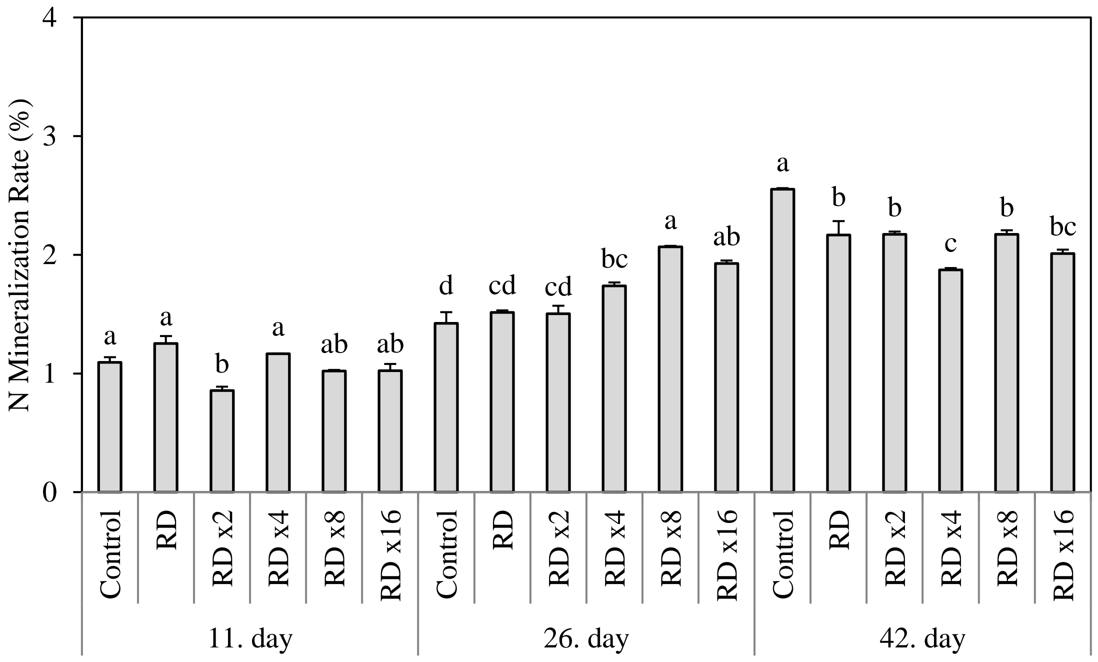


Fig 3 Soil nitrogen mineralization rates (%, means ± standard error, a, b and c indicate significant differences between means for each day separately, n=3)

Discussions

It was known that microbial activity stimulate the decomposition of soil organic matter and influence the soil nutrient dynamics. Therefore, this process in the solid phase of soil has been called nutrient mineralization [22]. Microorganisms in soil have a key role in ecosystem functions like suppression of pathogens, production of phytohormones, decomposition of pesticides including herbicides, bioremediation, application of wastewater, stimulation of plant growth [23, 24]. In general, adaptations of microorganisms in soil to exposition of agricultural herbicides occur in 3 ways: 1) increase in the metabolization of these pesticides by microorganisms [25, 26], 2) increase in negative effects on soil biota, 3) no effect on vital processes in soil biota [27-29].

Recommended field dose of herbicide containing indaziflam was similar with control in soil carbon mineralization and differences between control and these doses were non-significant. These results indicated that these doses of herbicide had no positive or negative effect on soil microbes. In contrast, higher doses of indaziflam (RD x4, RD x8 and RD x16) clearly and significantly increased soil carbon mineralization. It was

possible to conclude that soil microorganisms could use these herbicide doses for energy source and their activity.

Soil microorganisms can decompose natural and synthetic organic compounds while their decomposition products may increase or decrease microbial activity [30, 31]. As a result, when any environmental change like the application of herbicides for weed control occurred, this can affect soil carbon cycling by altering metabolic activity and community structure [22]. It was reported that soil microorganisms that were not affected by different soil moistures could use imazamox (a herbicide) as both carbon and nitrogen source and recommended field dose and its 2 fold dose of this herbicide were found similar with control in soil carbon mineralization [32]. In another study, it was noted that recommended field dose, its 2 and 4 folds of another herbicide named glyphosate-amine salt had no negative or positive effect on soil carbon mineralization compared to its non-herbicide exposed soil [33].

Diversity of microorganisms is in a wide range in soil ecosystem and therefore they were classified as photolithotrophic, photoorganotrophic, chemolithotrophic and chemoorganotrophic based on nutritional status. Phototrophic microorganisms (photolithotrophic and photoorganotrophic) were found rare in soil but chemotrophic microorganisms can be found in a wide range and numbers. All fungi species are chemoorganotrophic and can use organic molecules for carbon and energy source while bacterial and actinomycetes are originated from different groups. Nitrification (conversion of NH_4 to NO_3) and biological nitrogen fixation were maintained by chemolithotrophic organisms and these organisms can use CO_2 as a carbon source and obtain energy from the oxidation of inorganic compounds [34].

Most microorganisms play an active role in nitrification are chemolithotrophic and they can show the metabolic decomposition of herbicides [35]. It was indicated that when glyphosate and glufosinate (herbicides) applied in two different soils (haplustox and quartsmanet), NO_3-N contents were decreased in quartpsament soil but had no change in haplustox soil [36]. The reason of this decrease was explained by the higher amounts of clay and organic matter in quartpsament soil than haplustox soil [36]. Same authors reported that the avaliability of NH_4-N to NO_3-N microorganisms were lower in haplustox soil than quatzpsament and this decrease was caused by higher ion absorption in soil colloidal fraction [36]. In addition, it was found that glyphosate had a great

interest in iron and aluminium oxides that are common in oxisol soils [37]. Therefore, it was reported that this strong connection between glyphosate and these compounds decreased the bioavailability of glyphosate for microorganisms [35]. A decrease in nitrification may indicate a damaging factor for soil microbiota while an increase in conversion of ammonium to nitrate may cause nitrate contamination in soil and underground waters. It is important to indicate that these events can decrease the amount and availability of this nutrient [35].

Effects of recommended field dose of indaziflam (RD) on soil ammonium and nitrate contents with nitrogen mineralization rates were generally similar with control for 42 days of laboratory incubation in this study. It was possible to conclude that RD dose had no negative or positive effect on soil microorganisms that play an active role in ammonification and nitrification. In contrast, higher doses of indaziflam generally decreased ammonium contents but increased nitrate contents in this study. Mineral nitrogen contents were generally increased in all treatments as time progressed (11th day<26th day<42nd day). In general, indaziflam had no negative or positive effects on the soil nitrogen mineralization rate on 11th day. In contrast, higher doses of indaziflam (RD x4, RD x8 and RD x16) on 26th day significantly increased but all treatments on 42nd day decreased nitrogen mineralization rate ($P<0.05$).

It was reported that applications of recommended field dose and its 2 and 4 folds of imazamox (a herbicide) showed similarities with control in ammonium contents while increasing doses of imazamox decreased soil nitrate production [32]. Authors in the same research indicated that nitrate producing bacteria in soils were sensitive to addition of imazamox into soil [32].

Temporal results in nitrogen mineralization can be differed based on soil type. It was noted that mixation of a soil sampled from Adana (Turkey) with potassium bichromate at soil Cr levels increased in NH_4-N and NO_3-N contents after 42 days of incubation as following: 11th day< 26th day < 42nd day [38]. It was suggested that chromium affected bacteria that take role in nitrogen mineralization [38]. In contrast, it was indicated that NH_4-N contents and nitrogen mineralization of gypsum, marl and serpentine soils were higher on 11th day than 26th and 42nd days becuase of the availability of biodecomposable organic matter [39].

Conclusions

Recommended field dose of herbicide containing indaziflam had generally no negative effect on soil carbon and nitrogen mineralizations in this study. Higher doses of this herbicide (RD x4, RD x8 and RD x16) stimulated carbon mineralization after 42 days of incubation. In general, all herbicide doses had generally no negative or positive effect on soil nitrogen mineralization rate on 11th day but it was positive on 26th day. In contrast, all doses of herbicide had negative effect on soil ammonium and nitrate producing microorganims on 42nd day.

Funding

Funding was received from Cukurova University Scientific Research Projects Department (Project number: FBA-2018-10572) and all authors thank this department for their contribution for this study.

References

1. OIV Statistical report on world vitiviniculture. International Organisation of Vine and Wine. 2019. Available from: http://www.oiv.int/public/medias/6782/oiv-2019-statistical-report-on-world-vitiviniculture.pdf
2. TUIK. Meyveler, içecek ve baharat bitkilerin üretim miktarları (Seçilmiş ürünlerde), 2001-2019. 2019.
3. Kaçan, K. and Ö. Boz, Ege bölgesi geleneksel ve organik bağ alanlarında yabancı ot tür yoğunluk ve rastlama sıklıklarının belirlenmesi ve karşılaştırılması. Ege Üniversitesi Ziraat Fakültesi Dergisi, 2015. 52(2): p. 169-179.
4. Keller, M., The science of grapevines. 2015, 2 edn. Elsevier, Oxford
5. Mandl, K., et al., Effects of glyphosate-, glufosinate- and flazasulfuron-based herbicides on soil microorganisms in a vineyard. Bulletin of Environmental Contamination and Toxicology, 2018. 101: p. 562–569.
6. Kraehmer, H., et al., Herbicides as Weed Control Agents: State of the Art: I. Weed Control Research and Safener Technology: The Path to Modern Agriculture. Plant Physiology, 2014. 166(3): p. 1119-1131.
7. TUIK. Tarımsal ilaç kullanımı, 2006-2019. 2019.
8. Zaller, J.G., et al., Herbicides in vineyards reduce grapevine root mycorrhization and alter soil microorganisms and the nutrient composition in grapevine roots, leaves, xylem sap and grape juice. Environmental Science and Pollution Research, 2018. 25: p. 23215–23226.
9. van Hoesel, W., et al., Single and combined effects of pesticide seed dressings and herbicides on earthworms, soil microorganisms, and litter decomposition. Frontiers in Plant Science, 2017. 8:215.
10. Zaller J.G., et al., Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. Scientific Reports, 2014. 4:5634.
11. Alonso, D.G., et al., Sorption-Desorption of Indaziflam in Selected Agricultural Soils. Journal of Agricultural and Food Chemistry, 2011. 59(24): p. 13096-13101.
12. Gonzalez-Delgado, A.M., et al., Effect of application rate and irrigation on the movement and dissipation of indaziflam. Journal of Environmental Sciences, 2017. 51: p. 111-119.
13. EPA U. S., Pesticide fact sheet, indaziflam. 2010.

14. Jones, P.A., et al., Effect of Reed-Sedge Peat Moss on Hybrid Bermudagrass Injury with Indaziflam and Prodiamine in Sand-Based Root Zones. Weed Technology, 2013. 27(3): p. 547-551.
15. Jones, P.A., et al., Soil Type and Rooting Depth Affect Hybrid Bermudagrass Injury with Preemergence Herbicides. Crop Science, 2013. 53(2): p. 660-665.
16. Kacar, B., Toprak Analizleri. Vol. 484. 2012, Ankara: Nobel Akademik Yayıncılık.
17. Alef, K., Soil Respiration, in Methods in Applied Soil Microbiology and Biochemistry, K. Alef and P. Nannipieri, Editors. 1995, Academic Press Inc.: New York. p. 214-215.
18. Koçak, B., Atrazine ve Glyphosate'ın Toprak Karbon Mineralizasyonuna Olan Etkileri. International Journal of Life Sciences and Biotechnology, 2020. 3: p. 108-116.
19. Lemee, G., Investigations sur la Mineralisation de L'azote et son Evolution Annuelle Dans des Humus Forestiers in Situ. Oecıologia Plantarum, 1967. 2: p. 285-324.
20. Sağlam, M.T., Toprakta Mevcut Bazı Azot Formlarının Tayini Ve Azot Elverişlilik İndeksleri Vol. 18. 1979, Erzurum: Atatürk Üniversitesi Ziraat Fakültesi Yayınları.
21. Aka, H. and C. Darici, Carbon and nitrogen mineralization in carob soils with Kermes oak and Aleppo pine leaf litter. European Journal of Soil Biology, 2005. 41(1-2): p. 31-38.
22. Mendes, K.F., et al., Glucose mineralization in soils of contrasting textures under application of S-metolachlor, terbuthylazine, and mesotrione, alone and in a mixture. Bragantia, 2018. 77(1): p. 152-159.
23. Bottomley, P.J., Microbial Ecology, in Principles and Applications of Soil Microbiology, D.M. Sylvia, et al., Editors. 2005: New Jersey. p. 463-488.
24. Saleem, M. and L.A. Moe, Multitrophic microbial interactions for eco- and agro-biotechnological processes: theory and practice. Trends in Biotechnology, 2014. 32(10): p. 529-537.
25. Reis, M.R., et al., Atividade microbiana em solo cultivado com cana-de-açúcar após aplicação de herbicidas. Planta Daninha, 2008. 26: p. 323-331.
26. Hussain, S., et al., Bioremediation and Phytoremediation of Pesticides: Recent Advances. Critical Reviews in Environmental Science and Technology, 2009. 39(10): p. 843-907.
27. Pereira, J.L., et al., Effects of glyphosate and endosulfan on soil microorganisms in soybean crop. Planta Daninha, 2008. 26: p. 825-830.
28. Mahia, J., et al., Microbial biomass and C mineralization in agricultural soils as affected by atrazine addition. Biology and Fertility of Soils, 2008. 45(1): p. 99-105.
29. Blume, E. and J.M. Reichert, Banana leaf and glucose mineralization and soil organic matter in microhabitats of banana plantations under long-term pesticide use. Environmental Toxicology and Chemistry, 2015. 34(6): p. 1232-1238.
30. Schmidt, M.W.I., et al., Persistence of soil organic matter as an ecosystem property. Nature, 2011. 478(7367): p. 49-56.
31. Hussain, S., et al., Impact of Pesticides on Soil Microbial Diversity, Enzymes, and Biochemical Reactions. Advances in Agronomy, Vol 102, 2009. 102: p. 159-200.
32. Aka Sagliker, H., et al., Effects of Imazamox on Soil Carbon and Nitrogen Mineralization under Two Different Humidity Conditions. Ekoloji, 2014. 23(91): p. 22-28.
33. Sagliker, H.A., Carbon Mineralisation in Orange Grove Soils Treated with Different Doses of Glyphosate-Amine Salt. Journal of Environmental Protection and Ecology, 2018. 19(3): p. 1102-1110.
34. Moreira, F.M.S. and J.O. Siqueira, Microbiologia e bioquímica do solo, ed. UFLA. 2006: Universidade Federal de Lavras (UFLA). 729.
35. Damin, V. and P. Trivelin, Herbicides Effect on Nitrogen Cycling in Agroecosystems, in Herbicide and Environment, A. Kortekamp, Editor. 2011, InTech: Rijeka, Croatia.
36. Damin, V., P.C.O. Trivelin, and T.G. Barbosa, Mineralização do nitrogênio da stubble de milheto dessecado com herbicides. Revista Brasileira de Ciência do Solo, 2009. 33: p. 925-934.

37. Prata, F., et al., Influência da matéria orgânica na sorção e dessorção do glifosato em solos com diferentes atributos mineralógicos. Revista Brasileira de Ciência do Sol, 2000. 24(4): p. 947-951.
38. Kızıldağ, N., Doğu Akdeniz Bölgesindeki Krom Maden Alanı Topraklarında Azot Mineralizasyonu. Toprak Su Dergisi, 2017. 6(1): p. 52-57.
39. Sagliker, H.A., et al., Is parent material an important factor in soil carbon and nitrogen mineralization ? European Journal of Soil Biology, 2018. 89: p. 45-50.

Research article

Hamid, T.H.T.A. and N.F.A.M. Fuzi, Lactic Acid Bacterium with Antimicrobial Properties from Selected Malay Traditional Fermented Foods International Journal of Life Sciences and Biotechnology, 2021. DOI: 10.38001/ijlsb.781522

Lactic Acid Bacterium with Antimicrobial Properties from Selected Malay Traditional Fermented Foods

Tengku Haziyamin Tengku Abdul Hamid[1*], Nur Fatin Amysya M. Fuzi[1]

ABSTRACT

Traditional or local fermented foods have been the favourite sources for Lactic acid bacteria (LAB) used for starter cultures. Traditional food such as fermented cassava '*tapai ubi*', fermented glutinous rice '*tapai pulut*' and fermented shrimp paste '*belacan*' have been local heritage consumed as Malay delicacies. In this work, 33 LAB samples were isolated from *tapai ubi, tapai pulut and belacan* and out of these only 5 LAB isolates (PG, PH, BG, UG and UL) showed inhibitory properties against selected indicator organisms (*Bacillus subtilis, Escherichia coli, Salmonella typhimurium,* and *Staphylococcus aureus*). Morphologically, isolate PG, PH, BG are cocci, UL is rod and UG is coccobacillus. Biochemically, isolates (PG, PH, BG UL and UG) are found to be Gram positive, non motile, lactose fermenter and catalase negative. The 16s Ribosomal RNA gene sequencing was carried out and each was identified with an accession number (PB: MT645488, PH: MT645489; BG: MT645490 UG: MT645491 and UL MT645492). Isolates PG and PH from *tapai pulut* belonged to *Pediococcus pentosaceus* (at 99% and 98%, respectively). Meanwhile, isolate from *Belacan* BG belonged to *Enterococcus faecium* (99%), and those from fermented shrimp, UG and UL belonged to *Weissella confusa* (99%), *and Lactobacillus fermentum* (98%), respectively. Majority of the isolates demonstrated broad spectrum inhibition against both Gram positive and negative indicator strains. Compared to the rest of isolates, PH exhibited the highest antibacterial activity against *Bacillus subtilis.* These results suggested that isolate PH are the most potent isolates which is producing antimicrobial agent with potential as food preservatives.

ARTICLE HISTORY
Received
17 August 2020
Accepted
15 September 2020

KEYWORDS
Lactic acid bacteria, malay traditional food, bacterial antagonism

Introduction

Recent consumer awareness and perception have renewed interest in the consumption of healthy food; food with no additives or preservatives; or so called 'natural' or 'traditional' foods. The consumption of fermented food can offer many benefits that it was suggested to be included in food consumption guides [1]. Fermented foods exist in many cultures and lactic acid bacteria (LAB), or yeast are the common starter cultures used in food fermentation aimed at enhancing the texture and flavour of the desired products [2].

[1] Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Kuantan Campus, Jalan Istana, Bandar Indera Mahkota, 25200, Kuantan, Pahang, Malaysia
*Corresponding Autor: Tengku Haziyamin Tengku Abdul Hamid[1] e-mail: haziyamin@iium.edu.my

Fermentation of carbohydrates produces product such as lactic acid which reduces the pH thereby creating condition unfavourable for the growth of harmful or food spoilage organism.

As a result of urbanisation and commercial demand, the production of traditional food need to up-scaled to ensure its benefit or relevance. However, the large scale production of traditional food is still a challenge. According to Anal et al [3], fermented food which is usually prepared in a small scale set up is subjected to safety risk, especially due to microbial contamination following bad agricultural practises. Therefore, the use of selected microflora or a more define starter cultures is highly recommended in minimizing this risk. For years, works have been carried out to isolate useful LAB in an attempt to screen for the best strain to be used as starter culture. Lactobacillus, Lactococcus, Streptococcus, Pediococus and Leuconostac are among the commonest group of LAB which have been used as starter cultures [4]. Despite LAB can be found widely in other non-food sources such as gastrointestinal tract of human or animals, fermented food was still considered the favourite target of many works on isolation for LAB. LAB produces ribosomally synthesized antimicrobial peptides known as bacteriocins which were considered as potential alternative to chemical food preservatives and antimicrobial drugs [5]. The diversity of LAB, and their target spectrum of their antimicrobial activities have made them interesting target for bacteriocin isolation.

There have been several studies on the isolation LAB from several types of Malay traditional foods. These include *tapai* (fermented tapioca), *tempoyak* (fermented durian flesh), chilli puree and fresh goat's milk [6]; fermented fish; Chili bo (chili puree) [7]; bambangan [8]; *pekasam* (fermented fish), *jeruk maman* (fermented vegetable), *tapai* (fermented glutinous rice) and *tempoyak* (fermented durian)[9]; and a local vinegar [10]. In one of our previous study, *Staphylococcus piscifermentans* was isolated from Cincaluk (Malaccan fermented shrimp) [11]. These studies indicated that different or diverse groups of LAB are continually being isolated even from similar food sources. Moreover, the same type of food may not be similarly prepared or following a standard ingredients. In this study, Malay traditional fermented food such “tapai pulut”, “tapai ubi” and “belacan” were selected as the sources for lactic acid bacterium (LAB). This LAB strains were tested for their probiotic properties and their ability to produce antimicrobial

activities against selected pathogenic strains. The properties of this isolates could make them suitable candidate to be used as starter culture in food fermentation.

Materials and Methods

Media and reagents preparation

Isolation and culturing of lactic acid bacteria (LAB) was carried out using De Man Rogosa and Sharpe (MRS) media. Pathogenic strains used as indicator bacterium *Bacillus subtilis, Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus* were grown in nutrient agar (NA). Nutrient agar (NA) media was also used for the antimicrobial activities. Pathogenic strains *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium* were obtained from the Kuliyyah of Sciences collection at International Islamic University Malaysia, Kuantan Campus.

Sample sources

Three types of Malay traditional food, *tapai pulut* (fermented glutinous rice), *tapai ubi* (fermented cassava) and *belacan* (fermented shrimp paste) were purchased from local fresh market in Kuantan. About 10 g of each food sample was added with 90 ml of peptone water. Samples were homogenised and serially diluted up to 1×10^{-3} with 0.1% peptone water. The diluted samples were vortexed and spread on (MRS) agar plates. The plates were incubated anaerobically in gas pack for 3 days (72 hours), at 37°C. New formed colonies were sub-cultured on new MRS agar and incubated anaerobically for 24 hours at 37°C, and the procedure was repeated until single colony was obtained for each isolate. The new LAB isolates were maintained in MRS broth with 40 % glycerol and stored at -80 °C

Identification of lactic acid bacteria (LAB)

Preliminary identification and characterization of selected isolates were based on lactose utilization test, Gram staining and catalase test. Carbohydrate fermentation was tested using bromocresol lactose agar which contained bromocresol purple (purple indicator) (0.025 g), peptone (5.0 g), beef extract (3.0 g), lactose (10.0 g), and nutrient agar (15.0 g) per 1 liter water. The gram staining were carried out on the isolates using standard protocols and examined under light microscope (Nikon) at 100X magnification (immersion oil).

Screening of antimicrobial activities of LAB

In this work, preliminary screening for inhibitory activities from LAB were detected using disk diffusion methods according to Kirby method [12] and *Bacillus subtilis, Eschericia coli, Staphylococcus aureus and Salmonella typhimurium* were used as indicator bacterium. The strains were grown in Mueller-Hinton broth until turbidity reached at 0.5 McFarland standard and spread evenly using sterile cotton swab on NA agar plates to make bacterial lawn. The test LAB isolates were incubated anaerobically in MRS broth medium at for 48 hours at 37°C. Filter paper disks (Whatman, 9.0 mm diameter) were loaded with 20 µl of fresh cultures and allowed to dry. The filter discs were then laid on NA plates pre-streaked with indicator strains with gentle press. Then, all of the plates were incubated at 37°C for 24 hours. Tetracycline was used as positive control while MRS broth was used as negative control. The antimicrobial activities of the selected strains were determined by inhibitory zones around the wells and diameter of zone inhibition was measured (in mm).

Cell free extraction (CFE) and agar well diffusion assay

Following disk diffusion method, inhibitory activities from cell free extract (CFE) were detected using agar well diffusion assay [13]. Each LAB was propagated in MRS broth and incubated at 37°C for 48 hours. The broths were centrifuged (12 000x g) for 5 minutes at 4°C. Collected supernatant was filtered (Millipore, 0.22 µm pore size) to form LABs' CFE. About 35 ml of molten NA was poured into 50 ml falcon tube and mixed with 30 µl of indicator strains. This mixture was then poured on sterile plate and left to solidify, in which holes were later punched on solidified agar using sterile glass dropper. Each well was filled with 70 µl of prepared CFE and incubated at 37°C for 24 hours. MRS broth was used as a negative control. Following incubation, the zone on inhibitions around the well were observed and measured (in mm).

Genotypic characterisation

To all five LAB strains, the genomic DNA were extracted (GF-1, Vivantis) according to manufacturer's protocols. The gene for 16S rRNA were amplified using a pair of universal primer (forward: 5' – AGA GTT TGA TCC TGG CTC AG – 3' and reverse 5' – CCG TCA ATT CCT TTG AGT TT- 3') [14]. PCR reaction mixture contained 25 µl of master mix, 3 µl of DNA template, 5 µl of each primer and made up to 50 µl reaction volume with distilled H_20. The PCR runs were as follows: Initial denaturation at 94°C (2

minutes), denaturation at 94°C (1 minute), annealing at 55°C (1 minute), and extension at 72°C (1 minute). The amplification was repeated in 25 cycles followed by a final extension at 72°C (7 minutes). In this work, the DNA concentrations were checked using Nanodrop Spectrophotometer (NanoDropTM 2000, USA). The gel was visualised using 1% agarose gel electrophoresis and viewed using UV transiluminator (AlphaImager). The PCR products were electrophoresed and purified (Clean-Up kit,Vivantis) and sent to a sequencing agency (Apical scientific Sdn. Bhd.). The generated sequences were analysed using BLASTn which available at NCBI website (*http://blast.ncbi.nlm.nih.gov*). Selected sequences were aligned using ClustalX2, and MEGA 6 software were used to build up a phylogenetic tree. The 16S rRNA gene sequences of representative LAB strains were analysed using Neighbour-joining methods with bootstrap values based on 500 replications.

Results and Discussion

Morphological and biochemical characterisations

From 33 colonies, only five isolates (PG, PH, BG, UG and UL) were able to show lactose utilisation on MRS agar plate and these isolates were further subjected to biochemical, morphological and genotypic characterizations. As shown in **Fig 1**, the results of Gram staining, all isolates are gram positives in which three of the isolates (PG, PH and BG) are cocci. Meanwhile isolate UL appears rod-shaped and UG is coccobacillus (see to **Table 1**). The lactose fermentation was indicated by the changes in the colour of bromocresol dye from purple to yellow. Fermentation of lactose produces lactic acid as the main metabolite which in turn reduces the pH of media, a common feature exhibited by LAB [15]. LAB ferment sugar for carbon and energy sources while producing a variety of compounds such as organic acids, aromatic compounds and other substances beneficial to health. As shown in **Table 1**, all of these isolates were found to be catalase negative. Since LAB are adapted to anaerobic environment, they are lacking in hydrogen peroxide scavenging enzyme such as catalase [16]. Therefore, in catalase test there would be no bubble due to evolution of oxygen gas. Overall results from biochemical tests were consistent with other characterizations featured for LAB which are Gram positive, catalase negative, coccus, non-spore former, non-motile and anaerobic organisms.

Table 1 Morphological and biochemical tests on LAB

Characteristics	Lactic acid bacteria (LAB)				
	PG	PH	BG	UG	UL
Lactose test	+	+	+	+	+
Gram staining	+	+	-	+	-
Cell morphology	coccus	coccus	coccus	coccobacillus	bacillus
Catalase test	-	-	-	-	-

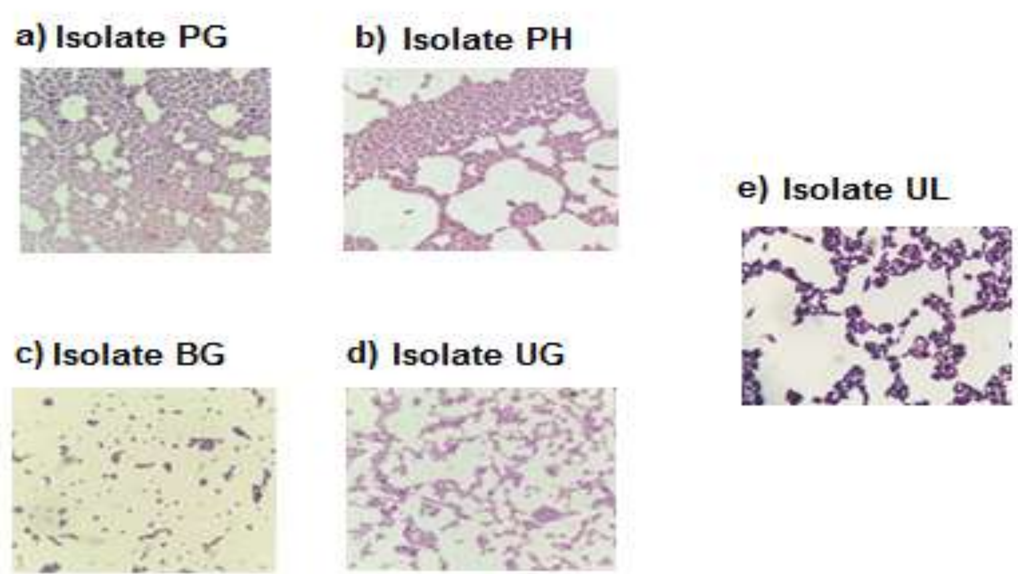


Fig 1 Morphologies of LAB isolates from Malay traditional food after Gram staining method, and viewed under light microscopic (Nikon, 100X magnification with oil immersion)

Antimicrobial activities of LAB

Based on disc diffusion assay, five isolates showed antimicrobial activities against Gram positive and Gram negative indicator bacterium (see **Table 2** and **Fig 2**). Both isolate PG and PH were able to show inhibitions against all indicator strains tested. Isolate PH showed strong inhibitions against almost all strains except a moderate inhibition against *S. thyphimurium.* Isolate BG was only active against gram negative strains (*E .coli* and *S. thyphimurium*). Meanwhile, Isolate UG and UL were lacking of inhibitory properties except against gram negative *E. coli.*

Table 2 Antimicrobial activity of the isolates against selected pathogenic bacteria using the disc diffusion assay

Pathogens	Inhibitory activities				
	PG	PH	BG	UG	UL
Bacillus subtilis	++	++	-	-	-
Eschericia coli	+++	+++	+++	++	+++
Salmonella typhimurium	+++	++	++	-	-
Staphylococcus aureus	++	+++	+	-	-

Sign denotes the degree of inhibition: '+' indicates low inhibition zone (0.9±05 mm); '++' moderate inhibition zone (1.0-1.2±05 mm); '+++' strong inhibition zone (1.3-1.6±05 mm); and '-' no inhibition zone.

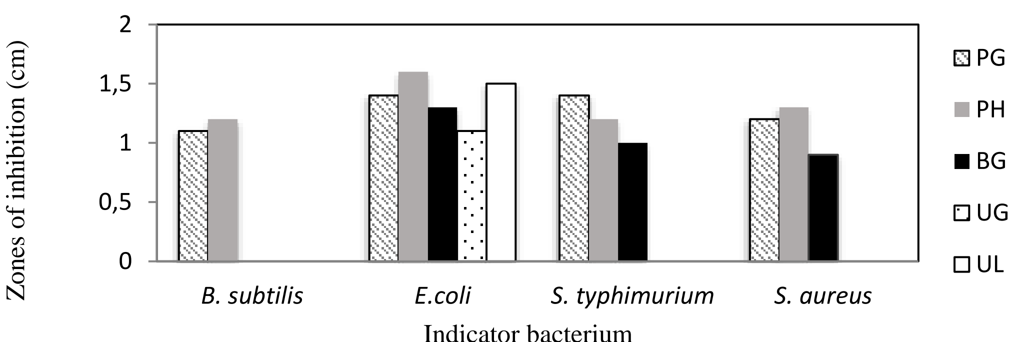


Fig 2 Zones of inhibition produced by LAB against selected pathogenic strains using agar disk diffusion methods

Table 3 Inhibitory activities using agar well diffusion assays

Pathogens	Inhibition activities				
	PG	PH	BG	UG	UL
Bacillus subtilis	+++	+++	+++	+++	+++
Eschericia coli	+++	+++	++	++	+++
Salmonella typhimurium	++	+++	+++	+++	++
Staphylococcus aureus	+++	++	++	-	-

Sign denotes the degree of inhibition: '+' indicates low inhibition zone (0.9±05 mm); '++' moderate inhibition zone (1.0-1.2±05 mm); '+++' strong inhibition zone (1.3-1.6±05 mm); and '-'no inhibition zone.

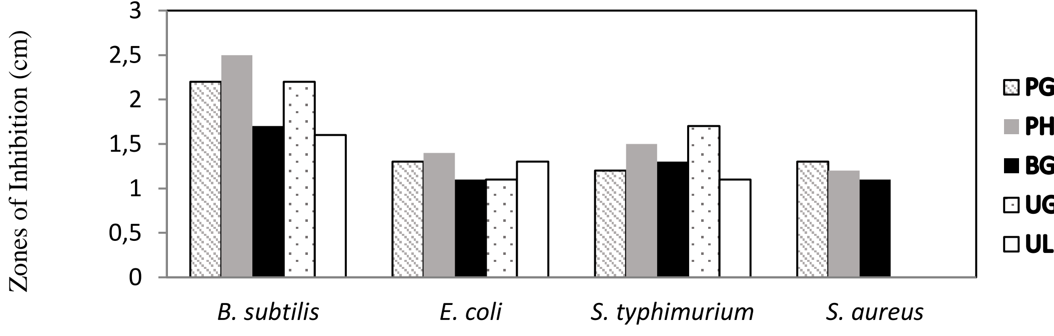


Fig 3 Zones of inhibition produced by LAB against selected pathogenic strains using agar well diffusion methods

Both agar disk diffusion and well diffusion methods were commonly used in studying antimicrobial activities. Due to its simplicity and cheap, disk diffusion method is commonly employed in antimicrobial screening work [17]. The trend of inhibition observed using disk diffusion method was however not necessarily replicated when tested in agar well diffusion method. In agar well diffusion assay (**Table 3** and **Fig 3**), all pathogenic strains showed inhibitions except for *Staphylococcus aureus* which showed resistance against isolates UG and UL. However, some of the zero inhibitions observed in disk diffusion method were able to shown inhibition in agar well method. Agar well diffusion methods were shown to be more sensitive than disk methods [18]. Disk diffusion method sometime produces smaller inhibition zones compared to agar well methods [19], and even the type of agar media used may affect its sensitivity [20]. The discordant we observed here were due to the phyco-chemical environment of these two methods and differences in the nature of inhibitory compounds produced by microorganism. For instance, inhibition due to low pH may be ineffective or not favourable when using disk diffusion method, a condition which is more effective for small organic or bacteriocin. Nevertheless, ability to show antagonisms is one of the desirable properties of LAB. Based on inhibitory strength and number of antagonised strains, the degree of antagonistic property among the isolates was in the order of PH > PG > BG > UG > UL. In term of spectrum of inhibition, Isolates PG, PH and BG are notable based on their ability to inhibit both Gram positive (*Bacillus subtilis, Staphylococcus aureus*) and Gram negative (*Escherichia coli and Salmonella typhimurium*) bacterium. Some of these inhibitions were due to the ability of LAB strains to produce bacteriocin or antimicrobial peptides.

Genotypic characterisations

The PCR amplification results for all isolates generated 1.5 kb fragment of 16s RNA (see **Fig 4**). Each of these sequence was assigned with an accession number (MT645488 for PB; MT645489 for PH; MT645490 for BG; MT645491 for UG; and MT645492 for UL) following gene submission at NCBI registery. The 16s ribosomal sequencing for the 5 LAB isolates indicated the following similarities: PG and PH with *Pediococcus pentosaceus* (at 99% and 98%, respectively); BG with *Enterococcus faecium* (99%), and UG with *Weisella confusa* (99%) and UL with *Lactobacillus fermentum* (98%), respectively. A phylogram was constructed as shown in **Fig 5,** incorporating the 16S

rRNA sequences of LAB strains from diverse genera such as Pediococcus, Lactobacillus, Enterococcus and Weisella.

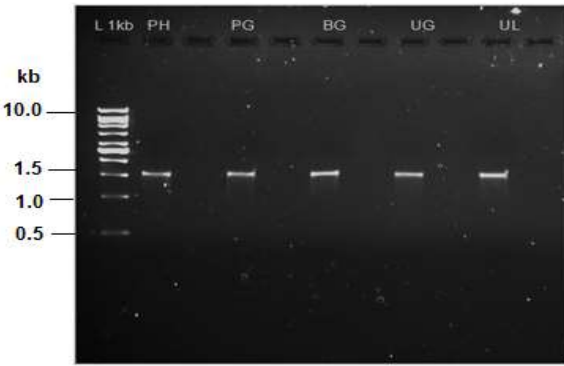


Fig 4 Ethidium bromide stained 1 % agarose gel displaying the bands of purified PCR amplification fragments product of 16s rRNA genes from LAB.

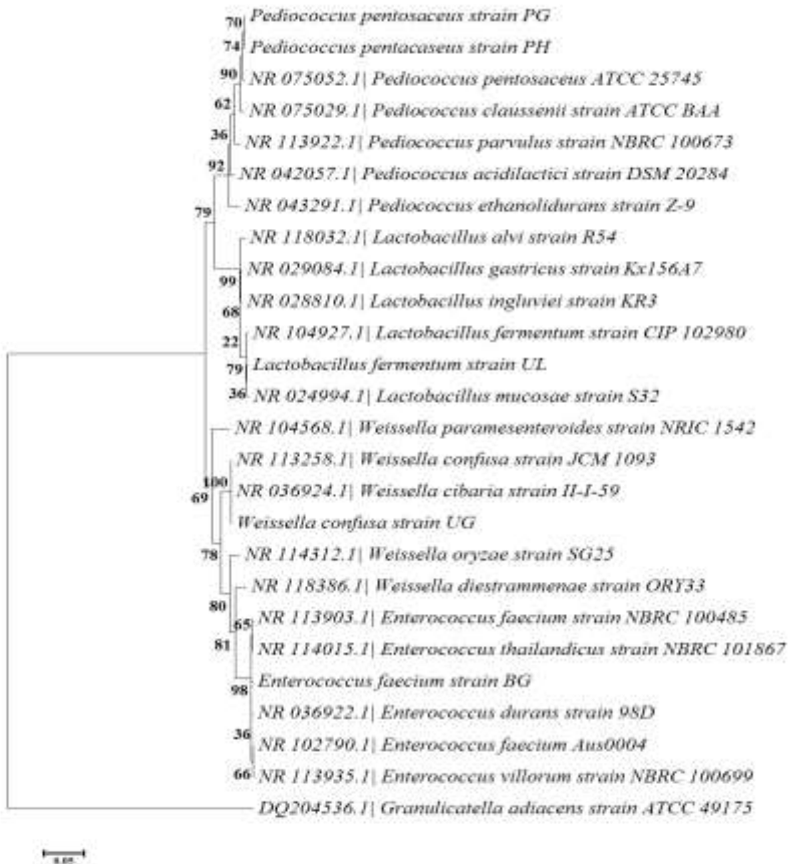


Fig 5 Phylogenetic tree was constructed using Neighbour-joining method (MEGA 6 software). The tree was constructed incorporating five 16S RNA sequences for five of the LAB isolates from Malay traditional fermented food (PG, PH, UL, UG and BG) and other 16s RNA sequences for LAB from other genera (Pediococcus, Lactobacillus, Enterococcus and Weissella). *Granulicatella adiacens* was selected as an out group in this tree construct.

All of the 5 isolates belonged common LAB isolated from various fermented foods throughout the world. For instance, *Pediococcus pentosaceus* were isolated from Kimchi (Korean fermented vegetable) [21], Korean sea food [22] and several Indonesian [23] and Tanzanian [24] fermented food. The isolation of *Lactobacillus fermentum* were common from fermented food product in many countries such as China [25, 26]; Turkey [27]; Tanzania [24] and Iran [28]. Diversity of LAB is notable as some of the strains could belong to opportunistic pathogen of which these were still being debated for being considered as probiotic. These include *Weissella confusa* [29] and nosocomial pathogen *Enterococcus faecium* [30]. *Weisella confusa* can be isolated from Indian fermented food [31]; Tanzanian [24] and Kimchi (Korea) [32]. Meanwhile, *Enterococcus faecium* are ubiquitous organism which can be isolated from cheese [33]; and other food sources [34].

Conclusion

In this study, five lactic acid bacteria strains designated as isolates PG, PH, BG, UG and UL have been successfully isolated from three types of traditional Malay food i.e., "tapai pulut", "tapai ubi" and "belacan". The phenotypic identifications indicated that these isolates showed typical properties of LAB with the abilities to antagonize selected pathogens. Antimicrobial activities studies showed varying degree of inhibition against pathogenic strains and most of these isolates showed broad spectrum inhibition. Based on 16S rRNA sequencing analysis, with more than 98% similarity; isolate PG was identified as *Pediococcus pentasaceus*, isolate UG as *Enterococcus faecium,* isolate UL *as Weissella confusa* and isolate BG as *Lactobacillus fermentum*. These isolates can be potential starter culture for traditional food preparation. Inhibitory studies may indicate that these isolates can be a potential sources for antibacterial agent such as bacteriocins which has potential to be used as natural bio-preservatives and in preventing the growth of spoilage pathogens in various food products.

Acknowledgements
The author wishes to thank to International Islamic University for facilities in completing this work.

Conflict of Inerest
The authors declare no conflict of interest.

Abbreviations
LAB: lactic acid bacteria, CFE: cell free extract, rRNA: ribosomal ribonucleic acid

References

1. Chilton, S.N., J.P. Burton, and G. Reid, Inclusion of fermented foods in food guides around the world. Nutrients, 2015. 7(1): p. 390-404.
2. Tamang, J.P., et al., Fermented foods in a global age: East meets West. Comprehensive Reviews in Food Science and Food Safety, 2020. 19(1): p. 184-217.
3. Anal, A.K., et al., Food safety risks in traditional fermented food from South-East Asia. Food Control, 2020. 109: p. 106922.
4. Altieri, C., Ciuffreda, E., Di Maggio, B. and Sinigaglia, M., Lactic acid bacteria as starter cultures, in Starter Cultures in Food Production. p. 1-15.
5. Perez, R.H., T. Zendo, and K. Sonomoto, Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microbial Cell Factories, 2014. 13(1): p. S3.
6. Mohd Adnan, A.F. and I.K. Tan, Isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential. Bioresour Technol, 2007. 98(7): p. 1380-5.
7. Leisner, J.J., et al., Identification of Lactic Acid Bacteria from Chili Bo, a Malaysian Food Ingredient. Applied and Environmental Microbiology, 1999. 65(2): p. 599-605.
8. Ng, S.Y., et al., Evaluation of probiotic potential of lactic acid bacteria isolated from traditional Malaysian fermented Bambangan (Mangifera pajang). CyTA - Journal of Food, 2015. 13(4): p. 563-572.
9. Siti Nasiroh, I.a.N.S., Azmi and Makky, Essam A. (2018) I), 7-9 August 2018 ,. Antibiotic susceptibility of Lactic Acid Bacteria (LAB) Isolated from Malaysian Fermented Foods. in International Food Science and Agrotechnology Conference (IFoSAC 2018). 2018.
10. Agnes Lee Chiu Nee, et al., Lactic acid bacteria isolated from locally produced vinegars and their antibacterial activity against foodborne bacteria. Universiti Malaysia Terengganu Journal of Undergraduate Research 2019. 1(2): p. 1-7.
11. Hajar, S. and T.H.T.A. Hamid, Isolation of lactic acid bacteria strain Staphylococcus piscifermentans from Malaysian traditional fermented shrimp cincaluk. International Food Research Journal 2013. 20(1): p. 125-129.
12. Hudzicki, J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. 2009. 1-23.
13. Schillinger, U. and W.H. Holzapfel, Antibacterial activity of carnobacteria. Food Microbiology, 1990. 7(4): p. 305-310.
14. Edwards, U., Rogall, T., Blocker, H., Emde, M. and Bottger, E.C., Isolation and direct complete nucleotide determination of entire genes. Nucleic Acid Research 1989. 17: p. 7843-7853.
15. Bintsis, T., Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. AIMS microbiology, 2018. 4(4): p. 665-684.
16. Hertzberger, R., et al., H_2O_2 Production in Species of the Lactobacillus acidophilus Group: a Central Role for a Novel NADH-Dependent Flavin Reductase. Applied and Environmental Microbiology, 2014. 80(7): p. 2229-2239.
17. Uwizeyimana, J.D., et al., Determination of Colistin Resistance by Simple Disk Diffusion Test Using Modified Mueller-Hinton Agar. alm, 2020. 40(4): p. 306-311.
18. Valgas, C., et al., Screening methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology, 2007. 38: p. 369-380.
19. Yesim, E., S. Nur, and M. Mustafa, Antimicrobial Activity of Essential Oil Against Rhizobium (Agrobacterium) vitis Using Agar Well and Disc Diffusion Method. Bacteriology Journal, 2018. 8: p. 1-11.
20. Nassar, M.S.M., W.A. Hazzah, and W.M.K. Bakr, Evaluation of antibiotic susceptibility test results: how guilty a laboratory could be? Journal of the Egyptian Public Health Association, 2019. 94(1): p. 4.

21. Jang, S., et al., The Culture of Pediococcus pentosaceus T1 Inhibits Listeria Proliferation in Salmon Fillets and Controls Maturation of Kimchi. Food Technol Biotechnol, 2015. 53(1): p. 29-37.
22. Lee, K.W., et al., Probiotic properties of Pediococcus strains isolated from jeotgals, salted and fermented Korean sea-food. Anaerobe, 2014. 28: p. 199-206.
23. Endang, S.R., Lactic Acid Bacteria in Fermented Foods of Indonesian Origin. Agritech, 2003. 23(2): p. 75-84
24. Mugula, J.K., et al., Microbiological and fermentation characteristics of togwa, a Tanzanian fermented food. International Journal of Food Microbiology, 2003. 80(3): p. 187-199.
25. Nawaz, M., et al., Characterization and Transfer of Antibiotic Resistance in Lactic Acid Bacteria from Fermented Food Products. Current Microbiology, 2011. 62(3): p. 1081-1089.
26. Bao, Y., et al., Screening of potential probiotic properties of Lactobacillus fermentum isolated from traditional dairy products. Food Control, 2010. 21(5): p. 695-701.
27. Sengun, I.Y., et al., Identification of lactic acid bacteria isolated from Tarhana, a traditional Turkish fermented food. International Journal of Food Microbiology, 2009. 135(2): p. 105-111.
28. Vasiee, A.R., et al., Isolation, identification and characterization of probiotic Lactobacilli spp. from Tarkhineh. International Food Research Journal, 2014. 21(6): p. 2487-2492.
29. Fairfax, M.R., P.R. Lephart, and H. Salimnia, Weissella confusa: problems with identification of an opportunistic pathogen that has been found in fermented foods and proposed as a probiotic. Frontiers in Microbiology, 2014. 5(254).
30. Gao, W., B.P. Howden, and T.P. Stinear, Evolution of virulence in Enterococcus faecium, a hospital-adapted opportunistic pathogen. Current Opinion in Microbiology, 2018. 41: p. 76-82.
31. Sharma, S., et al., Probiotic characterization and antioxidant properties of Weissella confusa KR780676, isolated from an Indian fermented food. LWT, 2018. 97: p. 53-60.
32. Su-Bin Ahn, H.-E.P., Sang-Myeong Lee, So-Young Kim, Mi-Yae Shon, Wan-Kyu Lee. , Characteristics and immuno-modulatory effects of Weissella cibaria JW15 isolated from Kimchi, Korea traditional fermented food, for probiotic use. Journal of Biomedical Research, 2013. 14(4): p. 206-211.
33. Amaral, D.M.F., et al., Enterococcus faecium and Enterococcus durans isolated from cheese: Survival in the presence of medications under simulated gastrointestinal conditions and adhesion properties. Journal of Dairy Science, 2017. 100(2): p. 933-949.
34. Ben Braïek, O. and S. Smaoui, Enterococci: Between Emerging Pathogens and Potential Probiotics. BioMed Research International, 2019: p. 5938210.

Research article

Tomic, N., et al., Delphinidin, Luteolin and Halogenated Boroxine Modulate *CAT* Gene Expression in Cultured Lymphocytes. International Journal of Life Sciences and Biotechnology, 2021.
DOI: 10.38001/ijlsb.789990

Delphinidin, Luteolin and Halogenated Boroxine Modulate *CAT* Gene Expression in Cultured Lymphocytes

Nikolina Tomic [1], Maida Hadzic [1], Naida Lojo-Kadric[1]*, Jasmin Ramic[1], Lejla Pojskic[1]

ABSTRACT

Luteolin and delphinidin are the flavonoids with known protective roles. They inhibit genotoxic effects induced by halogenated boroxine (HB) *in vitro*. Statistically significant decrease in the number of micronuclei and nuclear buds and decrease in proportion of abnormal cells were observed before, but mechanism of their anti-genotoxic activity is still not clear. In our experiment we aimed to quantify HB effects on the relative expression of *CAT* (*catalase*) gene and explore antioxidative effects of luteolin and delphinidin via restoration of *CAT* gene activity. Cell cultures from peripheral blood lymphocytes of five healthy donors were established and treated with single and combined treatments of HB with luteolin or delphinidin. Total RNA was isolated from harvested cells and reverse-transcribed. SYBR based Real-Time PCR amplification method was used. Relative gene expression measurements were done using normalization of ratio of target (*CAT*) and housekeeping (*GAPDH*) genes. Intergroup variance analysis was done with REST® software. Luteolin itself lead to downregulation of relative *CAT* gene expression as well as HB. But simultaneous treatment of HB and bioflavonoids lead to upregulation. Delphinidin as indenpendent treatment and as simultaneous treatment caused upregulation of relative *CAT* gene expression. Obtained results may suggest protective role of delphinidin and luteolin to oxidative-stress damage caused by HB, and also that new approaches to the treatment applications of HB should include bioflavonoids and monitoring corresponding antioxidant system. Our findings indicate that there is a quantifiable effect of luteolin and delphinidine on antioxidant genes which could be used in exact monitoring of oxidative stress related events.

ARTICLE HISTORY
Received
3 September 2020
Accepted
18 October 2020

KEYWORDS
bioflavonoids, dipotassium-trioxohydroxytetra fluorotriborate, catalase, antioxidants

Introduction

Oxidative stress presents the imbalance in cellular oxidation-reduction (redox) reactions in favor of the oxidation, leading to oxidative damage [1]. Oxidative-stress damage may influence the structure and function of numerous biomolecules (nucleic acids, proteins, carbohydrates, polyunsaturated lipids), which results in changes in the structure and

[1] University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Zmaja od Bosne 8 (Kampus), Sarajevo, Bosnia and Herzegovina
*Corresponding Author: Naida Lojo-Kadric, E-mail: naida.lojo@ingeb.unsa.ba

function of cells, tissues and organs [2]. The resulting damage may disturb ion homeostasis, signal transduction in cells, gene transcription, and thus lead to other disorders. Oxidative stress has a significant role in the etiopathogenesis of cardiovascular and infectious disorders, cancer, fibrosis and the aging process [3].

Antioxidant enzymes fulfill the major role in antioxidant defense [1]. Catalase has a key antioxidant enzymatic role in the bodies defense against oxidative stress. It catalyses degradation of the reactive oxygen species (ROS) hydrogen peroxide to water and oxygen and thereby protects cells against ROS toxic effects [4]. Catalase is encoded by the *CAT* gene. It is reported that various polymorphisms in this gene cause decrease in catalase activity and confer to various diseases [4]. The levels of catalase are strongly affected by *CAT* gene expression modulation [4].

Bioflavonoids present natural, polyphenolic compounds with numerous protective antioxidant, antimutagenic and anticarcinogenic properties in human cells. These properties of selected bioflavonoids (delphinidin and luteolin) have been addressed in several studies before. Luteolin showed antiproliferative and anticarcinogenic activity against various cancers [5-7]. It has also increased level of other antioxidative enzymes i.e. glutathione-S-transferase, glutathione reductase or superoxide dismutase [8]. Delphinidin prevented muscle atrophy and upregulated miR-23a expression, inhibited tumor transformation of mouse skin JB6 P+ cells and also showed cytotoxic effects in human osteosarcoma cells and protective effects against ROS-induced injuries in epithelial cells of human retina [9-12].

Halogenated boroxine (HB; dipotassium-trioxohydroxytetrafluorotriborate) is a compound with suggested activity in treatment of benign and malignant skin changes [13]. It has been shown to have suppressive role in division of various cell types both *in vitro* and *in vivo*, and at higher concentrations exhibit proven genotoxic effects [14-15]. It’s suggested that it might act as a pro-oxidant at higher concentrations [16]. It’s observed that it can also inhibit activity of catalase [17].

In treatment of HB in combination with bioflavonoids (delphinidin and luteolin), its genotoxic effects were significantly decreased. In the presence of the HB, luteolin showed more potent to decrease the number of micronuclei and nuclear buds. In similar setting

delphinidin suppressed the occurrence of aberrant cells [18]. It was also observed that the expression of *hTERT* gene was upregulated in samples treated in combine with HB and selected bioflavonoids (luteolin and delphinidin) [19]. Still the mechanism of their anti-genotoxic activity is not clear. Since HB is recognized as inhibitor of catalase activity as a major antioxidant enzyme, we designed experiment to assess antioxidative effects of luteolin and delphinidin on level of relative expression of *CAT* (*catalase*) gene.

Materials and Methods

Sample collection, cell cultures and treatments

Five healthy female volunteers donated blood samples. They were informed about the study details and they signed informed consent forms. Total volume of 400 µL heparinized (BD Vacutainer Systems, Plymouth, UK) peripheral blood was mixed with 5 mL of the PB-MAX karyotyping medium (GIBCO Invitrogen) and cultured for 72 h at 37 °C. Each culture was treated with luteolin, delphinidin and halogenated boroxine—$K_2(B_3O_3F_4OH)$ (HB) individually, and with two substances simultaneously. So, six different treatments were conducted for each blood sample.

Treatments preparation

HB, a white powder soluble in water, dimethyl sulfoxide (DMSO) and ethanol, was synthesized according to modified protocol which was described by Ryss and Slutskaya [20]. In our experiment HB was dissolved in the PB-MAX karyotyping medium (GIBCO-Invitrogen, Carlsbad, CA, USA) at a definitive concentration of 0.1 mg/mL (397.4 µM), for which has been shown to exhibit genotoxic potential. Bioflavonoids delphinidin, -delphinidin chloride (96.70%; HPLC) and luteolin (98.34%; HPLC) (Phyto-Lab GmbH & Co. KG, Germany) were also dissolved in DMSO (Panreac Quimica, Barcelona, Spain). Their final concentration added in cultures was 50 µM.

RNA isolation and reverse transcription

After the cultivation, Quick-RNA™ Mini Prep Plus kit (Zymo Research) was used for isolation of total RNA from harvested cell cultures. Isolated RNA was quantified on Qubit 2.0. Fluorometer (Invitrogen, Life Technologies), using a high-sensitivity RNA assay kit

and then reverse transcription of total RNA (10 ng) was done using Proto Script First-Strand cDNA Synthesis kit (New England BioLabs).

Relative gene expression analysis

SYBR based Real-Time PCR amplification method was used to analyze relative gene expression level in treated cultures. Realtime PCR was conducted in the 7300 Real-time PCR System (Applied Biosystems) and power SYBR Green Master Mix (Applied Biosystems,USA) was used according to the manufacturer's instructions. The following cycling conditions were set: 50 °C for 2 min, 95 °C for 10 min, and then 40 cycles of 95 °C for 15 s and 60 °C for 1 min following standard program for dissociation curve. For the amplification of *CAT* target and *GAPDH* housekeeping gene highly purified primers obtained from BioTeZ Berlin-Buch GmbH (Germany) were used (Table 1.).

Relative Expression Software Tool (REST®) was used for the analysis of results - normalization of ratio of target (*CAT*) and housekeeping (*GADPH*) gene and statistical analysis (Pair Wise Fixed Reallocation Randomisation Test©) were done. Based on calculated relative expression ratio REST© gives us regulation factor. For up-regulation, the regulation factor is equal to the value of expression ratio and for down-regulation, the regulation factor is presented as a reciprocal value [21].

Table 1 Primers used for amplification of target and housekeeping gene in Real Time PCR

Gene	Forward	Reverse
GAPDH	TGAAGGTCGGAGTCAACGGA	CATCGCCCCACTTGATTTTGG
CAT	GAACTGTCCCTACCGTGCTC	GAATCTCCGCACTTCTCCAG

Result and Discussion

In luteolin-treated cultures *CAT* gene expression was down regulated when compared against control and HB-treated cultures, although not statistically significant. In all other cultures, when they were compared against culture treated with luteolin individually, *CAT* gene expression was statistically significant up regulated. It was observed that *CAT* expression was significantly upregulated in delphinidin-treated cultures and cultures treated simultaneously with HB and bioflavonoid (HB+delphinidin and HB+luteolin) when compared to cultures treated with HB only. In combined treatments of HB and antioxidants,

when compared against delphinidin-only treated cultures, relative *CAT* gene expression was up-regulated but not statistically significant. Also, there was no significant difference in *CAT* gene expression level between simultaneously treated cultures but it was up regulated in cultures treated with HB and luteolin. The results of our study have not detected any significant differences of *CAT* gene expression level in any treatment, single or combined, when compared to untreated cell-cultures (control).

Table 2 Results of comparative analysis of relative *CAT* gene expression. Regulation factor-Direction of change in expression-P-value are shown in each cell, respectively**.**
(* statistically significant change observed; ↓ - down regulated expression of *CAT* gene, ↑ - up regulated expression of *CAT* gene)

Treatment	NC	HB	LU	DE	HB+DE	HB+LU
HB	7,226 (↓) 0,817					
LU	23,778 (↓) 0,336	3,092 (↓) 0.194				
DE	5,315 (↑) 0,659	38,409 (↑) 0,005*	118,764 (↑) 0,001*			
HB+LU	12,201 (↑) 0,420	88,173 (↑) 0,007*	272,636 (↑) 0,003*	2,296 (↑) 0,163	1,115 (↑) 0,832	
HB+DE	10,946 (↑) 0,498	79,1 (↑) 0,005*	244,584 (↑) 0,001*	2,059 (↑) 0,249	-	-

In comparison with control, expression of *CAT* gene in any treatment wasn't significantly different. But, in luteolin-single tratment and HB-single treatment *CAT* expression was down-regulated, while in every other treatment it was up-regulated. In higher concentrations, HB exhibits genotoxic effect [14] and might act as a pro-oxidant [16]. Also, it can inhibit catalase activity [17]. So, the results obtained in this study for HB are kind of expected, as for the luteolin it could be surprising considering that bioflavonoids as

antioxidants are directly engaged in the suppression of damage of genetic structures [22] and that antigenotoxic activity of selected bioflavonoids *in vitro* has been confirmed [18]. However, luteolin also showed genotoxic effects and inhibited proliferation of human lymphocyte culture [23]. Furthermore, in the previous study of Hadzic *et al.* [19] in combined treatment of HB and luteolin, when compared to treatment with luteolin only, *hTERT* gene was significantly upregulated. This is in a way in correlation with our results as when we compared individual luteolin treatment and simultaneous HB and luteolin treatment, in cultures treated with simultaneous treatment *CAT* gene expression was significantly up regulated and by highest observed factor in the study.

In almost every culture treated with delphinidin, simultaneously or individually, *CAT* gene expression was up regulated, except when compared against culture treated with HB and luteolin simultaneously. It is familiar from previous studies that delphinidin reduces the proportion of micronuclei *in vivo* [24], has cytostatic [25] and concentration-dependent cytotoxic effects [11]. Concerning its genotoxic effects, in some studies it showed neither aneugenic nor clastogenic activity [23], while in others it showed significant inhibitory potential to the frequencies of total defect cells in the presence of genotoxins, but also induced them in individual treatments with delphinidin [26]. In this study, delphinidin did not cause down regulation of expression of *CAT*, as was the case with luteolin. Nevertheless, the upregulation in cultures treated with simultaneous treatment of HB and delphinidin compared to those treated with delphinidin respectively as well as higher regulation factors associated with simultaneous treatment than those associated with individual treatment, may indicate that this antioxidant also shows stronger antioxidant activity in the presence of prooxidants.

Non-concordant results from our study concerning luteolin effects could be explained by the fact that in numerous studies it has been shown that some well-known antioxidants such as vitamin C, flavonoid myricetin, hormone melatonin and various carotenoids can exhibit bimodal effects, both antioxidant and prooxidant, depending on the conditions [27-30]. Hence, though delphinidin and luteolin in combined treatment with HB have showed positive effect on *CAT* gene expression, there is a need for additional studies to better

understand the precise role of these flavonoids and to elucidate whether they could be double-edge sword in certain conditions.

Conclusion

Obtained results may indicate protective role of delphinidin and luteolin in combined treatment with HB, and also that potential treatment use of HB should include bioflavonoids and monitoring corresponding antioxidant system. Still, for more precise determination of luteolin and delphinidin antioxidative effects in combined treatments, more treatments and effector genes should be explored.

Acknowledgments
This research was partly supported by the Ministry of Education and Science of the Federation of Bosnia and Herzegovina [grant number 05-39-5613-1-2/11].

References

1. Sies, H., Oxidative stress: a concept in redox biology and medicine. Redox biology, 2015. 4: p. 180–183.
2. Pisoschi, AM. and A. Pop, The role of antioxidants in the chemistry of oxidative stress: A review. European Journal of Medicinal Chemistry, 2015. 97: p. 55–74.
3. Ghezzi, P., et al., The oxidative stress theory of disease: levels of evidence and epistemological aspects. British Journal of Pharmacology, 2017. 174: p. 1784–1796.
4. Kodydková, J., et al., Human Catalase, Its Polymorphisms, Regulation and Changes of Its Activity in Different Diseases. Folia Biologica, 2014. 60: p. 153-167.
5. Gao, G., et al., Luteolin exhibits anti-breast cancer property through up-regulating miR-203. Artificial Cells, Nanomedicine, and Biotechnology, 2019. 47 (1): p. 3265-3271.
6. Imran, M., et al., Luteolin, a flavonoid, as an anticancer agent: A review. Biomedicine & Pharmacotherapy, 2019. 112: 108612.
7. Seelinger, G., et al., Anti-carcinogenic Effects of the Flavonoid Luteolin. Molecules, 2008. 13: p. 2628-2651.
8. Lin, Y., et al., Luteolin, a flavonoid with potentials for cancer prevention and therapy. Current Cancer Drug Targets, 2008. 8 (7): p. 634–646.
9. Murata, M., et al., Delphinidin Prevents Muscle Atrophy and Upregulates miR-23a Expression. Journal of Agricultural and Food Chemistry, 2017. 65 (1): p. 45-50.
10. Kuo, H.D., et alAnthocyanin Delphinidin Prevents Neoplastic Transformation of Mouse Skin JB6 P+ Cells: Epigenetic Re-activation of Nrf2-ARE Pathway. The AAPS Journal, 2019. 21 (5): p. 83.
11. Lee, D.Y., et al., Cytotoxic effects of delphinidin in human osteosarcoma cells. *Acta Orthopaedica et Traumatologica Turcica,* 2018. 52 (1): p. 58–64.
12. Ni, T., W. Yang, and Y. Xing, Protective effects of delphinidin against H2O2 induced oxidative injuries in human retinal pigment epithelial cells. Bioscience Reports, 2019. BSR20190689.
13. Galic, B., Removal of skin changes. European patent EP1996514, 2013.

14. Haverić, S., et al., Effects of dipotassium trioxohydroxytetrafluorotriborate ($K_2[B_3O_3F_4OH]$) on genetic material and inhibition of cell division in human cell cultures. *Drug and Chemical Toxicology*, 2011. 34 (3): p. 250-254.
15. Haveric, S., et al., Genotoxicity evaluation of dipotassium-trioxohydroxytetrafluorotriborate, $K_2(B_3O_3F_4OH)$, in human lymphocyte cultures and mice reticulocytes. Brazilian Archives of Biology and Technology, 2016. 59: e16160195.
16. Marasović, M., et al., Quantum Chemical and Biochemical Study on Antioxidant Properties of Halogenated Boroxine $K_2[B_3O_3F_4OH]$. Croatica chemica acta, 2017. 90 (2): p. 155–161.
17. Islamovic, S., B. Galic, and M. Milos, A study of the inhibition of catalase by dipotassium-trioxohydroxytetrafluorotriborat $K_2[B_3O_3F_4OH]$. Journal of Enzyme Inhibition and Medicinal Chemistry, 2014. 29 (5): p. 744–748.
18. Hadzic, M., et al., Inhibitory effects of delphinidin and luteolin on genotoxicity induced by $K_2(B_3O_3F_4OH)$ in human lymphocytes in vitro. Biologia, 2015. 70: p. 553–8.
19. Hadzic, M., et al., Bioflavonoids protect cells against halogenated boroxine-induced genotoxic damage by upregulation of hTERT expression. Zeitschrift fur Naturforschung. C, Journal of biosciences, .2018. 74 (5-6): p. 125-129.
20. Ryss, I.G., and M.M. Slutskaya, Report on the Platinum Sector. Akademia Nauka SSSR, 1951. 26: p. 216.
21. Pfaffl, M.W., G.W. Horgan, and L. Dempfle, Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Research, 2002. 30 (9): e36.
22. Luca, V.S., A. Miron, and A.C. Aprotosoaie, The antigenotoxic potential of dietary flavonoids. Phytochemistry Reviews, 2016. 15 (4): p. 591-625.
23. Ezić, J., et al., Analysis of delphinidin and luteolin genotoxicity in human lymphocyte culture. Journal of Health Sciences, 2015. 5 (2): p. 41-45.
24. Azevedo, L., et al., Differential response related to genotoxicity between eggplant (*Solanum melanogena*) skin aqueous extract and its main purified anthocyanin (delphinidin) *in vivo*. Food and Chemical Toxicology, 2007. 45: p. 5852-5858.
25. Lazzé, M.C., et al., Anthocyanins induce cell cycle perturbations and apoptosis in different human cell lines. Carcinogenesis, 2004. 25: p. 81427-81433.
26. Hadžić, M., Analysis of potential of bioflavonoids (luteolin and delphinidin) in inhibition of genotoxic and cytotoxic effects of halogenated boroxine in vitro. MSc Thesis, University of Sarajevo, 2013.
27. Chakraborthy, A., et al., Antioxidant and pro-oxidant activity of Vitamin C in oral environment. Indian Journal of Dental Research, 2014. 25 (4): p. 499.
28. Chobot, V., and F. Hadacek, Exploration of pro-oxidant and antioxidant activities of the flavonoid myricetin. Redox report Communications in free radical research, 2011. 16 (6): p. 242–247.
29. Wang, J., et al., Antioxidant and Pro-Oxidant Activities of Melatonin in the Presence of Copper and Polyphenols In Vitro and In Vivo. Cells, 2019. 8 (8): p. 903.
30. Ribeiro, D., et al., Antioxidant and pro-oxidant activities of carotenoids and their oxidation products. Food and Chemical Toxicology, 2018. 120: p. 681-699.

Research article

Al-kaf. H.A., F. Huyop, and N.A. Zainol, Growth Analysis of *Lactobacillus Acidophilus* Using Different Non-Digestible Carbohydrates. International Journal of Life Sciences and Biotechnology, 2021. 4(1)
DOI: 10.38001/ijlsb.796319

Growth Analysis of *Lactobacillus Acidophilus* Using Different Non-Digestible Carbohydrates

Haia Abobakr Al-kaf [1]* Fahrul Huyop[1] Noor Azwani Zainol[1]

ABSTRACT

Probiotics are live microorganisms and offer health benefits to the digestive system and used in the production of many fermented foods. Non-digestible carbohydrates are dietary fibers which cannot be digested and absorbed by the small intestine. Strains of *Lactobacillus*, are among the most common and popular group of probiotics and added to many dairy products and dietary supplements. Besides, *Lactobacillus acidophilus* can exhibit many useful benefits such as showing thermostability, maintaining the growth activity at a wide pH range, and offering strong inhibition actions against spoilage of food and pathogenic bacteria. Aims of this study are to analyse the ability of non-digestible carbohydrates to act as a carbon source in enhancing the growth activity of *L. acidophilus in vitro* and to determine which type of non-digestible carbohydrate sources contributed a high biomass production. *L. acidophilus* was grown on de Man, Rogosa and Sharpe (MRS) medium. The optical density and pH of the cell biomass produced were measured and cell dry weight was determined. The highest biomass production recorded was for barley 10.02 g. L^{-1} followed by yam 8.79 g. L^{-1}, 7.17 g. L^{-1} for garlic, 6.81 g. L^{-1} for banana and 4.86 g. L^{-1} for sweet potato, while positive control (glucose) recorded 4.20 g. L^{-1} of cell biomass. The results also showed a decreasing in the pH values which indicated the formation of lactic acids in the medium after 24 h of incubation at 37°C on rotary shaker set at 200 rpm. The overall results, confirmed that *L. acidophilus* helps in the hydrolysis of non-digestible carbohydrates and subsequent conversion of the sugars to cell biomass and decrease the pH compared to the negative control (without carbon source). This shows that in future, production of a synbiotic products using these non-digestible carbohydrates and probiotics strains is promising to offer many benefits to human's health.

ARTICLE HISTORY
Received
17 September 2020
Accepted
25 October 2020

KEYWORDS
Probiotics, *Lactobacillus acidophilus*, non-digestible carbohydrates, Rogosa and Sharpe (MRS), cell biomass

Introduction

Probiotics are live microorganisms that play an important role in the digestive system by keeping the gut healthy and balancing the beneficial microflora in the gut [1]. The use of probiotics in the last two decades has been increased significantly due to their ability in conferring many health benefits to human's digestive system such as protecting the host from different harmful microorganisms, and making the immune system stronger. In

[1] Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia,81310 Johor Bahru, Malaysia
* Correspondence: haiaalkaff10@gmail.com/ fzhutm@gmail.com

addition, probiotics are known for their ability in improving feed digestibility and decreasing the metabolic disorders [2]. Non-digestible carbohydrates are dietary fibres (prebiotics) and cannot be digested and absorbed by the small intestine. Prebiotic is defined as non-digestible food which helps in improving and balancing the growth of the beneficial microflora in the digestive system. Plants are considered the common source of these non-digestible carbohydrates and contain a mixture of polysaccharides which are the integral components in the cell wall of the plants [3]. Gut microbiota offer many positive effects to the function, metabolism and integrity of the intestine. Gut microbiota can also fight many pathogens by the induction of immunomodulatory molecules which have antagonistic properties and can be gained from the production of lactic acid bacteria for instance *Bifidobacterium* and *Lactobacillus* genera [3]. Choosing substrate specificities for probiotics is important for the optimization of the density of probiotics in the intestine. The increase of probiotics population is influenced by the composition of the monosaccharide of the non-digestible carbohydrates, the glycosidic linkage between the monosaccharide residues, and the degree of polymerization [4]. Many researchers proved the ability of prebiotics (non-digestible carbohydrates) in inhibiting the attachment of pathogens to epithelial cells and it is depending on the secretion of the peptides which are responsible in enhancing the absorption of minerals, stimulating the appetite, and preventing from diseases [3]. The term "synbiotic" was introduced by Gibson and Roberfroid (1995) which referred to a combination of both probiotics and prebiotics [5]. Consuming synbiotic can stimulate and activate the metabolism of a physiological intestinal microbiota [6]. Thus, using synbiotic can help in overcoming problems related to the survival of probiotics in the gastrointestinal tract. The combination of both probiotics and prebiotics as a single product can lead to great influence compared of using probiotic or prebiotic alone [7]. Thus, current studies are focusing on studying and selecting carbohydrates components which can be utilized by probiotic strains in high levels to produce synbiotic products to enhance the growth of beneficial bacteria in the gastrointestinal tract. Recently, many people prefer a good quality in purchasing food products and they search for specific features such as longer shelf-life products that had been produced using natural fermentation process. The process of natural food fermentation is carried out by using fermenting microbial

communities such as lactic acid bacteria (LAB) as an energy source to enhance the quality of the product [8]. LAB are among the most common and significant groups of probiotic used in food processing industries, particularly in dairy products. These microorganisms can boost the digestion of lactose and stimulate the immune system, and prevent from diarrhea [9]. Functional food ingredients offer many beneficial effects for humans and these benefits are gained from the bioactive components present in the products. These ingredients in functional food are probiotics, prebiotics, soluble fibers, and others. Nowadays, the demand for fermented probiotic foods is increasing significantly and > 500 of the probiotic products have been introduced in the world, since probiotic products offer many benefits to humans, therefore the most common way to consume probiotics is mostly through the intake of food products and some fermented products may not last longer than a month and the cells may die or not be active for a period of time [10]. Thus, it is important to maintain probiotics strains live longer and also indicates the importance of dietary fibers to be used as substrates for sustaining the growth and increasing the shelf-life of probiotic strains along with the processing and storage conditions [11]. Therefore, the aims of this study were, to prepare raw non-digestible carbohydrates and seeding with *L. acidophilus* in the prepared MRS media. The growth of the bacteria was analysed by measuring cell dry weight of *L. acidophilus* over specific time frame with pH.

Material and Methods

Preparation of non-digestible carbohydrates samples

Selected sources of non-digestible carbohydrates samples have been used in this study namely, sweet potato, yam, garlic, barley and banana. Garlic and barley are commercially available. For the preparation of sweet potato, yam and banana samples, 500 g of each was obtained as a raw material from a local market. The samples were washed, peeled and sliced thinly. Then, both sweet potato and yam samples were placed on a dehydrator's tray and dried at 68°C for 24 h. For banana sample the drying process was carried out at 45°C for 72 h. After the drying process, all the samples were ground into a fine powder. On the other hand, 100 g of fine powder of barley and garlic samples were obtained from commercially available product in the supermarket.

Microorganism

The *Lactobacillus acidophilus* (ATCC 4356) strain was provided by the Institute of Bioproduct Development, Universiti Teknologi Malaysia. Originally, it was purchased from a German Culture Collection Center (Leibniz Institute DSMZ, Braunschweig, Germany).

Seed culture of *L. acidophilus* and inoculum preparation

The de Man, Rogosa and Sharpe (MRS) medium was prepared according to the standard procedure [12]. For the preparation of the inoculum, the seed culture was grown into a 250 mL Erlenmeyer shake flask, containing 40 mL of the prepared MRS broth, 1 g of glucose (already dissolved in 10 mL of distilled water) and 1 mL of *L. acidophilus* stock culture. The inoculum preparation was carried out as first and second inoculum. The flask of the first inoculum was incubated for 24 h at 200 rpm and 37°C on a rotary shaker (Innova 4080, New Brunswick, NJ, USA). On the second day, 5 mL of the first inoculum seed cultures of *L. acidophilus* was transferred into the second inoculum using sterile tip and further incubated following the same incubation conditions as first inoculum. After the cultivation of the second inoculum, the production medium was prepared by adding 1 g of the prepared and the commercialized non-digestible carbohydrates samples mixed with 10 mL of distilled water into 250 mL Erlenmeyer shake flasks with a volume of 40 mL of the previous prepared liquid MRS broth. The experiment was carried out in duplicates and two controls been used; one as positive (glucose) and other one as negative (no carbon source). 5 mL of the cultivated second inoculum was pipetted into all the Erlenmeyer shake flasks containing the samples and then all the flasks were agitated for 24 h at 200 rpm at 37°C on a rotary shaker (Innova 4080, New Brunswick, NJ, USA).

Optical density and pH measurement

After 24 h of incubation, cell growth was observed by measuring the optical density (OD) of the cultivated stocks in a single beam spectrophotometer (DR 6000, Hach Co., Loveland, CO, USA) at Absorbance 600 nm. For better accuracy, the stocks were diluted into ratio of 1:100. The OD of the culture was converted to dry cell mass through a previously prepared linear correlation between OD and CDW. One $OD_{600\ nm}$ was almost equal to 0.3 g. L^{-1} for this culture. The pH was measured after 24 h of incubation period using sterilizable pH probe (TOLEDO, Delta 320 pH Meter).

Results and Discussion

Non-digestible carbohydrates and seeding of *L. acidophilus* in the growth media

In this study, growth of *L. acidophilus* in MRS medium was monitored for the production of biomass in every sources of non-digestible carbohydrate and commercially available carbon sources and compared to that of the positive control using glucose as a carbon source and negative control without any carbon source. *L. acidophilus* in MRS medium was grown in an orbital shaker over 24 h period at 37°C. The growth activity was recorded as shown in (Table 1). Based on (Table 1), the data showed that the values were significant ($p<0.05$) and this indicates that *L. acidophilus* was able to grow in the provided carbon source.

Table 1 Mean and standard deviation values of maximum $OD_{(600nm)}$ reading for the growth cultures over 24 h of incubation period

Carbon source	$OD_{600\ nm}$ reading
Negative control	0±0.00
Positive control	0.14±0.016
Yam	0.293±0.014
Sweet potato	0.162±0.039
Banana	0.227±0.014
Commercial Barley	0.334±0.002
Commercial Garlic	0.239±0.022

*Mean ± standard deviation of duplicates analysis
*Note: positive control = (glucose); negative control = (no carbon source)

Measurement of cell dry weight based on an OD reading and pH analysis

Cell dry weight (CDW) and pH were recorded in order to analyse the correlation between the two readings over 24 h of cultivation in shake flask culture. The OD of culture was converted to dry cell mass through a linear correlation standard curve. 1 $OD_{600\ nm}$ was almost equivalent to 0.3 g L^{-1} [13]. pH reading was taken and each pH was measured as in (Table 2). The pH values and CDW values were plotted using bar chart as presented in (Figure 1). The correlation between the two were compared. Based on (Figure 1) pH values are low except for negative control and the decrease in pH indicated that there was

a cell biomass accumulation and the provided carbon sources were fermented by *L. acidophilus* effectively.

Table 2 Mean and standard deviation values of pH for the growth cultures over 24 h of incubation

Carbon source	pH
Negative control	8.5±0.098
Positive control	5.17±0.028
Yam	4.81±0.056
Commercial Garlic	4.86±0.021
Banana	4.97±0.014
Commercial Barley	4.22±0.021
Sweet potato	4.96±0.014

*Mean ± standard deviation of duplicates analysis
*Note: Positive control = (glucose); Negative control = (no carbon source)

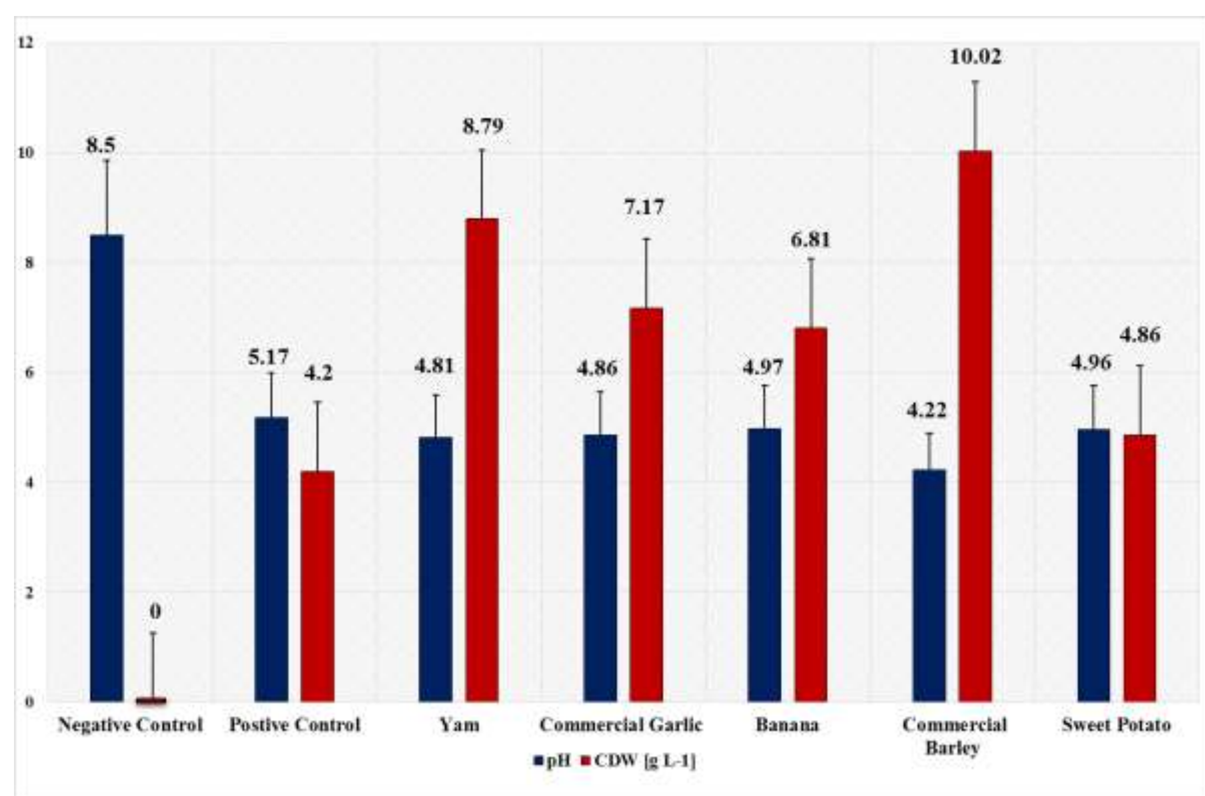


Fig 1 pH and cell dry weight (CDW) of the cultivated samples over 24 h of incubation period

From (Figure 1), we can conclude that using commercial barley allowed the highest production of the cell biomass recorded as 10.02 g L^{-1} and this is due to the structure of

barley since it is consisting of heterogeneous complex molecules and this leads in improving the growth and the activity of *Lactobacillus* strains [14]. Moreover, [15], stated the lactic acid bacteria can grow well in the presence of any cereal source especially barley, and this makes it a good substrate. In addition, the increase of the cell biomass could be due to the decrease in the content of starch during fermentation of barley with *Lactobacilli* as it was previously reported [16]. The approximate amount of starch content in barley is about 70%, this indicated that *L. acidophilus* had fermented the starch efficiently during the 24 h of cultivation. Moreover, the biochemical characteristics of β-1,3-1,4-glucanase; which is an endoglucanase enzyme and mainly found in *Lactobacillus* species played a role as well in increasing the production of cell biomass by hydrolyzing barley β-glucan compound completely [17]. According to one study [14] , β-glucans were reported to be fermented completely by the intestinal microbiota and were able to enhance the growth rate and the production of lactic acid bacteria. Yam, produced cell biomass of 8.79 g L^{1}. The high cell biomass produced can be due to α-amylase by *L. acidophilus* with yam. The production of α-amylase results in the breaking down of starch into fermentable carbohydrates and yield lactic acid as a final product [18]. Furthermore, yam generally is consist of 60-80% starch content and 20-30% of amylose; which makes it a good source for *L. acidophilus* to utilize it and used it as a carbon source [19]. The high content of starch leads to the accumulation of lactic acid, which had formed due to the use of α-amylase which degraded it effectively. According to the analysis done by [20] it was reported that yam when it was applied *in vivo* to mice it had inhibited the growth of pathogenic bacteria of *Clostridium perfringens*. The growth of *Lactobacillus* has been observed and it was reported that addition of yam as prebiotic source had increased and enhanced *Lactobacillus* in the gut of mice. Commercial garlic recorded 7.17 g L^{1} of cell biomass. The increase of the produced cell biomass is could be due to the fructan compound present in garlic which is an important storage carbohydrate and it was recorded that the fructan amount in garlic is more than 75% [21]. Fructooligosaccharides, shown in (Figure 2) can be found in an amount of 10-16% in garlic and it has a degree of polymerization from 3-50 which makes garlic a good source of prebiotic and promotes the survival of *L. acidophilus* [22]. Thus, to influence and enhance the growth of probiotics strains it is mainly depending on the length of degree

polymerization and it is believed that *L. acidophilus* uses the fructan as a source of carbon. The total amount of fibers in garlic is nearly 26% which makes it a good source to be utilized and used as a carbon source during fermentation process. Garlic fructan can work as prebiotic and energy source for *L. casei.* Hence, specific amount of garlic particularly those which are below the antimicrobial activity concentration can promote the growth of beneficial bacteria present in the gut [23].

Fig 2 General chemical structures for inulin and inulin-like oligosaccharides [24]

For banana, *L. acidophilus* records cell biomass of 6.81 g L^{-1}. It was concluded that *L. acidophilus* can produce cellulase and hemicellulase enzymes which leads in the utilization of the carbohydrates in the banana and increase the growth activity of the *L. acidophilus* [25]. In addition, the use of banana powder as non-digestible carbohydrate source, contributed in positive effects due to the high amounts of dietary fibers in banana and this makes it suitable for the growth of *L. acidophilus*. Furthermore, sucrose, fructose and glucose in banana also play a role in increasing the cell biomass and producing lactic

acid and pyruvic acids, the pathway used to produce these compounds is Embden-Meyerhoff-Parnas, which used NADH as the co-factor and the enzyme lactate dehydrogenase. The biomass produced for sweet potato sample was 4.86 g L^{-1} which is somewhat low value compared to barley, yam and garlic. The utilization of sweet potato as a carbon source by *L. acidophilus* is due to the capability of the strain in producing exogenous enzymes such as amylase, protease, and lipase in order to degrade the carbohydrates in the sweet potato such as sucrose, maltose, and glucose [26]. The degradation of these carbohydrates resulted in the production of lactic acid and promote the survival of *L. acidophilus* because of the nitrogen and carbon sources offered during the fermentation of the carbohydrates. Moreover, based on the data, it was indicated that the fermentation process was effective by the enzymes produced by *L. acidophilus* which utilized the fibers or starch in sweet potato as an energy source. On the other hand, for the positive control containing glucose, the biomass of cells produced was as low as 4.20 g L^{-1} and pH 5.17. This was due to *L. acidophilus* fermented glucose and the value of cell biomass decreased slightly due to the simple building blocks of glucose compound and the number of the molecules 1-2 in glucose [27]. The overall results indicated that non-digestible carbohydrates samples were utilized significantly and no growth of *L. acidophilus* in medium without sugar (negative control) was recorded. CDW and pH were correlated to each other. Low in pH value during growth was due to *L. acidophilus* since it is a lactic acid bacteria and used the provided carbohydrate and transformed it into the simplest form of glucose and then converted it back as lactic acid during the fermentation. The decrease of pH values indicates the formation of titratable acidity in the cultivated culture. Based on the results, the differences in the values of CDW for the samples is mainly depending on the chemical composition and structure for each carbon source used. According to Healthline American website (https://www.healthline.com/about) barley is consisting of 73.5 g of carbohydrates; mainly polysaccharides and 17.3 g of fibers. The different types of polysaccharides in barley such as cellulose and β-glucans are playing a critical role in enhancing the fermentation process and increasing the biomass production in *L. acidophilus* effectively and this is linked to β and α -glycosidic bonds [28]. Moreover, the amount of carbohydrates and fibers in yam are 37 g and 5 g respectively. Garlic contains 33 g of

carbohydrates and 3.1 g of fibers. While, banana contains 24 g of carbohydrates and 3.1 g of fibers. Lastly, for sweet potato it contains 20 g of carbohydrates and 3 g of fibers. All these different ranges of carbohydrates and fibers content in each sample indicated their ability to be utilized by *L. acidophilus* and increase the biomass production after 24 h of incubation and indicated the differences of the cell dry weight values and pH values. Based on the results, we can conclude that barley is considering one of the important source that can be used in future to produce fermented products and act as a natural supplier or substrate for enhancing the growth of probiotics or can be used in the production of synbiotics products because it helps in sustaining the growth and shelf-life of probiotics strains and the beneficial bacteria in the gut if it is consumed regularly.

In the future, it is recommended to study the chemical composition, structures and characteristics of non-digestible carbohydrates (barley, yam, garlic, banana and sweet potato) to understand how *L. acidophilus* can utilize these non-digestible carbohydrates effectively. This study focuses on using selected non-digestible carbohydrates in food industries to act as a carbon source for probiotic strains to increase shelf-life and enhance the growth activity. In addition, it will potentially help in producing symbiotic products which consist of both probiotics and prebiotics and this will help in providing many health benefits to the consumers and used as a treatment for any disorder in human health.

Conclusion

Thus, combining non-digestible carbohydrates and *L. acidophilus* can gain great benefits for human's health because probiotics can survive the acidic gastric environment in the gut, whereby non-digestible carbohydrates (barley) can enhance the growth activity and produce high cell biomass. In future, production of synbiotic products using non-digestible carbohydrates and *L. acidophilus* can help in sustaining the product's quality and increase the shelf-life.

Abbreviations

LAB: lactic acid bacteria; CDW: cell dry weight; OD: optical density; MRS: de Man, Rogosa and Sharpe; dp: degree of polymerization; NADH: Nicotinamide adenine dinucleotide (NAD) + hydrogen (H); rpm: Rotary per minute; nm: Nanometers; h: Hours.

Acknowledgements

We sincerely thankful for the lab staff of Biosciences Department, University Teknologi Malaysia and Institute of Bioproduct Development (IBD), Johor Bahru.

Funding
We are grateful to the Fundamental Research Grant Scheme (FRGS) R.J130000.7854.5F189 (Ministry of Higher Education Malaysia) and RUG-UTM-HR-Q.J130000.2414.08G59 for partial financial assistance.

References

1. Sharifi, M., et al., Kefir: a powerful probiotics with anticancer properties. Medical Oncology, 2017. 34 (11): p. 1-7.
2. Zielińska, D., D. Kołożyn-Krajewska, and M. Laranjo, Food-origin lactic acid bacteria may exhibit probiotic properties: review. BioMed Research International, 2018. p. 1-15.
3. Davani-Davari, D., et al., Prebiotics: Definition, types, sources, mechanisms, and clinical applications. Foods, 2019. 8 (3): p. 92.
4. Zartl, B., et al., Fermentation of non-digestible raffinose family oligosaccharides and galactomannans by probiotics. Food & Function, 2018. 9 (3): p. 1638-1646.
5. Gibson, G. and M.B. Roberfroid, Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition, 1995. 125 (6): p. 1401–12.
6. Śliżewska, K. and A. Chlebicz-Wójcik, The in vitro analysis of prebiotics to be used as a component of a synbiotic preparation. Nutrients, 2020. 12 (5): p. 1272.
7. Markowiak, P. and K. Ślizewska, Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients, 2017. 9 (9): p. 1021.
8. Srivastava, R.K, Enhanced shelf life with improved food quality from fermentation processes. Journal of Food Technology and Preservation, 2018. 2 (3): p. 1–7.
9. Colombo, M., et al., Beneficial properties of lactic acid bacteria naturally occurring in dairy production systems. BMC Microbiology, 2018. 18 (1): p. 1-12.
10. Dinkçi, N., V. Akdeniz, and A.S, Akalin, Survival of probiotics in functional foods during shelf life. Food Quality and Shelf Life, 2019. p. 201-233.
11. Capozzi, V., et al., Stressors and Food Environment: Toward strategies to improve robustness and stress tolerance in probiotics. Probiotics, Prebiotics, and Synbiotics: Bioactive Foods in Health Promotion, 2016. p. 245-256.
12. Atlas, R.M., 2006, The Handbook of Microbiological Media for the Examination of Food. Boca Raton, Fla, USA. p. 246.
13. Kepli, A. N., et al., Medium optimization using response surface methodology for high cell mass production of Lactobacillus acidophilus. Journal of Scientific and Industrial Research, 2019. 78 (9): p. 608–14.
14. Arena, M., et al., Barley β-glucans-containing food enhances probiotic performances of beneficial bacteria. International Journal of Molecular Sciences, 2014. 15 (2): p. 3025-3039.
15. Suman, D., Barley: A cereal with potential for development of functional fermented foods. Intl. J. Ferment. Food, 2019. 8 (1): p. 1–13
16. Liptáková, D., Z. Matejčeková, and L. Valík, Lactic acid bacteria and fermentation of cereals and pseudocereals. Fermentation Processes, 2017.
17. Lee, J.M., et al Improvement of thermostability and halostability of β-1,3-1,4-glucanase by substituting hydrophobic residue for Lys48. International Journal of Biological Macromolecules, 2017. 94. p. 594-602.
18. Batista, N.N., et al., Fermentation of yam (Dioscorea spp. L.) by indigenous phytase-producing lactic acid bacteria strains. Brazilian Journal of Microbiology, 2019. 50 (2): p. 507-514.

19. Li, T., et al., The beneficial effects of purple yam (: Dioscorea alata L.) resistant starch on hyperlipidemia in high-fat-fed hamsters. Food & Function, 2019. 10 (5): p. 2642-2650.
20. Hsu, C.C., et al., Effect of yam (Dioscorea olata compared to Dioscorea japonica) on gastrointestinal function and antioxidant activity in mice. Journal of Food Science, 2006. 71 (7): p. S513-S516.
21. Zhang, N., et al., Study on prebiotic effectiveness of neutral garlic fructan in vitro. Food Science and Human Wellness, 2013. 2 (3-4): p. 119-123.
22. Martinez-Gutierrez, F., et al., Potential use of Agave salmiana as a prebiotic that stimulates the growth of probiotic bacteria. LWT, 2017. 84. p. 151-159.
23. Shalini, R., et al., Growth of selected probiotic bacterial strains with fructans from Nendran banana and garlic. LWT - Food Science and Technology, 2017. 83: p. 68-78.
24. Pramono, Y.B., et al., Utilization of lesser yam (Dioscorea esculenta L.) flour as prebiotic in yogurt to total lactic acid bacteria (LAB), sugar reduction, and organoleptic properties. Digital Press Life Sciences, 2020. 2: p. 00011.
25. Powthong, P., et al., study of prebiotic properties of selected banana species in Thailand. Journal of Food Science and Technology, 2020. 57 (7): p. 2490-2500.
26. Putra, A.N, Sweet potato varieties sukuh potential as a prebiotics in tilapia Feed (Oreochromis niloticus). International Conference of Aquaculture Indonesia, 2014. (1989): p. 254–58.
27. Ikuta, D., et al., Conformationally supple glucose monomers enable synthesis of the smallest cyclodextrins. Science, 2019. 8 (5): p. 674-677.
28. Bello, B., et al., Evaluation of the effect of soluble polysaccharides of palm kernel cake as a potential prebiotic on the growth of probiotics. 3 Biotech, 2018. 8 (8): p. 1-14.

Research article

Poyraz, F.Ş., et al., 2,4-Dichlorophenoxyacetic Acid Loaded Polymeric Nanoparticle Synthesis and Its Effect on Biomass of Medicago sativa Cell Suspension Cultures. International Journal of Life Sciences and Biotechnology, 2021. 4(1). DOI: 10.38001/ijlsb.789851

2,4-Dichlorophenoxyacetic Acid Loaded Polymeric Nanoparticle Synthesis and Its Effect on Biomass of *Medicago sativa* Cell Suspension Cultures

F. Şayan Poyraz[1*], Edibe Ece Abacı[1], Caner Ertürk[1], Tayfun Acar[2], Serap Derman[2], Semiha Erişen[1], Banu Mansuroğlu[1]

ABSTRACT

Nanoparticular systems, which have made great advances with the development of nanotechnology, have been used extensively recently in pesticide, herbicide plant growth regulators, fertilizer applications, gene transfer technologies and agriculture. It was aimed to synthesize and characterize 2,4-D loaded PLGA nanoparticles and investigate their biological activity in comparison with its free form. Here, the effects of 2,4-D loaded poly (lactic-co-glycolic) acid (PLGA) nanoparticles on biomass in Medicago sativa cell suspension cultures were investigated. Single emulsion solvent evaporation method is used in nanoparticle synthesis. As a result of the characterization of nanoparticles, 63.82% encapsulation efficiency, 60.73% reaction efficiency and 10.51% drug loading capacities were calculated. Particle size was measured as 181.7 ± 3.74 nm, zeta potential -18.3 ± 1.48 and polydispersity index as 0.081. Compared with the free 2,4-D molecule, it was observed that the addition of 2,4-D to the medium using the nanoparticles drug release system increased the growth of plant cells and the yield of biomass in *M. sativa* cell suspension cultures.

ARTICLE HISTORY
Received
03 September 2020
Accepted
10 November 2020

KEY WORDS
2,4-D,
polymeric nanoparticle,
Medicago sativa

Introduction

Herbicides disrupt the hormonal balance that regulates plant metabolism, such as cell division, cell growth, protein synthesis, and respiration. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a synthetically available, hormone-like, translocable, systemic selective herbicide [1]. It has an enzyme activity in plant metabolism that influences plant growth [2]. For this reason, it is used as a synthetic auxin and helps to induce rooting of scions and fruit drop [3]. Additionally, it ensures to regeneration of callus and root formation at low doses in *in vitro* cultures. Gopi and Vatsala stated that, maximum callus growth was

[1] Yildiz Technical University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, Istanbul, Turkey
[2] Yildiz Technical University, Faculty of Chemical and Metallurgical Engineering, Department of Bioengineering, Istanbul, Turkey
*Corresponding Author: Fatma Şayan Poyraz e-mail: sayanpoyraz@gmail.com

obtained from 2,4-D, compared to 1-Naphthaleneacetic acid (NAA), Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA) in *Gymnema sylvestre* [4]. In a study conducted on effects of kinetin and 2,4-D over callus formation in various mediums, kinetin was not effective alone while 2,4-D induced callus regeneration were reported [5]. Malik et al. stated that high 2,4-D levels inhibit callus proliferation, but low concentrations of 2,4-D involve morphogenesis [6]. Besides these positive effects of 2,4-D, it has toxic effects at higher concentrations was reported by another research for rooting of vine rootstock cuttings [7]. Additionally, 2,4-D concentration affect germination and initial development of *Regnellidium diphyllum* Lindman (Monilophyta, Marsileaceae). The development ratio of megaspores were high at lowest concentration of 2,4-D, it was negatively affected as the concentration increased [8]. Overall, previous studies show that the concentration of 2,4-D has important roles in plant growth and development.

Cell suspension cultures are one of the methods used for the production of medically and economically valuable secondary metabolites [9]. The production of secondary metabolite from whole plants is limited owing to cultivation and environmental limitations. The cells in the suspension medium have many advantages since they are physiologically more homogeneous and controllable. Plant cell cultures may also use as model systems for the study of metabolite pathways because the initiation of cell growth in suspension culture leads to rapid increase of biomass and a condensed biosynthetic cycle. Whereas, secondary metabolite synthesis may occur in specific cell and organ types and at a certain time period in whole plants. Because of these advantages, cell suspension cultures are frequently used in plant tissue culture studies [10].

In recent years, there has been a significant increase in studies on nanoparticular systems in areas such as medicine, food and agriculture. In these systems, the continuous release of active chemicals from nanomaterials can increase the effectiveness of them by offering better results with lower doses and number of applications [11]. Although different nanoparticular systems (metalic, polymeric, protein etc.) have various features, especially polymeric nanoparticles with high stability in biological fluids are preferred. This is because of polymeric nanoparticles which are obtained from biodegradable materials and it makes them possible to release the active ingredient in the target tissue by biodegradation. The Poly- (D,L-lactic-co-glycolic acid) (PLGA) is the most commonly used polymer in the production of polymeric nanoparticles. It has been determined that

the systems created by loading the active substances into the PLGA nanoparticle have increased the pharmacokinetic properties, therapeutic indices and biocompatibilities of the active substances compared to the free form [12-15].

European Medicine Agency (EMA) and Food and Drug Administration (FDA) approved that PLGA nanoparticles can be used for the preparation of DNA, RNA, peptide and protein carrier systems in many different structures. Additionally, having long-term clinical experience, being biocompatible and biodegradable are among the advantages of PLGA [13, 16]. For these reasons, PLGA is accepted and used as the gold standard of biodegradable polymers [17].

Although there are many studies about nanoparticular systems of PLGA [11, 18, 19], a controlled delivery system where 2,4-D is used as an active ingredient have not been found in the literature.

Thus, in this study whose originality was demonstrated, the characterization and *In vitro* release of the 2,4-D loaded nanoparticular system which prepared by a single emulsion solvent evaporation method using PLGA copolymer was investigated. Then, the optimized nanoparticles were added to the nutrient medium in *Medicago sativa L.* suspension cultures and their effect on time-dependent biomass yield was investigated.

Materials and Methods

Materials

PLGA (lactide:glycolide = 50:50; inherent viscosity 0.45–0.60 dL/g, Mw ~ 38-54 kDa,), polyvinyl alcohol, 2,4-dichlorophenoxyacetic acid, ethanol, dichloromethane (DCM), NaCl, NaOH, HCl, $Na_2HPO_4.2H_2O$, NaH_2PO_4, Murashige and Skoog (MS) medium were purchased from Sigma Aldrich (St. Louis, USA). All the chemicals and solvents used in nanoparticle preparation were of analytical grade and used without further purification.

Methods

Preparation of nanoparticles

2,4-D loaded PLGA nanoparticles were prepared with a single emulsion solvent evaporation method according to the literature [20]. Firstly, 2,4-D and PLGA were dissolved in ethanol and DCM, respectively. After that, solutions were mixed for the preparation of the homogeneous mixture. Mixture was added to 3% (w/v) polyvinyl alcohol (PVA) solution drop by drop. Sonication was done to emulsify the aqueous phase and the organic phase. The emulsified solution was added to 0.1% (w/v) PVA solution so

that nanoparticles were formed in the solution. Organic solvents (DCM and ethanol) were evaporated incubating at room temperature overnight, then the nano-suspensions were centrifuged at 10,000 g for 40 minutes at 4 °C (Beckman Coulter Allegra X-30R Centrifuge, Germany). The supernatant was collected and the nanoparticles were washed three times with 35 mL of distilled water. The collected supernatants were used in indirect measurement to determine encapsulation efficiency. The prepared 2,4-D loaded PLGA nanoparticles were freeze-dried for 48 hours at 0.01 mbar at -70 °C without any cryoprotectant. All lyophilized nanoparticles were stored at -80 °C until use.

UV-Vis spectroscopy, size and zeta potential, FT-IR and *In vitro* drug release analyses were used for characterization of nanoparticles. Reaction yield (RY), encapsulation efficiency (EE) and drug loading capacity (DL) were determined indirectly by spectrophotometric UV measurement of the supernatant obtained after centrifugation. Particle size and zeta potential values of 2,4-D loaded PLGA nanoparticles were determined by dynamic light scattering and electrophoretic light scattering techniques, using Zetasizer (Nano ZS, Malvern Instruments, UK). Functional groups present on the surface of nanoparticles were analyzed by Fourier-transform infrared spectroscopy in universal attenuation total reflectance (ATR) mode. *In vitro* release studies were performed at 37 °C at pH 7.4 during 30 days.

Characterization of 2,4-D loaded PLGA nanoparticles

Reaction yield (RY), encapsulation efficiency (EE), drug loading capacity (DL)

Reaction yield is obtained by calculating the ratio of solid nanoparticles obtained by freeze-drying to the total amount of 2,4-D and PLGA used. It is calculated as given in Equation 1. The amount of 2,4-D in the nanoparticle was found by subtracting the amount of 2,4-D found in the supernatant from first 2,4-D amount used. Encapsulation efficiency was calculated with indirect method according to Equation 2. Drug loading capacity was calculated with the ratio of the amount of substance in the nanoparticle calculated by the encapsulation efficiency to the total amount of nanoparticle obtained (Equation 3).

$$Reaction\ Yield(\%) = \frac{Obtained\ NPs\ (mg)}{Amounts\ of\ Total\ Material\ (2{,}4D+PLGA)}\ x\ 100 \qquad (1)$$

$$Encapsulation\ Efficiency\ (\%) = \frac{2{,}4-D\ into\ NP\ (mg)}{Amounts\ of\ Total\ 2{,}4-D\ (mg)}\ x\ 100 \qquad (2)$$

$$Drug\ Loading\ (\%) = \frac{2{,}4-D\ into\ NP\ (mg)}{Amounts\ of\ Total\ NP\ (mg)}\ x\ 100 \qquad (3)$$

Particle size and zeta potential

The dynamic light scattering and electrophoretic light scattering techniques were used for the analysis of particle size and zeta potential values of 2,4-D loaded PLGA nanoparticles, using Zetasizer (Nano ZS, Malvern Instruments, UK) [21]. 2,4-D loaded PLGA nanoparticles solution diluted with ultra-pure water was used for the measurements. Each measurement was done in triplicate at 25 °C.

FT-IR analysis

Fourier-Transform Infrared spectroscopy was used for the functional groups analysis on the surface of nanoparticles by in universal attenuation total reflectance (ATR) mode [22]. The FTIR spectra of 2,4-D, PLGA and 2,4-D loaded PLGA nanoparticles were obtained with 32 scans per sample and resolution of 4 cm^{-1} in the region of 650 to 4000 cm^{-1}.

In vitro drug release study

The *in vitro* release study of 2,4-D loaded PLGA nanoparticles was carried out according to the modified version of the dissolution method [23]. Firstly, 5 mg of lyophilized 2,4-D loaded PLGA nanoparticles were suspended in 10 mL of phosphate buffer saline at pH 7.4 by vortexing until particles were fully dispersed. 10 mL samples were collected at a specific period of time then, centrifuged at 10,000 g, +4 °C for 20 min. Supernatants were analyzed by UV-spectrophotometer for the measurement of 2,4-D per mL. The 10 mL fresh buffer was added to the solution after each sampling.

Callus culture of alfalfa

The surface sterilization of alfalfa seeds was performed by using 50% commercial bleach (5% (v/v) NaOCI) for 10 minutes and then rinsing with sterile distilled water three times. After that, they were placed on MS medium (Murashige & Skoog) with 3% (w/v) sucrose and 0.8% (w/v) agar. The pH of the medium was adjusted to 5.6 before sterilization at 121 °C for 25 min. Hypocotyls were removed from 7-day-old aseptic seedling and used as the explant. The explants (0.5-1.0 cm) were cultured on MS medium with 3 mg/L 2.4-D and 0.5 mg/L BAP for callusing. Cultures were incubated at 25 °C in the dark. After 6 weeks, cell suspension cultures were initiated with 1 g of friable callus.

Effect of 2,4-D loaded PLGA on cell biomass

Callus were transferred to 30 mL MS liquid medium containing 4 mg/L 2,4-D or 2,4-D loaded PLGA nanoparticles in Erlenmeyer flasks. The cultures were shaken continuously at 110 rpm in an orbital shaker and were kept in the growth chamber at 24±2 °C in the

dark. Twelve replicates were used for each treatment. The cell biomass was determined during 28 days by measuring every 7 days. Three replicates were used to each measurement. The cell biomass yield was measured in term of fresh weight and was determined using the formula in Equation 4.

$$The\ cell\ biomass\ yield = \frac{Final\ weight - Initial\ weight}{Initial\ weight}\ x\ 100 \quad (4)$$

Statistical Analyses

The data were presented as means ± standard deviation (SD) of at least three separate experiments performed in triplicate. Statistical analysis was performed using GraphPad Prism software version 6 (GraphPad Software, La Jolla, CA).

Results

Nanoparticle analysis

Characterization studies of synthesized and optimized nanoparticles were carried out by size analysis, zeta potential measurement, FT-IR and *in vitro* release studies. Particle size distribution and zeta potential measurement of the 2,4-D loaded PLGA nanoparticles were performed with the Zeta Sizer (Malvern ZEN 3600 Nano iS10). The average particle size of the nanoparticles were measured as 181.7±3.74 nm, the mean zeta potential value was -18.3±1.48 mV and the multiple distribution indices determining the size distribution were measured as 0.081. EE was found as a 63.82%, RY 60.73% and DL 10.51 %. Table 1 shows the results regarding the optimized 2,4-D loaded nanoparticles.

Table 1 Physicochemical characterization results of 2,4-D loaded PLGA nanoparticles

	RY (%)	**EE (%)**	**DL (%)**	**Size (nm)**	**Zeta (mV)**	**PDI**
2,4-D Loaded PLGA Nanoparticles	60.73	63.82	10.51	181.7±3.74	-18.3±1.48	0.081

FT-IR spectra of free 2,4-D molecule, PLGA and 2,4-D loaded PLGA nanoparticles were plotted to determine polymer interactions with 2,4-D in the nanoparticle formation process. As a result of the examination of the FT-IR spectra , FT-IR spectra of the nanoparticles were similar to the PLGA spectrum and there is no extra band (belonging

to any adsorption or a newly formed bond) observed. The result indicate that the 2,4-D molecule is effectively loaded to nanoparticles (Figure 1).

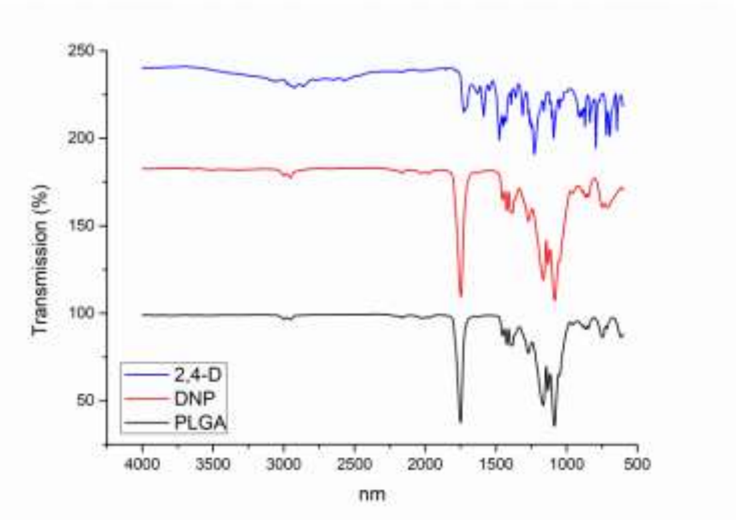


Figure 1 FT-IR Spectra of 2,4-D, PLGA and 2,4-D loaded PLGA nanoparticles (DNP)

The release experiment of the nanoparticles, which were suspended in PBS buffer (pH 7.4) and allowed to shake horizontally at 200 rpm at 37 °C, were performed for a period of 30 days. The samples were centrifuged at 10,000 g for 20 minutes at certain times and the 2,4-D amount released calculated by reading the UV values of the upper phases at 284 nm in the UV spectrophotometer. As a result of the release experiments it is observed that the 2,4-D contained in the nanoparticles was released slowly and in a controlled manner and released a total of 37.10% after 30 days.

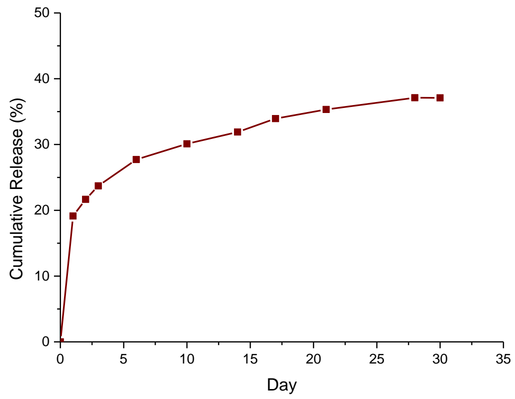


Figure 2 *In vitro* drug release of 2,4-D loaded PLGA nanoparticles

Preparation of nanoparticular systems was carried out by single emulsion (o/w) solvent evaporation method [24]. Reaction yield (RY), entrapment efficiency (EE), drug loading

(DL), particle size (Z-Ave), polydispersity index (PDI) and zeta potential (mV) analyzes were examined, and FT-IR and *in vitro* release analyzes were performed for characterization. Various syntheses have been made for nanoparticle characterization and used in tissue culture studies by mixing particles of similar properties that provide ideal results for all parameters. In optimized nanoparticles; RY 63.16%, EE 46.26%, DL 10.51%, size 181.7 nm, zeta potential value -18.3 mV and PDI value as 0.081 was determined. The analysis of the FT-IR spectra obtained, it was observed that the optimized nanoparticles have PLGA properties and this result shows that, the 2,4-D is effectively encapsulated in to the nanoparticular system. In the emission study, it was observed that the 2,4-D emission occurred slowly for 30 days and at the end of this period, it released 37.10%.

Evaluation of cell biomass yield

One of the most important disadvantages of PGR application in agriculture is that these compounds degrade rapidly under high light and temperature conditions, leading to a loss of biological activity. Additionally, most of the PGRs can be phytotoxic when used in high concentrations. Therefore, development of controlled release systems for PGRs are extremely important [25]. In this study, the effects of 2,4-D loaded PLGA nanoparticles on biomass yield in alfalfa suspension cultures were evaluated. Changes in biomass yield in cell suspension cultures were determined 4 times (every 7 days) until day 28. Biomass yields were calculated using the formula in Equation 4 by data fresh weight of biomass obtained from cell suspension culture every 7 days. As a result of the first week of the treatment, the average biomass yield in MS medium containing free 2,4-D was measured as 104%. The average biomass yield was measured as 148% in MS medium with 2,4-D loaded PLGA nanoparticles. At the second week of culture, the average amount of biomass in MS medium with free 2,4-D was 154% while biomass yield in 2,4-D loaded PLGA nanoparticles was 231%. Biomass yield values obtained at the end of the third week was 113% for free 2,4-D; It has been determined as 264% for 2,4-D loaded PLGA nanoparticles treatments. The biomass yields in the fourth week were determined as 138% and 304% in free 2,4-D and 2,4-D loaded PLGA nanoparticles treatments, respectively.

The results showed that the increase of biomass yield in free 2,4-D reached the highest value in the 2nd week. The increase of biomass was lower in the 3rd and 4th weeks compare with the 2nd week. However, the increase of biomass yield has continued in 2,4-

D loaded PLGA nanoparticles applications regularly. As a result of these calculated values, it was determined that the growth rate of 2,4-D loaded PLGA nanoparticles was more effective than free 2,4-D (Fig 3).

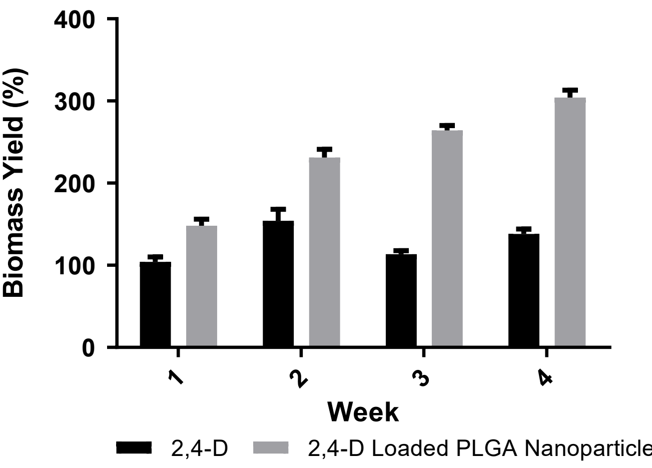


Figure 3 Effects of 2,4-D and 2,4-D loaded PLGA nanoparticles on *M. sativa* biomass

During cell culture measurements, it was observed that the bulk of the cells obtained from the medium with free had a darker colour (Fig 4). Supernatant could be darker due to the cell death. While the cells were slowly exposed to 2,4-D in the nanoparticle release system, they were exposed directly 2,4-D in the medium with free 2,4-D. It is known that high concentrations of 2,4-D may have a toxic effect. The results thought that free 2,4-D caused cell death in MS medium.

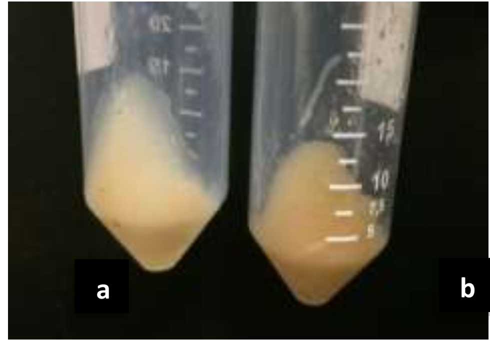


Figure 4 Biomasses obtained from media containing 2,4-D loaded PLGANP (a) and free 2,4-D (b)

Discussion

As it is known, polymeric nanoparticles have advantages such as increasing the solubility of the hydrophobic molecules in water, increasing their biocompatibility, enabling them to be active in the biological system for a longer time and at lower dose [26]. Due to these

advantages, it has been reported that many hydrophobic molecules are loaded into nanoparticular systems, thus increasing their biological activities [15, 27-29]. There are many studies on nanoparticular systems in mammalian cell cultures and nanoparticles have been used quite successfully until now in mammalian cell cultures and drug targeting systems [30, 31]. Recently, applications of nanoparticles on plants have gained popularity. The use of nanotechnology in agriculture aims to minimize the use of herbicides, pesticides and fertilizers while increasing agricultural production [25]. By encapsulating various chemicals used in agriculture into polymeric nanoparticles, undesirable toxic effects on organisms can be reduced and the thermal and photochemical stability of the encapsulated active ingredient can be increased [32]. Polymeric nanocarriers are able to control the delivery of chemicals to plants and reduce excess run-off [33]. In addition, nanoparticles are ideal materials due to their low cost, biocompatibility and biodegradability [25].

Callus culture is considered as an important starting material for the production of large amounts of biomass in plant biotechnology studies [34]. However, in some studies on PGRs, it is known that PGRs given after optimum dose negatively affect biomass. For example, Ma et al. reported the negative effects of GA3 over 50 µM in *Leymus chinensis* [35]. Therefore, the use of various PGRs in nanocarriers can positively affect plant growth and biomass by preventing the achievement of supra-optimal levels by enabling the slow and sustained release of the active substance [11].

The effect of various plant regulators and particulate systems on cell culture studies in recent years has been studied. In a study using methyl jasmonate-loaded PLGA nanocarriers to improve natural defences in *Vitis vinifera*, Chronopoulou et al. showed that methyl jasmonate-loaded PLGA NPs react faster compared to free MeJA, encapsulation in PLGA NPs significantly increased MeJA cell uptake and activation of MeJA-induced responses [36]. In another study where PLGA-block-PEG Copolymers were used as carriers for controlled release of herbicides into the soil, in the 3-month release test, it was observed that almost 90% of the loaded herbicides were slowly released into the soil. This is very important for agriculture because the average growing season of cultivated plants is about a few months [37]. Studies on *Phaseolus vulgaris* have been performed in chitosan-based nanoparticles prepared as carrier systems of GA3. In the study, GA3, Alginate/Chitosan-GA3 NP and Chitosan/Tripolyphospathe-GA3 NP

were compared and ALG/CS-GA3 showed stronger effects in terms of leaf development and carotenoid levels [38]. Pereira et al. synthesized GA3 nanoparticles with γ-PGA and Chitosan as a plant hormone carrier. The nanoparticular system was more effective than free hormone in *Phaseolus vulgaris* seeds and accelerates germination within 24 hours after treatment and increases leaf area and root development (including the formation of lateral roots) [39].

There are a lot of studies on the loading of molecules with low solubility and stability in the physiological environment in to the nanoparticular systems [26, 40-43]. These systems can improve the distribution of the molecule more effectively and more efficiently in nanoparticles that can be controlled and can release for a long time. A few promising studies have evaluated the use of PLGA nanoparticle in plants. Atrazine (ATZ), was encapsulated into PLGA to prevent damage to the environment. When the release profiles of ATZ loaded PLGA-NPs were examined, it was observed that they showed a much slower release compared to the pure herbicide [19]. In another study with ATZ, potato plants were used as biological models and herbicidal activity of designed PLGA nanoherbicides were evaluated. As a result of in vitro findings, it was determined that as the ATZ concentration increased in PLGA nanoparticles, potato plants experienced a significant decrease in root length, fresh weight, dry weight and leaf number. It was observed that the root length was affected the most among these parameters. Thus, it has been proposed that PLGA nanoherbicides can be used as an alternative method to prevent weed growth [44]. Tong et al. stated that nanoparticles obtained in the formulation in which metolachlor was encapsulated into mPEG-PLGA increased the solubility of hydrophobic metolachlor in water and did not contain any active substance on its surface. Nanoparticular systems have been proven to have a higher biological effect on *Oryza sativa* and *Digitaria sanguinalis* compared to free metholachlor. In addition, in the cytotoxicity test, it was observed that the NP toxicity decreased on the preosteoblast cell line [18]. These studies showed that polymeric nanoparticles caused less environmental damage when used as a pesticide carrier and contributed to plant growth by eliminating various damages of herbicides.

Considering unique properties of nanoparticles and the lack of nanoparticular systems with 2,4-D in the literature; 2,4-D, which is a water-insoluble and toxic molecule, was added to nanoparticular systems, thus making it soluble in tissue culture studies. At

present study, free 2,4-D and 2,4-D loaded PLGA nanoparticles were applied to *M. sativa* cell cultures and their effects on biomass yield were compared. The addition of 2,4 D loaded PLGA nanoparticles to the medium was much more effective compared to the free 2,4-D added medium, thanks to the controlled release of 2,4-D in the particulate system.

Conclusion

In this study, it is aimed to reduce the adverse effects of 2,4-D molecule, which is known to show a high concentration level of toxic effects, by nanoparticular systems. We also intended to increase its effectiveness by remaining intact for a longer time thanks to the controlled release mechanism of the substance. It is known that nanoparticle release systems increase the effect of active subtances on cells. However, a study on the application of 2,4-D using nanoparticular release systems in plant cell cultures has not been reached. In this study, the effect of applying 2,4-D with nanoparticle release systems to plant cell cultures was demonstrated for the first time. After reaching the optimized formulation of the 2,4-D loaded PLGA nanoparticles system, the effect of 2,4-D loaded PLGA NPs on plant cell growth was investigated. At the end of the study, it was observed that the addition of 2,4-D to the medium using the nanoparticles drug release system was increased the amount of cell biomass. It has been observed that the particulate system was much more effective than the free substance at the study.

It is thought that the optimized system, which was loaded into the particular system and whose water solubility increased and positive results were obtained in plant cell culture studies, may be a reference for drug delivery systems. These systems can be used as an alternative method for the addition of compounds with a high concentration of toxic effects to the medium. In nanoparticle systems, the amount of oscillation can be adjusted and time-dependent manipulation of cells can be performed without the need for subculture.

Abbreviations

2,4-D; 2,4-Dichlorophenoxyacetic Acid, PLGA; Poly (lactic-co-glycolic) acid, EE; Encapsulation efficiency, RY; Reaction yield, DL; Drug loading, FT-IR; Fourier-transform infrared spectroscopy, MS medium; Murashige and Skoog medium, DNP; 2,4-D loaded PLGA nanoparticles.

Funding

There is no fundings for this project.

References

1. Sezer, K., Şeker Pancarı Küspesinden Elde Edilen Aktİf Karbonun Atiksulardaki 2,4-D Ve Metribuzin Pestisitlerinin Adsorpsiyonda Kullanilabilirliğinin Araştırılması, in Kimya Mühendisliği Ana Bilim Dalı. 2010, Hacettepe Üniversitesi.
2. Turan, G.T., 2,4-Diklorofenoksiasetik Asitin (2,4-D) Kılıçkuyruk (*Xiphophorus Hellerii*) Balıklarının Bazi Dokularında Asetiklorinesteraz (AChE) Aktivitesi Üzerine Etkileri, in Biyoloji Ana Bilim Dalı. 2012, Marmara Üniversitesi.
3. Yalçınkaya, M., Bir Herbisit Olan 2,4-D (Diklorofenoksiasetik Asit)'nin *Poecilia reticulata* P.,1859'da Medulla Spinalis Üzerine Etkileri, in Biyoloji Ana Bilim Dalı. 2006, Ege Üniversitesi.
4. Gopi, C. and T. Vatsala, In vitro studies on effects of plant growth regulators on callus and suspension culture biomass yield from Gymnema sylvestre R. Br. African Journal of Biotechnology, 2006. 5(12).
5. Dalila, Z.D., H. Jaafar, and A.A. Manaf, Effects of 2, 4-D and kinetin on callus induction of Barringtonia racemosa leaf and endosperm explants in different types of basal media. Asian Journal of Plant Sciences, 2013. 12(1): p. 21-27.
6. Malik, S.I., et al., Effect of 2, 4-dichlorophenoxyacetic acid on callus induction from mature wheat (Triticum aestivum L.) seeds. International Journal of Agriculture & Biology [Internet], 2003: p. 156-159.
7. Tofanelli, M.B.D., P.L. Freitas, and G.E. Pereira, 2, 4-dichlorophenoxyacetic acid as an alternative auxin for rooting of vine rootstock cuttings. Revista Brasileira de Fruticultura, 2014. 36(3): p. 664-672.
8. Cassanego, M., A. Droste, and P. Windisch, Effects of 2, 4-D on the germination of megaspores and initial development of Regnellidium diphyllum Lindman (Monilophyta, Marsileaceae). Brazilian Journal of Biology, 2010. 70(2): p. 361-366.
9. Surmuş Asan, H., Hypericum Retusum Aucher'in Kallus Ve Hücre Süspansiyon Kültürlerinde Hiperisin Türevlerinin Üretilmesi. 2013, Dicle Üniversitesi: Fen Bilimleri Enstitüsü.
10. Phillips, G.C., J.F. Hubstenberger, and E.E. Hansen, Plant regeneration by organogenesis from callus and cell suspension cultures, in Plant Cell, Tissue and Organ Culture. 1995, Springer. p. 67-79.
11. Santo Pereira, A.d.E., H.C. Oliveira, and L.F. Fraceto, Polymeric nanoparticles as an alternative for application of gibberellic acid in sustainable agriculture: a field study. Scientific reports, 2019. 9(1): p. 1-10.
12. Alimohammadi, Y.H. and S.W. Joo, PLGA-based nanoparticles as cancer drug delivery systems. Asian Pac J Cancer Prev, 2014. 15: p. 517-535.
13. Danhier F, A.E., Silva JM, Coco R, Le Breton A, Preat V., Plga-Based Nanoparticles: An Overview Of Biomedical Applications. Journal of Controlled Release, 2012. 161: p. 505-522.
14. Panyam, J. and V. Labhasetwar, Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Advanced drug delivery reviews, 2003. 55(3): p. 329-347.
15. Duranoğlu, D., et al., Synthesis of hesperetin-loaded PLGA nanoparticles by two different experimental design methods and biological evaluation of optimized nanoparticles. Nanotechnology, 2018. 29(39): p. 395603.
16. Makadia HK, S.S., Poly Lactic-Co-Glycolic Acid (Plga) As Biodegradable Controlled Drug Delivery Carrier. Polymers, 2011. 3: p. 1377-1397.
17. Kutlu, C., Beyİn Tümörlerİnİn Tedavİsİ İçİn Çİft Etkİlİ Doku İskelesİ-Nanopartİkül Sİstemlerİnİn Gelİştİrİlmesİ in Biyomühendislik. 2011, Hacettepe Üniversitesi.
18. Tong, Y., et al., Polymeric nanoparticles as a metolachlor carrier: water-based formulation for hydrophobic pesticides and absorption by plants. Journal of agricultural and food chemistry, 2017. 65(34): p. 7371-7378.

19. Chen, X.-t. and T. Wang, Preparation and characterization of atrazine-loaded biodegradable PLGA nanospheres. Journal of Integrative Agriculture, 2019. 18(5): p. 1035-1041.
20. Kızılbey, K., Optimization of Rutin-Loaded PLGA Nanoparticles Synthesized by Single-Emulsion Solvent Evaporation Method. ACS Omega, 2019. 4(1): p. 555-562.
21. Kamaly, N., et al., Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. Chemical reviews, 2016. 116(4): p. 2602-2663.
22. Van de Voort, F., Fourier transform infrared spectroscopy applied to food analysis. Food Research International, 1992. 25(5): p. 397-403.
23. Heng, D., et al., What is a suitable dissolution method for drug nanoparticles? Pharmaceutical research, 2008. 25(7): p. 1696-1701.
24. G Nava-Arzaluz, M., et al., Single emulsion-solvent evaporation technique and modifications for the preparation of pharmaceutical polymeric nanoparticles. Recent patents on drug delivery & formulation, 2012. 6(3): p. 209-223.
25. Pascoli, M., et al., State of the art of polymeric nanoparticles as carrier systems with agricultural applications: a minireview. Energy, Ecology and Environment, 2018. 3(3): p. 137-148.
26. Makadia, H.K. and S.J. Siegel, Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers, 2011. 3(3): p. 1377-1397.
27. Men, K., et al., Nanoparticle-delivered quercetin for cancer therapy. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 2014. 14(6): p. 826-832.
28. Arasoglu, T., et al., Synthesis, characterization and antibacterial activity of juglone encapsulated PLGA nanoparticles. Journal of applied microbiology, 2017. 123(6): p. 1407-1419.
29. Bishayee, K., A.R. Khuda-Bukhsh, and S.-O. Huh, PLGA-loaded gold-nanoparticles precipitated with quercetin downregulate HDAC-Akt activities controlling proliferation and activate p53-ROS crosstalk to induce apoptosis in hepatocarcinoma cells. Molecules and cells, 2015. 38(6): p. 518.
30. Stella, B., et al., Design of folic acid-conjugated nanoparticles for drug targeting. J Pharm Sci, 2000. 89(11): p. 1452-64.
31. Bilemsoy, E., Development Of Nonsurfactant Cyclodextrin Nanoparticles Loaded With Anticancer Drug Paclitaxel. Journal of Pharmaceutical Sciences, 2007. 97(4): p. 1519–1529.
32. Cicek, S. and H. Nadaroglu, The use of nanotechnology in the agriculture. Advances in Nano research, 2015. 3(4): p. 207.
33. Chhipa, H., Nanofertilizers and nanopesticides for agriculture. Environmental chemistry letters, 2017. 15(1): p. 15-22.
34. Khalil, S.A., N. Ahmad, and R. Zamir, Gamma radiation induced variation in growth characteristics and production of bioactive compounds during callogenesis in Stevia rebaudiana (Bert.). New Negatives in Plant Science, 2015. 1: p. 1-5.
35. Ma, H.-Y., et al., A Multi-year Beneficial Effect of Seed Priming with Gibberellic Acid-3 (GA 3) on Plant Growth and Production in a Perennial Grass, Leymus chinensis. Scientific reports, 2018. 8(1): p. 1-9.
36. Chronopoulou, L., et al., Microfluidic synthesis of methyl jasmonate-loaded PLGA nanocarriers as a new strategy to improve natural defenses in Vitis vinifera. Scientific Reports, 2019. 9(1): p. 1-9.
37. Lewicka, K., et al., Biodegradable Blends of Grafted Dextrin with PLGA-block-PEG Copolymer as a Carrier for Controlled Release of Herbicides into Soil. Materials, 2020. 13(4): p. 832.
38. Santo Pereira, A.E., et al., Chitosan nanoparticles as carrier systems for the plant growth hormone gibberellic acid. Colloids and Surfaces B: Biointerfaces, 2017. 150: p. 141-152.
39. Pereira, A., et al., γ-Polyglutamic acid/chitosan nanoparticles for the plant growth regulator gibberellic acid: Characterization and evaluation of biological activity. Carbohydrate polymers, 2017. 157: p. 1862-1873.

40. Kumar, M., Nano and microparticles as controlled drug delivery devices. J. Pharm. Pharm. Sci, 2000. 3(2): p. 234-258.
41. Pal, S.L., et al., Nanoparticle: An overview of preparation and characterization. Journal of Applied Pharmaceutical Science, 2011. 1(6): p. 228-234.
42. Danhier, F., et al., PLGA-based nanoparticles: an overview of biomedical applications. Journal of controlled release, 2012. 161(2): p. 505-522.
43. Mora-Huertas, C., H. Fessi, and A. Elaissari, Polymer-based nanocapsules for drug delivery. International journal of pharmaceutics, 2010. 385(1-2): p. 113-142.
44. Schnoor, B., et al., Engineering atrazine loaded poly (lactic-co-glycolic acid) nanoparticles to ameliorate environmental challenges. Journal of agricultural and food chemistry, 2018. 66(30): p. 7889-7898.

Research article

Ozata, E., Yield and Quality Stabilities of Waxy Corn Genotypes using Biplot Analysis. International Journal of Life Sciences and Biotechnology, 2021. 4(1): p. 61-89. DOI: 10.38001/ijlsb.811966

Yield and Quality Stabilities of Waxy Corn Genotypes using Biplot Analysis

Erkan ÖZATA [1]*

ABSTRACT

This study was conducted to determine the stability of the potential genotypes of waxy Corn (10 candidates, 2 standard varieties) in terms of yield, yield components and quality characteristics in various environments over a period of two years under Samsun conditions. Biplot analysis of GGE and AMMI was used to determine the stability of genotypes. Mean grain yields of genotypes ranged from 8560.6 to 17290.6 kg ha^{-1}, number of days to flowering from 71.3 to 77.5 days, plant height from 251.7 to 295.0 cm, the height of first ear from 85.3 to 98.3 cm, grain ear ratio from 81.3 to 85.5%, grain moisture content from 20.5 to 25.0%, single ear weight from 145 to 286.3 g, the number of ears per plant from 0.9 to 1.0 ear plant^{-1}, 1000 grain weight from 317.7 to 402.2 g, hectoliter from 76.9 to 79.3%, crude protein ratio from 9.4 to 10.4%, crude oil content from 3.3 to 5.0%, total starch ratio from 57.5 to 60.0%, carbohydrate ratio from 69.6 to 71.6%, and energy value from 383.8 to 393.7 kcal. The result of the analysis of variance showed that yield, yield components and chemical composition of corn varieties significantly (p>0.01) different between genotypes (G), environments (E) and genotype x environment (GE) interactions. A significant difference in yield, yield components, and quality characteristics of waxy Corn genotypes was shown by the biplot (AMMI and GGE) multivariate analysis. Compared with other genotypes evaluated, the yield and stability of the ADAX11 and ADAX18 genotypes were higher. In addition, in the scientific and precise assessment of the high yield, stability, and adaptations of the waxy Corn hybrids, the AMMI model and GGE biplot analysis provide great ease for Corn breeders.

ARTICLE HISTORY
Received
17 October 2020
Accepted
20 December 2020

KEYWORDS
Waxy Corn,
GGEBiplot,
AMMIBiplot,
Stability,
Waxy quality

Introduction

Waxy Corn (Zea mays L. ceritina) is one of the seven subspecies included in the Corn plant; according to USDA records, this type of Corn was discovered in China in 1908 and was first described by Collins in 1909[1]. Fresh waxy Corn is consumed in most Asian countries (China, Korea, Taiwan, Vietnam, Laos, Myanmar and Thailand) and the growing area continues to expand rapidly [2].

Starch, a complex carbohydrate produced by plants for long-term storage of excess glucose, is found in tiny granular structures in the roots, tubers and seeds of plants. Starch is produced in almost all plants and can be obtained by means of many plants for

[1] Blacksea Agrıculture Research Instıtute, Department of Field Crops, Samsun / Turkey
*Corresponding Author: Erkan ÖZATA, e-mail: erkan.ozata@tarimorman.gov.tr

commercial purposes, one of which is obtained by means of corn kernels (dent corn). The starch content in wet corn milling is around 66% of the dry weight. Starch can be measured as a percentage of raw Corn production, which makes starch a profitable material. The yield of starch can be obtained from the wet milling of dent corn, a variety widely grown for starch production. However, starch obtained from waxy Corn by wet milling method has a starch yield of about 90% of dry weight [3].

Some of the starches known as "waxy starches" derive from the endosperm tissue, which is waxy and contains a small amount of amylose in the granular composition (< 15%). Due to its crystalline nature, the energy requirements of waxy starches for gelatinization are very high [4]. In comparison to waxy starch, the amylose content in other types of starch is more than 30%. In addition, researchers have reported that this starch contains other polysaccharide molecules and is slightly deformed from other starches [5].

The starch content present in waxy Corn has a high molecular weight so that it is digested faster than starches of other types. The starch of waxy Corn can be extracted through a wet milling process. Heat treatment causes a loss of starch viscosity in common Corn, but since waxy Corn does not produce amylase, starch maintains its stickiness. Waxy Corn starch can therefore be modified and used in the food industry, in particular in the production of corn snacks with sauce [6]. Apart from food production, waxy Corn starch can be used in the textile industry, the production of glue, corrugated carton, the paper industry as well as animal nutrition due to its high feed quality [7,8].

The main objective of plant breeding is to develop new superior and high quality varieties. Therefore, plant breeders must look for the most appropriate breeding materials and methods to clearly present the results of scientific studies. A graphic technique is a tool commonly used to demonstrate findings obtained in Corn breeding experiments. The visual presentation of the results of the breeding study is essential to better convey the message to readers. Various graphic methods have been introduced and used by researchers as alternatives to traditional methods. The biplot is one of the most preferred techniques for visualizing the results of a breeding experiment.

The additive main effects and multiplicative interaction (AMMI) and genotype main effect (G) and genotype by environment (GE) interaction (GGE) biplot model are excellent tools for visual assessment to demonstrate the combining abilities of parents and crosses. The multivariate models have been frequently used by plant breeders to

estimate the adaptability and stability of Corn hybrids in breeding experiments [9,10,11,12,13,14,15].

The genotype of waxy Corn has been intensively cultivated in the production of roasted corn snacks and amylopectin starch in Turkey. However, the waxy Corn varieties used in agricultural production all come from abroad and are not officially registered in Turkey. Consequently, the breeding of new varieties of waxy Corn is required to meet domestic demand for waxy Corn. Research on waxy Corn is relatively new in Turkey and few studies have investigated the stability of yields and the genotypic quality characteristics of waxy Corn. This research was conducted to identify genotypes with high stability in yield, yield components and quality characteristics of potential waxy Corn genotypes in different environments in Samsun during the 2018 and 2019 Corn growing seasons.

Materials and Methods

The materials used in the experimental study consisted of 10 high-yield waxy Corn hybrids provided by the Corn Research Institute (MAEM) and 2 control varieties. The information on experimental plant material is given in (Table 1). Since no genotype of waxy Corn was recorded in Turkey, 2 genotype hybrid Corn varieties which were very common in Turkey were used as control treatments to compare yields of MAEM hybrid waxy Corn.

The soil in the experimental field had clay loamy texture, mildly alkaline, non-salty, mildly calcareous, and low organic matter content. The available phosphorus content for plants is low, while the potassium content is high (Table 2).

The experimental field was located in Samsun province which is characterized by a rainy and temperate climate. The relative humidity and temperature values during growing seasons (2018-2019) were relatively close to each other, whereas differed from the long term averages. Average temperature values in 2018 and 2019 were 1.0 and 1.5 °C higher than the long term averages. Total and monthly precipitation values of the study area were significantly different from the long term precipitation data (Figure 1)

Table 1 Descriptive characteristics of waxy Corn and dent corn hybrids investigated

Genotypes Code	Pedigrees		Origin	Cycles (Maturity)/Colors	Grain texture	Type
	Female Line	Male Line				
ADAX-2	915A	915E	MAEM/ Turkey	Yellow-Portugal/Medium	Waxy	Single Hybrid
ADAX-9R	DH-682	DH-680	MAEM/ Turkey	Yellow/Medium	Waxy	Single Hybrid
ADAX-11	DH-680	DH-684	MAEM/ Turkey	Yellow/Medium	Waxy	Single Hybrid
ADAX-11R	DH-684	DH-680	MAEM/ Turkey	Yellow/Medium	Waxy	Single Hybrid
ADAX-15	DH-681	DH-684	MAEM/ Turkey	Yellow/Medium	Waxy	Single Hybrid
ADAX-16	DH-681	915A	MAEM/ Turkey	Yellow-Portugal/Medium	Waxy	Single Hybrid
ADAX-17	DH-683	DH-682	MAEM/ Turkey	Yellow/Medium	Waxy	Single Hybrid
ADAX-18	DH-684	915A	MAEM/ Turkey	Yellow-Portugal/Medium	Waxy	Single Hybrid
ADAX-19	DH-685	DH-686	MAEM/ Turkey	Yellow/Medium	Waxy	Single Hybrid
Kalumet	Unknown		DU PONT/USA	Yellow/Late	Dent corn	Single Hybrid
P2088	Unknown		DEKALB/ USA	Yellow/Late	Dent corn	Single Hybrid

Table 2 Some of soil physical and chemical properties of the experimental field

Parameter	Bafra-2018	Bafra-2019
pH	7.57	7.65
P_2O_5 (kg da^{-1})	7.92	8.2
K_2O (kg da^{-1})	80	82
Organic Material (%)	2.29	2.34
Lime (%)	4.4	4.4
Electrical Conductivity (%)	0.03	0.03

The experimental layout was a Randomized Complete Block Design with three replications. The seeds were sown in May by hand as placing two seeds in each seed bed. Each plot had four rows with 5 m length and area of each plot was 14 m^2. The interrow and intrarow spacings were 0.7 and 0.18 m, respectively. The seedlings were thinned removing the weak plants when the plants reached V4-V6 leaf stage (40-50 cm). Fertilizer application rate, determined based on soil analyses, was 80 kg P ha^{-1} and 220 kg N ha^{-1}. All phosphorus and half of nitrogen were applied at planting in bands, and the rest of the nitrogen was given at V4-V6 stage [16]. The harvest was carried out by hand in each harvest period.

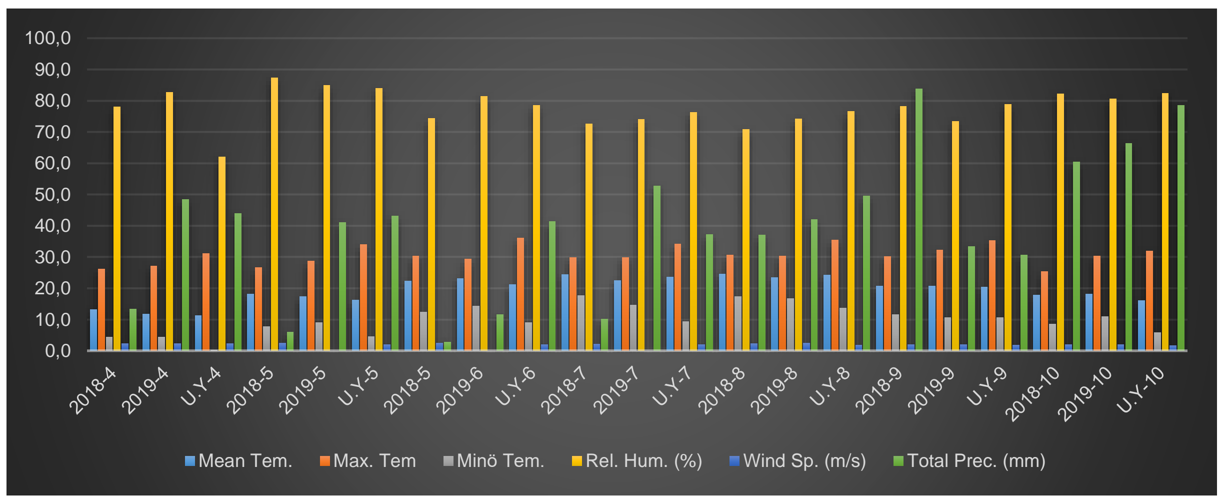


Fig 1 Some climate data for 2018 and 2019 growing seasons and long term (1985-2019)

Physical analyzes (1000 grain weight, hectoliter, grain moisture content, and grain/ear ratio) and morphological observations (plant height, height of the ears, number of ears per plant, flowering) were performed following the Corn Technical Instructions [16] The indicated an important role of pollen effect (xenia) in modifying biochemical compositions of Corn kernels [17]. The selected plants were covered using a kraft paper during the flowering period to prevent changes in the quality characteristics of waxy Corn genotypes due to the pollen effect. The iodine test, that is the most convenient method to determine the purity of the waxy, was used to determine the waxy characteristic of each inducer line and hybrid [18]. The purity of waxy was determined by counting 100 kernels in two replications. The kernels were moistened overnight, and a small portion was cut from the crown end of each kernel. The kernels were sprayed with a 0.5% iodine solution. The color of amylopectin starch in the waxy Corn temporarily turns to a brownish color following the exposure to iodine. In contrast, iodine solution causes a permanent blue or violet color in Corn containing amylose starch. Moisture content of grains was determined by drying the grains in an oven at 130±2ºC temperature under atmospheric pressure, until reaching a constant weight.

Dry matter weight, crude protein, crude oil, crude cellulose, crude ash and starch contents of Corn grains were determined by using both chemical and near infrared spectroscopy (NIRS) methods (FOSS, XDS). Carbohydrate content of samples were calculated using the following equation:

CAR (%) = 100 - (U% + PB %+ GB % + Cel.B % + Cen.B %)

In the equation, CAR (%) is the percent carbohydrates; U (%) is the moisture content of grains; PB (%) is the crude protein content; GB (%) is the crude oil content; Cel.B (%) is the crude fiber content and Cen.B (%) is the crude ash content [19].

The energy value was calculated using the Atwater general factor system Conversion factors were taken as 4.0 kcal g^{-1} for protein and carbohydrates, and 9.0 kcal g^{-1} for oil [20].

Energy = (Protein x 4) + (Oil x 9) + [(Carbohydrate- Dietary fiber) x4]

Statistical analysis

Statistical evaluations of the data were carried out using GenStat (12th Edition) software. Homogeneity and normality tests were applied prior to the combined variance analysis for the mean values obtained in different environments. The data was log-transformed in case of nonnormal distribution, before the analyses to linearize the relationships between the variables [21]. Multiple location test (MLT) data were analyzed without scaling ('Scala 0' option) to create environment centered (centering 2) GE biplots as described by Yan and Tinker 2006. For GE genotype assessment, GE genotype-oriented singular value segmentation (SVP = 1) was used along with the 'Mean versus stability' option of the GE biplot software, whereas environment-oriented singular value segmentation (SVP = 2) (Relation among testers) was used for the evaluation of the locations. The 'The-Which-won-where' option was used to identify the ideal genotype in a given environment and define the mega environments [22]. The significance in the dataset was assessed by analysis of variance (ANOVA) and the least significant difference (LSD) was used to differentiate the means in case ANOVA denoted significant differences.

Result

Yield, yield components and chemical composition performances of waxy hybrid corn genotypes

Grain yield is the most important selection criterion in Corn breeding. Variance analysis indicated that genotype (G), year (Y) and GY interaction had statistically significant impact ($p \leq 0.01$) on grain yield (Table 3). Grain yields ranged from 6481 (ADAX-2) to 17986 (Kalumet) kg ha^{-1} in the first year, and from 7518 (Adax17) to17623 (Kalumet) kg ha^{-1} in the second year. Flowering time is one of the selection criteria used to determine the maturation groups of genotypes (Table 3). Variance analysis showed that G, Y and GY interaction had a statistically significant effect ($p \leq 0.01$) on the number days to

flowering. Flowering time of genotypes ranged from 68.0 (ADAX17R) to 77.0 (Kalumet) days in 2018, and from 71.3 (ADAX17R) to 77.5 (Kalumet) days in 2019 (Table 3). Plant height is another important selection criteria in corn breeding. Variance analysis indicated significant ($p \leq 0.01$) differences in plant heights between G, Y and GY interaction. The plant height of the genotypes varied between 228.3 (ADAX17) and 286.7 (ADAX16) cm in the first year, and 251.7 (ADAX17R) and 295.0 (ADAX17) cm in the second year, and the mean plant length of genotypes was 264.2 cm (Table 3). Ear height is another morphological evaluation criterion in Corn breeding. Corn breeders co-evaluate two factors as the criteria in the ear height. The first one is that the ear height should be at the same height for all plants in a plot, and the second one is that the plant height and ear height ratio should be between 1/2 and 1/3. Higher or lower ear/plant ratio may cause plants to lie down and the harvesting becomes difficult. Variance analysis showed that the effect of Genotype, Year, and GY interaction ($p \leq 0.01$) on the ear heights was statistically significant (Table 3). The mean ear height varied between 68.3 (ADAX-11R) and 108.3 (ADAX-17) cm in the first year and between 81.7 (ADAX-2) and 110.0 (ADAX-18) cm in the second year. The mean first ear height in the experiment was determined as 90.9 cm. (Table 3). Grain/ear ratio value is another important characteristic determined in corn harvest, and is calculated together with the grain moisture content right after the harvest. The effects of Genotype and GY interaction on grain/ear ratio was statistically significant ($p \leq 0.01$), while the effect of year was insignificant. The grain/ear ratio in the experiment varied between 80.7 and 84.8% in the first year and between 79.9 and 86.8% in the second year, and the mean grain/ear ratio was determined as 82.3% (Table 4). Grain moisture content is of great importance in corn breeding and is one of the important selection criteria. Moisture content of grains is desired to be low in the Black Sea region, where the relative humidity is high. Therefore, corn breeders focus on genotypes with low grain moisture content or genotypes losing the grain moisture fast. The variance analysis showed that the effects of Genotype and GY interaction on grain moisture content was statistically significant at $p<0.01$ level, while the effect of year was important at $p<0.05$ level. The grain moisture content varied between 19.7 and 25.8% in the first year and between 21.2 and 25.8% in the second year (Table 4).

Ear weight is used as a selection criterion in Corn breeding. The ear weight ranged from 130.6 to 306.0 g in the first year, and from 105.5 to 266.7 g in the second year, and the mean ear weight for combined genotype was between 156.6 and 286.3 g (Table 4). The number of ears per plant varied depending on the subspecies of the Corn. The sugar corn and popcorn subspecies have the potential to form two or more ears in a plant, while dent corn and hard corn subspecies tend to form single ear per plant. The number of ears per plant in the experiment was between 0.89 and 1.01 ear $plant^{-1}$ in the first year, and between 0.84 and 1.01 ear $plant^{-1}$ in the second year, and mean number of ear per plant for the experiment was 0.97 (Table 4). On thousand grain weight is the common selection criterion used in all field crops by the plant breeders. The result of variance analysis showed that the differences in 1000 grain weight significantly ($p<0.01$) differed between Genotype, Year and GY interaction. The 1000 grain weight varied between 310.3 and 411.7 g in the first year of the experiment and ranged from 315.1 to 392.8 g in the second year. The mean 1000 grain weights of both years varied between 312.7 and 402.2 g, and the mean value for the experiment was calculated as 344.5 g (Table 5). Hectoliter weight refers to the weight of 100 L corn in kilogram, and is one of the most commonly used physical quality parameter which has a positive effect on corn quality. The shape, size and homogeneity of corn grains are the most important factors that determine the hectoliter weight of genotypes. Positive relationship has been reported between hectoliter weight and grain yield. The mean hectoliter weight of genotypes ranged from 77.2 to 79.6 kg h^{-1}, and the mean hectoliter weight in the experiment was determined to be 78.1 kg h^{-1} (Table 5). Carbohydrate content is one of the widely used grain chemical content properties in assessing the quality criteria in recent years. Low-energy and nutritious products have become highly preferred in the daily diet. The mean carbohydrate content of genotypes varied between 69.6 and 71.6%, with an average of 70.5% (Table 5). The energy value, considered a criteria to determine the consumption rates of the products in the daily diet, has become an important selection criterion for popcorn, sugar corn and waxy corn which are used in direct consumption. In general, the products with low calorie and high fiber content are recommended to include in the daily diet. The energy values of genotypes ranged from 383.8 to 393.7 kcal 100 g^{-1} (Table 5). The crude protein ratio is a selection criterion used to assess the quality of the product in all field crops. The mean crude protein ratio of genotypes varied between 8.7 and 10.5% in the first year and

between 9 and 10.4% in the second year (Table 6). Crude oil ratio varied depending on the subspecies of the waxy Corn. The mean crude oil ratios of genotypes varied between 3.21 and 4.73% in the first year, and between 3.31 and 3.98% in the second year, with a mean value of 3.60% (Table 6). Starch ratios of genotypes varied between 56.6 and 60.3% in the first year, and 57.7 and 60.0% in the second year, and the average starch ratio in the experiment was 58.8% (Table 6).

Table 3 The results of mean and multiple comparison tests for yield, flowering, plant height and ear heights of waxy Corn genotypes

	Yield			Flowering (day)			Plant Height			Ear Height		
Genotypes	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
ADAX-11	13772 bcd	10521 bcd	12147 bcd	68.0 e	72.5 d	74.0 bc	256.7 b-e	251.7	254.2 bcd	68.3 e	100.0 bc	84.2 c
ADAX-11R	11906 de	11171 bcd	11538 cde	68.0 e	72.8 cd	72.8 bc	256.7 b-e	278.3	267.5 ab	83.3 bcd	88.3 de	85.8 c
ADAX-15R	102.0 e	10009 cde	10110 ef	71.7 b	74.7 b	74.7 ab	246.7 d-g	273.3	260.0 abc	83.3 bcd	95.0 cd	89.2 bc
ADAX-16	15495 bc	10944 bcd	13220 bc	70.7 c	74.5 b	75.2 abc	286.7 a	258.3	272.5 a	86.7 bcd	108.3 ab	97.5 a
ADAX-17	1072.5 de	7518 f	9121 f	68.7 de	72.2 de	72.2 c	228.3 g	295.0	261.7 abc	108.3 a	88.3 de	98.3 a
ADAX-17R	123.2 de	9011 def	10662 def	68.0 e	71.3 f	73.5 bc	249.4 efg	251.7	250.6 cd	76.7 de	93.9 cd	85.3 c
ADAX-18	13116 de	12533 b	12824 bc	69.0 d	73.5 c	73.5 bc	276.7 abc	253.3	265.0 ab	80.0 cde	110.0 a	95.0 ab
ADAX-19	12516 cde	12223 bc	12370 bc	72.0 b	73.5 c	73.0 bc	251.3 d-g	270.0	260.6 a-d	81.7 bcd	96.3 cd	89.0 bc
ADAX-2	6481 f	10651 bcd	8566 f	69.0 d	72.5 d	73.3 bc	230.0 fg	253.3	241.7 d	91.7 bc	81.7 e	86.7 c
ADAX-9R	11702 de	78.9 ef	9795 ef	68.0 e	71.5 ef	72.8 bc	261.7 bcd	258.3	260.0 abc	80.0 cde	95.0 cd	87.5 c
KALUMET	17968 a	17623 a	17796 a	77.0 a	77.5 a	76.5 a	260.0 c-f	258.3	259.2 a-d	76.7 de	100.0 bc	88.3 bc
P2088	12400 ab	11585 bc	11993 b	72.0 b	74.7 b	74.3 abc	278.3 ab	268.3	273.3 a	93.3 b	103.3 abc	98.3 a
Mean	12384 A	10973 B	11678	70.7 B	76.7 A	73.8	257.9 B	264.2 A	261.0	84.2 B	97.6 A	
CV (%)	8.59	12.94	10.71	0.71	1.03	0.89	5.64	6.15	5.69	5.31	8.82	7.06
LSD (0.05) G	183.81**	240.12**	202.21**	0.83**	1.34	1.05**	24.3**	ns	24.54*	9.72**	12.56**	10.22**
LSD (0.05) Y		116.28*			0.49**			4.68**			4.60**	

Table 4 The results of mean and multiple comparison tests for grain/ear ratio, grain moisture, ear weight and NEPP of waxy Corn genotypes

	Grain/Ear ratio			Grain Moisture (%)			Ear Weight (g)			Number of Ear per plant (piece)		
Genotypes	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
ADAX-11	82.9 b	80.3 ef	81.6 cd	21.9 bcd	24.4 ab	23.2 bc	244.9 bc	157.8 bcd	201.3 b	0.94	0.97	1.0
ADAX-11R	80.7 d	81.8 cd	81.3 d	20.7 bcd	23.3 bcd	22.0 cd	205.8 cd	165.6 bcd	185.7 bc	1.03	0.96	1.0
ADAX-15R	82.9 b	81.8 cd	82.3 bcd	21.7 bcd	23.0 bcd	22.4 cd	180.6 de	142.4 cde	161.5 cd	0.96	0.98	1.0
ADAX-16	82.4 bcd	84.3 b	83.4 b	21.6 bcd	23.8 bcd	22.7 bc	263.1 ab	158.3 bcd	210.7 b	1.00	0.97	1.0
ADAX-17	83.0 b	79.9 f	81.5 cd	22.2 bc	23.4 bcd	22.8 bc	203.7 cd	105.5 f	154.6 d	0.89	1.01	1.0
ADAX-17R	83.1 bcd	81.3 de	82.2 cd	19.9 cd	23.1 de	21.5 de	197.0 cd	134.6 def	165.8 cd	0.95	0.95	0.9
ADAX-18	82.7 bc	82.4 c	82.5 bc	21.6 bcd	23.0 bcd	22.3 cd	205.6 cd	185.0 b	195.3 b	1.01	0.97	1.0
ADAX-19	82.5 bcd	82.3 cd	82.4 cd	22.8 b	22.7 cde	22.7 bc	214.6 bcd	182.2 b	198.4 b	1.00	0.94	1.0
ADAX-2	82.3 bcd	81.8 cd	82.0 cd	22.0 bcd	25.8 a	23.9 ab	130.9 e	159.1 bcd	145.0 d	0.94	0.98	1.0
ADAX-9R	82.7 bc	82.1 cd	82.4 bc	19.7 d	21.2 e	20.5 e	202.9 cd	116.6 ef	159.8 cd	0.96	0.97	1.0
KALUMET	84.8 a	86.2 a	85.5 a	25.8 a	24.1 abc	25.0 a	306.0 a	266.7 a	286.3 a	0.99	0.99	1.0
P2088	80.9 cd	82.6 c	81.8 cd	22.2 bc	23.1 bcd	22.7 bc	247.6 bc	173.9 bc	210.8 b	0.98	0.97	1.0
Mean	82.3 B	84.4 A	83.4	21.8 B	23.5 A		209.0 a	162.3 b	185.6	1.0	1.0	1.0
CV (%)	1.3	0.77	1.16	4.32	6.27	5.36	13.88	11.91	13.32	5.15	2.06	4.12
LSD (0.05) G	1.08**	1.46**	0.60**	1.69**	2.32**	1.91**	50.77**	32.68**	40.38**	ns	ns	ns
LSD (0.05) Y		ns			1.36*			19.97**			ns	

Table 5 The results of mean and multiple comparison tests of some quality and yield components of waxy Corn genotypes

	1000 grain weight (g)			Hectolitre (%)			Carbonhyrdrate (%)			Energy (kcal)		
Genotypes	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
ADAX-11	354.1 c	318.5 d	336.3 de	78.9 bc	78.5	78.7 abc	69.7 a-d	71.0 bcd	70.3	384.3 bc	387.4 bcd	385.9 de
ADAX-11R	374.1 b	343.8 c	358.9 c	80.1 a	79.1	79.6 a	70.6 abc	71.6 abc	71.1	392.3 ab	395.1 ab	393.7 a-d
ADAX-15R	331.5 d	325.2 d	328.3 efg	79.6 ab	78.2	78.9 abc	70.7 abc	70.3 bcd	70.5	385.0 a	387.9 bcd	386.5 abc
ADAX-16	349.8 c	315.8 d	332.8 def	78.7 bcd	76.9	77.8 cd	71.3 d	70.6 abc	70.9	389.5 ab	391.3 cd	390.4 b-e
ADAX-17	353.5 c	317.2 d	335.3 de	77.5 efg	77.2	77.3 d	71.1 d	70.3 cd	70.7	394.9 ab	387.6 bc	391.3 b-e
ADAX-17R	348.5 d	321.5 d	335.0 g	78.5 cde	78.1	78.3 bcd	69.2 bcd	70.6 cd	69.9	390.8 a	386.0 ab	388.4 ab
ADAX-18	354.1 c	322.7 d	338.4 d	77.7 d-g	78.1	77.9 cd	69.4 cd	69.8 abc	69.6	389.4 ab	389.6 cd	389.5 cde
ADAX-19	323.3 d	323.9 d	323.6 fg	79.2 ab	78.7	79.0 ab	70.0 cd	70.1 d	70.0	390.8 a	392.3 bcd	391.6 abc
ADAX-2	310.3 e	315.1 d	312.7 h	76.8 g	77.5	77.2 d	70.2 cd	70.9 abc	70.5	393.8 bc	385.3 bcd	389.6 ef
ADAX-9R	354.1 c	357.7 bc	355.9 c	78.7 bcd	77.2	77.9 cd	70.2 a-d	69.3 a	69.7	390.2 a	388.7 a	389.5 a
KALUMET	411.7 a	392.8 a	402.2 a	77.3 fg	78.8	78.1 cd	71.7 a	71.4 abc	71.6	385.2 c	388.2 d	386.7 f
P2088	378.8 b	370.7 b	374.7 b	78.0 c-f	79.3	78.7 abc	72.3 ab	70.7 ab	71.5	383.8 bc	383.8 bcd	383.8 de
Mean	353.6	335.4	344.5	78.4	78.1	78.3	70.5	70.5	70.5	389.2	388.6	388.9
CV (%)	1.39	3.07	1.82	0.81	1.7	1.34	1.35	1.04	2.04	0.99	0.78	0.89
LSD (0.05) G	8.30**	17.4**	12.82**	0.25**	ns	1.76**	1.61*	1.24*	ns	7.83*	5.13*	5.54**
LSD (0.05) Y		4.84**			ns			ns			ns	

Table 6 The results of mean and multiple comparison tests for some quality components of waxy Corn genotypes

	Crude Protein (%)			Crude oil (%)			Total Starch (%)		
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
ADAX-11	9.5 c	10.0 ab	9.7 c	3.25 fgh	3.45 bcd	3.4 efg	60.0 a	57.7	58.9 a-d
ADAX-11R	9.8 b	10.2 a	10.0 bc	3.15 gh	3.47 bcd	3.3 fg	59.9 a	58.6	59.3 abc
ADAX-15R	10.4 a	9.9 ab	10.2 ab	3.31 efg	3.41 cd	3.4 efg	57.8 c	58.8	58.3 b-e
ADAX-16	10.5 a	9.3 bc	9.9 bc	4.01 b	3.98 a	4.0 ab	58.3 bc	58.0	58.1 b-e
ADAX-17	10.4 a	10.4 a	10.4 a	3.68 cd	3.44 cd	3.6 de	59.3 ab	59.4	59.4 ab
ADAX-17R	10.2 a	10.4 a	10.3 a	3.64 bc	3.71 abc	3.7 c	59.2 ab	58.6	58.9 abc
ADAX-18	10.4 a	9.1 c	9.8 c	3.50 def	3.96 a	3.7 cd	59.2 ab	57.9	58.5 cde
ADAX-19	10.4 a	10.3 a	10.4 a	3.99 b	3.80 ab	3.9 bc	58.1 c	58.6	58.3 de
ADAX-2	9.2 d	9.5 bc	9.3 d	4.73 a	3.68 abc	4.2 a	56.6 d	58.4	57.5 e
ADAX-9R	10.3 a	10.2 a	10.3 ab	3.33 efg	3.40 cd	3.4 efg	58.5 bc	58.3	58.4 b-e
KALUMET	8.7 f	9.3 bc	9.0 e	3.21 h	3.31 d	3.3 g	60.3 a	59.6	59.9 a
P2088	9.0 e	9.5 bc	9.2 de	3.51 de	3.32 d	3.4 ef	60.0 a	60.0	60.0 a
Mean	9.9 A	9.8 bc	9.9	3.61 A	3.58 B	3.6	58.9	58.7	58.8
CV (%)	1.51	3.96	2.93	4.15	5.61	5.01	3.88	2.39	1.86
LSD (0.05) G	0.25**	0.64**	0.47**	0.26**	0.31**	0.6**	1.12**	ns	0.60**
LSD (0.05) Y		ns			ns			ns	

The additive main effects and multiplicative interactions (AMMI) analysis for various characteristics of corn hybrids

The results of AMMI variance analysis using the mean values of yield, yield components and quality characteristics of waxy Corn genotypes, and the statistical differences between genotype (G), environment (E), GE interactions were given in Table 7. The results indicated that 95.15% of the total variance in the experiment was attributable to environmental effect, followed by Genotype effect with 0.49% and GE interaction effect with 4.15%, respectively.

Table 7 Main and interaction effects for waxy Corn hybrids

Source	df	SS	MS	F	Explained %
Total	1079	97999153	90824		
Treatments	179	95427788	533116	235.68	
Genotypes	11	464687	42244	18.67**	0.49
Environments	14	90804025	6486002	689.85**	95.15
Block	75	705149	9402	4.16ns	0.74
G x E Interactions	154	4159077	27007	11.94**	4.36
IPCA1	24	4133151	172215	76.13**	99.38
IPCA2	22	17311	787	0.35ns	0.42
IPCA3	20	6305	315	0.14ns	0.15
IPCA4	18	1019	57	0.03ns	0.02
Residuals	70	1291	18	0.01ns	
Error	825	1866216	2262		

The effect of environment on grain yield, yield components and grain chemical composition was approximately 12 to 14 times higher than the effect of GE interaction. The influence level of factors was ranked as follow; Environment> GE interaction> Genotype.

Table 8 The means, variances and component scores obtained in AMMI model for waxy Corn genotypes

	Mean	Variance	IPCAe[1]	IPCAe[2]	IPCAe[3]	IPCAe[4]
1000 Grain Weight	344.5	0.00185	-0.09611	0.24876	-0.03726	0.00283
Carbohydrate	70.5	0.00002	0.06936	0.03084	0.03408	-0.00463
Crude Oil	3.6	0.00149	0.17588	-0.25927	-0.03515	-0.06068
Crude Protein	9.9	0.0005	0.1394	0.0652	0.05684	-0.1495
Ear Height	90.9	0.00064	0.04937	0.00018	-0.24318	0.02445
Energy	388.9	0.00005	0.08921	0.02713	0.03661	-0.03886
Ear Weight	185.6	0.00636	-0.35626	-0.06801	0.04159	0.00408
Flowering	73.8	0.00005	0.03915	-0.01087	0.03513	0.04158
Grain/Ear Ratio	83.4	0.00001	0.07223	0.0018	0.05001	0.01559
Hectoliter	78.1	0.00002	0.06335	0.01543	0.05787	0.00016
Grain Moisture	22.7	0.00035	0.02081	-0.05217	0.04757	0.19705
NEPP	1	0.00005	0.04591	0.00696	0.02588	0.00973
Plant Height	261	0.00022	0.04516	0.03882	-0.07455	-0.0016
Total Starch	58.8	0.00003	0.04776	0.02605	0.02264	0.02713
Yield	1167.8	0.00795	-0.40522	-0.07086	-0.01807	-0.06734

Table 9 The first four AMMI selections based on data for yield, yield components and chemical analysis of Corn grains

	Mean	Score	1	2	3	4
1000 Grain Weight	344.5	-0.0961	KALUMET	P2088	ADAX-11R	ADAX-9R
Carbohydrate	70.5	0.0694	ADAX-9R	ADAX-17	ADAX-11R	ADAX-15R
Crude Oil	3.6	0.1759	ADAX-2	ADAX-16	ADAX-19	ADAX-18
Crude Protein	9.9	0.1394	ADAX-17R	ADAX-9R	ADAX-11R	ADAX-19
Ear Height	90.9	0.0494	P2088	ADAX-16	ADAX-17	ADAX-18
Energy	388.9	0.0892	ADAX-17R	ADAX-9R	ADAX-19	ADAX-17
First Ear Weight	185.6	-0.3563	KALUMET	P2088	ADAX-11	ADAX-16
Flowering	73.8	0.0392	KALUMET	ADAX-16	ADAX-19	P2088
Grain/Ear Ratio	83.4	0.0722	ADAX-19	ADAX-11R	ADAX-16	ADAX-17R
Hectoliter	78.1	0.0634	ADAX-19	ADAX-11R	ADAX-17R	ADAX-9R
Grain Moisture	22.7	0.0208	KALUMET	ADAX-2	ADAX-11	P2088
NEPP	1	0.0459	KALUMET	ADAX-16	ADAX-19	P2088
Plant Height	261	0.0452	P2088	ADAX-17	ADAX-16	ADAX-18
Total Starch	58.8	0.0478	KALUMET	P2088	ADAX-17	ADAX-11R
Yield	1167.8	-0.4052	KALUMET	P2088	ADAX-16	ADAX-19

Table 10. The mean values of waxy Corn genotypes and IPCA scores

Genotype	Mean	IPCAg[1]	IPCAg[2]	IPCAg[3]	IPCAg[4]
ADAX-11	1214.7	-0.07685	0.00438	0.12004	0.03505
ADAX-11R	1153.8	-0.02137	0.11491	0.08404	-0.02764
ADAX-15R	1011.0	0.11136	0.09746	0.02358	0.02871
ADAX-16	1322.0	-0.03758	-0.11744	-0.11207	-0.0736
ADAX-17	912.1	0.18681	0.1252	-0.12574	0.06129
ADAX-17R	1066.2	0.11602	-0.01047	0.06936	-0.11679
ADAX-18	1282.4	-0.03918	-0.06727	-0.06989	-0.06177
ADAX-19	1237.0	-0.03304	-0.11776	0.05195	-0.10904
ADAX-2	856.6	0.27709	-0.22028	0.03686	0.14907
ADAX-9R	979.5	0.12037	0.17368	0.0229	-0.00755
KALUMET	1779.6	-0.44136	-0.00651	0.03854	0.10155
P2088	1199.3	-0.16228	0.02412	-0.13956	0.02072

The additive variance can be successfully separated from the multiplicative variance using the AMMI analysis which evaluates the major component axis’s together. The impact level of each variable in GE interaction can be determined by AMMI which captures a large part of the GE sum of squares (Gauch, 2006). The result indicated that

the first principal component (PC1) explained 99.38% of the total variation in the GE interaction ($p<0.01$) and the rest was explained by PC2 (0.42%), PC3 (0.15%) and PC4 (0.02%). Since the first two PCs accounted up more than 60% of the variance in the data (Yan et al. 2001), the AMMI model is considered adequately elaborating the variability in the GE interaction.

The AMMI model for genotype x environment interactions

The AMMI model is a bi-directional variance analysis in which the main effect of genotype and environment factors is explained in the x axis and the interaction effect is given in the y axis (Fig. 1). The stability of genotypes is interpreted by the distance to the x axis. Closer to the x axis means more stable the genotype. The genotypes are considered stable when they are located close to the x axis, in contrast, they are unstable when located far from the x axis. The AMMI analysis indicated that the genotypes had a high variation and scattered on different regions of the graph.

The AMMI analysis showed a positive interaction with grain yield and single ear weight, grain ratio and 1000 grain weight. In addition, a negative correlation was determined between grain yield and grain moisture, crude protein and crude oil contents (Figure 2). The results revealed that ADAX11, ADAX 9R and ADA18 waxy genotypes had well yield stability, while the stability of ADAX2 and ADAX17R waxy genotypes was low. Kalumet dent genotype was the most productive genotype, however the stability of Kalumet genotype was low, in contrast the stability of ADAX9 genotype was high, while the yield of ADAX9 was low.

The AMMI analysis demonstrated the first four genotypes to be preferred in terms of grain yield, yield components and chemical properties (Table 9). The first preference according to the grain yield and yield components of Corn varieties should be the standard dent corn varieties (Kalumet and P2088). Waxy genotypes were ranked the first places in terms of protein, oil and starch contents. The ADAX11, ADAX 17, ADAX19 and ADAX18 waxy genotypes were identified as genotypes that can be preferred in the first place (Table 9 and 10).

Adaptability analysis of tested genotypes based on GGE biplot

The polygons in a scatter plot (which-won-where) correlates genotypes with each other and the environment with respect to grain yield obtained in multiple environmental conditions and shows which genotype is more compatible with which environment. If the

genotypes and environments are placed in the same polygon, this indicates a positive interaction between genotype and environment. In contrast, if the genotype and environment are located in different polygons, this indicates a negative relationship between genotype and environment [22]. The lengths of the vectors and the angle between the vectors provide important clues in the interpretation of the graph [23]. The longer the vector length of the genotypes related to AEC abscess, the more stable the environment or the genotype. If the angle between the vectors is more than 90° the relationship is considered negative, and if the angle is less than 90° the relationship is considered positive. Positive interaction was determined between the grain yield and 1000 grain weight, grain moisture and single ear weight, while a negative relationship was obtained with grain yield and crude oil and crude protein contents (Figure 3 and 4).

The 'which-won-where' biplot is an important biplot graph explaining the mega environment and sector-genotype relationship. The polygonal corners drawn in the graph indicate that genotypes are the most preferred genotypes for that sector. The researcher stated that a positive interaction when genotypes and environments are placed in the same sector, a negative interaction when they are placed in different sectors, a mixed interaction when all are placed in the same sector [23]. The environments consisted of 4 intertwined mega circles in the scatter biplot, drawn using the mean values of grain yield, yield components and grain chemical compositions. Some characteristics located in a mega environment are also located in the clusters of other mega environments (Figure 4 and 5). This intersection cluster indicates an important relationship between the yield and the quality components.

The environments were divided into 5 sectors. The first sector composed of Kalumet> P2088> ADAX11 genotypes with grain yield, single ear weight, 1000 grain weight, grain moisture content, grain/ear ratio, flowering, plant height and first ear weight. The second sector included ADAX-9R>ADAX17>ADAX11R>ADAX15 genotypes and flowering characteristics. The crude protein content and ADAX2>ADAX16>ADAX19 genotypes were included in third sectors. The 4th sector included the oil content and the sector 5 included only the ADAX18 genotype. The BBE plot indicated that stability of the Kalumet variety considering the grain yield and yield components was high, while the stability of ADAX-2 genotype was low. The high number of sectors in a BBE plot is important in the breeding studies to assess the reliability of the selected environments.

Analysis of the high yield and stability of the tested varieties based on GGE biplot

The assessment of yield and stability of genotypes in the GGE biplot (Figure 6) was determined using the average environment (tester) coordinate methods [24]. The ideal genotype should have an average environment coordinate, indicated by the first two components [25]. The mean yield and stability of an ideal genotype, located in the center of the concentric circle, should be high [26]. Ranking biplot graphs showed that the stabilities of genotypes in terms of grain yield, yield components and chemical compositions were different (Figure 7).

Analysis of ideal varieties based on GGE biplots

The comparison of genotypes in biplot model showed the ideal region (indicated by the arrow) representing the mean grain yield values in all locations, and indicated the information on the waxy Corn hybrids located in the ideal region. The most stable characteristics in terms of grain yield, yield components and quality composition were yield and single ear weight, and followed by 1000 grain weight and grain moisture content, respectively. Crude protein and crude oil contents were determined as the low quality properties for stability of waxy Corn genotypes (Figure 7).

Discussion

The coefficient of variations for the yield, yield components and grain chemical characteristics determined in the experiment showed that the reliability of the experiment was within the acceptable limits for Corn experiments, and the values were in consistent with the literature [27,28].

The mean grain yield of 4 candidate waxy Corn genotypes (ADAX-11, ADAX16, ADAX18 and ADAX 19), when years evaluated together, was higher than mean value obtained for the experiment (11678 kg ha^{-1}). The mean grain yields of waxy corn genotypes were 20 to 40% lower than the dent corn varieties (Table 3). The researcher reported that waxy Corn genotypes had a yield of 18 to 27% lower than dent corn varieties [7,29]. The results obtained in the experiment are consistent with the results of previous studies. Control varieties in both years were the latest flowering genotypes with the lowest number of days for flowering. The number of days to flowering recorded in this study complies with the results of previous studies carried out in similar locations [30,31,32].

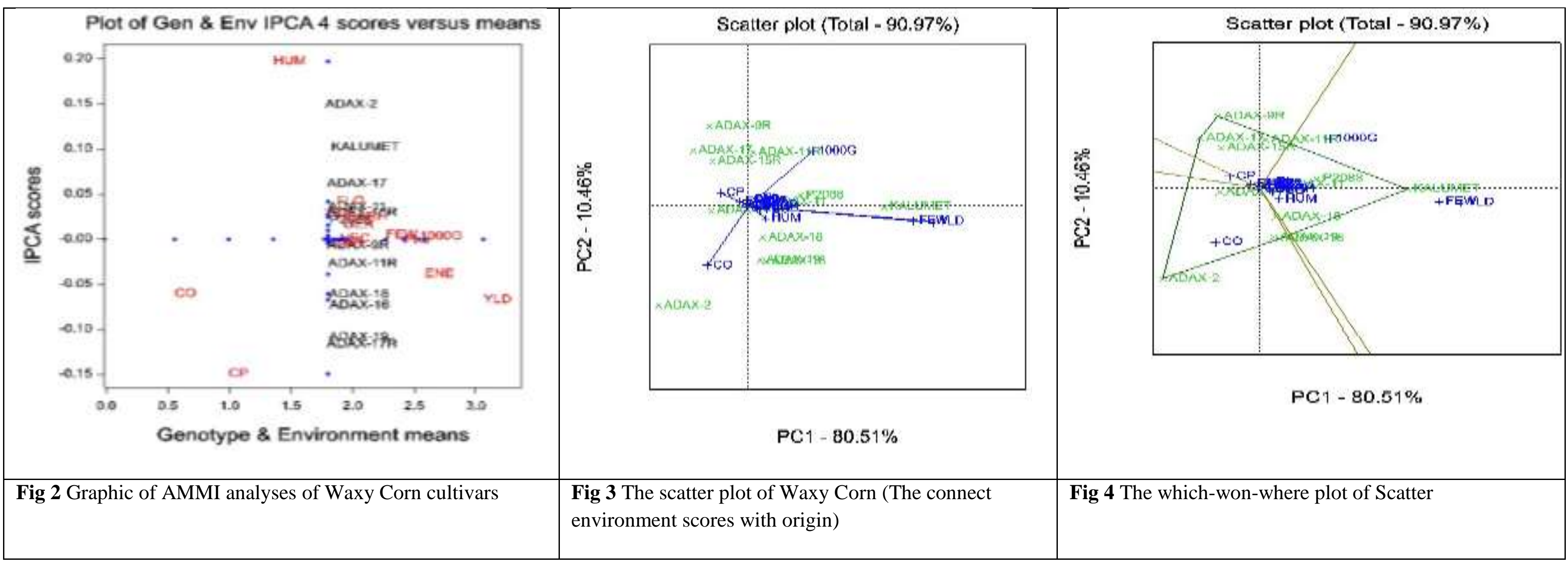


Fig 2 Graphic of AMMI analyses of Waxy Corn cultivars

Fig 3 The scatter plot of Waxy Corn (The connect environment scores with origin)

Fig 4 The which-won-where plot of Scatter

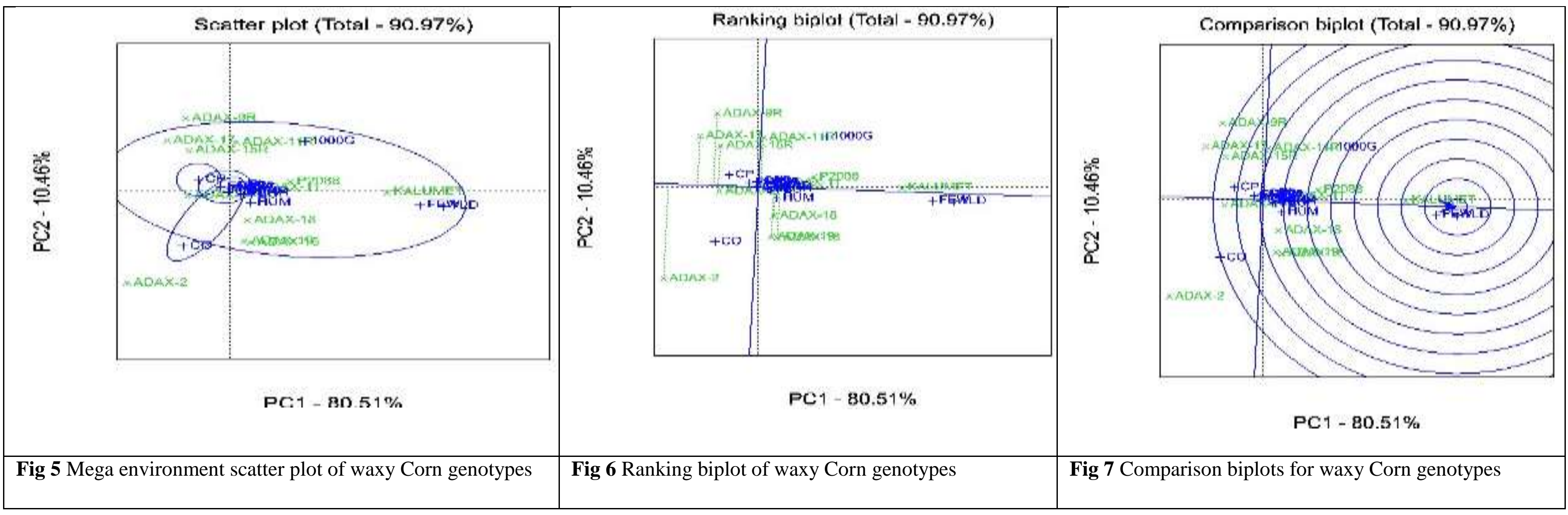


Fig 5 Mega environment scatter plot of waxy Corn genotypes	**Fig 6** Ranking biplot of waxy Corn genotypes	**Fig 7** Comparison biplots for waxy Corn genotypes

The researchers reported that the number of days to flowering between 58.5 and 77.5 days. In addition, the variation in flowering period over the years shows that although the flowering period is a genetic feature, it is also affected by the environmental factors (mean temperature during sowing-flowering period and precipitation regime).

The mean plant heights of 4 waxy Corn genotypes (ADAX11R, ADAX15R, ADAX17 and ADAX 19) were higher than the mean plant height recorded in the experiment (Table 7). In addition, the plant heights of waxy Corn genotypes were similar to the plant heights of dent corn (control) varieties. The results obtained in the experiment are similar to the plants heights (255-335.8 cm) reported in the studies conducted under Samsun conditions [30,31,32]. Plant height is a genetic characteristics, though significantly affected by the environmental factors. The lower average temperature and higher precipitation during the vegetation period of the second year caused higher plant heights of the genotypes compared to the first year of the experiment.

The mean ear heights of 3 waxy Corn genotypes (ADAX-16, ADAX-17 and ADAX-18) were higher than the mean value recorded in the experiment. The ear height of waxy corn genotypes was 10 to 20% lower than that of the dent corn varieties. The many researcher reported that the ear height of corn varieties varied between 98.3 and 145 cm, which are partially lower than the ear heights obtained in the experiment [31,32, 33]. The results in the experiment indicated that the ear height is not only affected by the genetic characteristics but also by the environmental factors. The differences in ear height may be related to low ear formation in waxy corn genotypes, and higher sensitivity of waxy corn genotypes to plant density compared to the dent corn.

Grain/ear ratios of 3 waxy Corn genotypes (ADAX-16, ADAX19 and ADAX-2R) were higher than the mean grain/ear ratio of the experiment. Corn breeders desire to have a grain/ear ratio higher than 85%. Waxy genotypes had relatively lower grain/ear ratios than the control varieties. The results obtained in the experiment are in harmony with the grain/ear ratios (78.0-85.8%) reported by [34,31]. The results of the study revealed the strong influence of genetic structure on grain/ear ratio of waxy Corn genotypes. The mean grain moisture content of the genotypes was 22.7% and mean grain moisture content of 5 waxy Corn genotypes (ADAX11R, ADAX15R, ADAX17R, ADAX-18 and ADAX9R) was lower than

the mean moisture content of the experiment. The waxy genotypes in both years had lower grain moisture content than the standard varieties (Table 8). The results obtained in the experiment are compatible with the grain moisture contents (19.30-30.9%) recorded in a similar location by [34,31]. In addition, the grain moisture content of waxy Corn genotypes in a five year study varied between 20.7 and 24.0%, which were relatively lower compared to grain moisture contents of dent corn [7].

One thousand grain weight is a genetic trait but also affected by the environmental factors. Positive correlation was obtained between 1000 grain weight and grain yield. Many researcher reported that 1000 grain weights of dent corn varied between 302.7 and -489.0 g. [35,36]. In addition, Hao and Wu (2008) reported that 1000 grain weights of waxy Corn pure lines ranged from 142 to 192 g, while Edy and Baktiar (2017) reported that waxy Corn hybrids ranged from 312 to 332 g. The values obtained in the experiment are higher than those reported [37]. 100 grain weight can be attributed to the genetic structure of hybrid genotypes used in the experiment. The shape, size and homogeneity of corn grains are the most important factors to determine the hectoliter weight of genotypes. The mean hectoliter weights of genotypes was determined as 78.1 kg h^{-1}. Sayaslan et al. (2016) and Saygı and Toklu (2017) reported that hectoliter weights ranged between 65.1 and 80.1 kg h^{-1}. The results showed that the hectoliter weights of waxy Corn genotypes were similar to hectoliter weights of dent corn varieties, and the genetic structure has a significant effect on hectoliter weight of corn varieties. The products with low energy and nutritious have recently become more preferred. The mean carbohydrate content of the genotypes was 70.5% (Table 5). The results revealed that the genetic (genotype potential) effect has predominant influence on carbohydrate content. The carbohydrate content of dent corn varieties varied between 67.9 and 81.2%, and waxy Corn genotypes ranged between 71.5 and 74.5% [37,19,39]. The carbohydrate content obtained in this study are similar to the results reported in previous studies.

Energy content varied depending on Corn genotypes. Ma et al. (2019) reported that the mean energy values of dent corn and waxy corn varieties were 378.5 and 380.5 kcal 100 g^{-1}, respectively, which were slightly higher than the energy values calculated in this study. The difference is probably related to the higher oil and protein contents of waxy Corn genotypes

used in the experiment compared to those used by [38]. The energy values, similar to the grain chemical composition, are under the influence of the genetic structure.

Single ear weight was significantly different among Corn genotypes. The researcher reported that single corn weight of dent corn varied between 177 and 224 g, while the other reported that single ear weights of waxy corn genotypes ranged between 98 and 282 g [40,41,42]. The results are similar to the finding reported in the previous studies. The number of ears per plant recorded for waxy and dent Corn genotypes were similar. The number of ears per plant for grain corn was reported between 1.12 and 1.28, for waxy Corn genotypes from 1 to 3 [40,43,44]. The number of ears per plant obtained in this experiment were lower than those reported by Souvandouane et al. (2010), while in harmony with the values given in the previous studies. This difference can be attributed to the difference in genetic structure (waxy x super sweet hybrid) of the waxy genotypes used in the experiment and the differences in fertilizer applications and the sowing density (60x25 cm).

Crude protein ratio is one of the most important quality traits and varies depending on the corn subspecies. The protein ratios in various experiments reported varying between 8 and 13% [19,45]. In addition, the protein ratio of waxy corn varies reported varying between 7.89 and 9.4% [46,41,7,38]. The protein ratios reported in previous studies are compatible with the dent corn, while higher than the waxy corn genotypes. The oil content of waxy and dent Corn genotypes were different. The oil ratio of dent corn varieties was reported varying between 3.4 and 5.0% [17,19,31].

The crude oil ratio of waxy Corn genotypes reported in the previous studies ranges from 4.4 to 5.1% [7,47,41,38]. The crude oil ratios obtained in the experiment were relatively lower compared to those reported in the literature. The difference is related to the low crude oil ratios of the parents of the genotypes used in the experiment, and the crude oil ratios of waxy Corn genotypes were higher than the crude oil ratios of the control varieties. In addition, the results showed that the genetic structure of genotypes significantly affects the crude oil content. Similar to the crude protein and oil contents, the grain starch content slightly differed between the genotypes. The results indicated that starch content of waxy genotypes is significantly affected by the genetic structure.

The starch ratios of waxy corn genotypes have been reported between 69.01 and 73.2% [7,47,38]. The differences between starch ratios reported in the literature and those obtained in this study can be attributed the difference in the genotypes used.

The significance of GY interaction is related to the differences in climate and soil conditions between the years. The significance of GE interaction ($p<0.01$) in both years can be explained by changes in the stabilities of genotypes under different environments. The variation of yield potentials for corn genotypes depending on the environment have been reported also by [48,49,27].

The AMMI biplot is a comprehensive and effective method to classify the genotypes based on their levels in combination with target environments, and graphically ranks the genotypes with their strengths and weaknesses in different environments [20]. GGE biplot method provides convenience in explaining the ideal genotype and environment relationship for breeders [50,51] Both biplot methods (AMMI and GGE) explain the grain yield, yield components and chemical composition interactions, and provide reliable information to the breeders about the candidate genotypes or halfway materials in the gene pool. The AMMI biplot analysis indicated that the highest effect on experimental variance was resulted from the environment (95.15%) followed by genotype (0.49%) and GE interaction (4.15%), respectively. The coassessment of grain yield, yield components and chemical composition showed that the effect of the environment was quite higher than genotype and GE interaction. Similar results on AMMI studies have been reported by [50,51]. The study to determine the yield stability of some corn hybrids, and explained that genotype effect on the experimental variance was 9.17%, the effect of year and environment was 77.13% and GE interaction was 13% [52]. The other reported that the environment effect on the agronomic properties of some corn varieties was 46.67%, the effect of the genotype was 22.26%, and the GE interaction was 31.06% [53]. The results obtained in this study differed from other studies. The difference can be attributed to the type of genotypes (waxy Corn) used in the experiment and narrow genetic diversity of the genotypes. The first four genotypes determined by the AMMI analysis in terms of yield, yield components and chemical compositions were given in Table 5. The dent corn variety (Kalumet) can be preferred in terms of 1000 grain weight, single ear weight, flowering, grain moisture content and number of ears per plant, while, the

variation was high in the waxy genotypes (Table 5). One thousand grain weight and single ear weight came to the fore as the most stable traits of the corn varieties (Figure 1). The ADAX11 and ADAX18 waxy genotypes are considered the most fertile and stabile genotypes. In addition, both waxy genotypes can be recommended as good candidates for similar ecologies.

The GGE biplot analysis is used extensively in different test environments to determine the stability of genotypes in terms of yield, yield components and quality characteristics. The representative stability graph of grain yield, yield components, and chemical composition plotted on the mean of the squares showed that Kalumet> P2088> ADAX16>ADAX19 were the first four genotypes which have average yields above the overall average in ideal environments and higher stability. The mean yields of ADAX-2>ADAX-9R>ADAX17 genotypes were lower than the mean yield of the experiment; thus, the stability of genotypes was considered low. Similar results have been reported many researcher [54,55,49,27]. The GGE biplot defines the relationships between all environments based on the general model of MET data, whereas the simple correlation coefficients describe the relationships between the two environments [20,21]. The GGE biplot is considered an efficient method to determine the best genotypes, which are representative across environments [50,51]. The most stable cultivars were Kalumet and P2088, while ADAX-11 and ADAX18 genotypes were determined as the most stable waxy Corn genotypes. Many researchers indicated that the comparison biplot model helps determine the ideal genotype based on the mean yield and quality values [27,49,56]. Placement of the varieties in different sectors indicated that these genotypes were genetically different in terms of yield. The ADAX-9R, ADAX17, ADAX11R and ADAX15 cultivar candidates were genetically similar to each other. Similar results have been obtained in the biplot studies conducted by [50,51]. Highly stable and efficient waxy genotypes (ADAX11 and ADAX18) identified by the GGE biplot analysis have the potential to be used in countries where the ecological conditions are similar.

Conclusion

Waxy Corn is an important Corn subspecies used in many areas of the starch industry and the consumption of fresh ear has recently increased due to the rich nutrient content. This

study was carried out in 2018 and 2019 to determine the grain yield, yield components and chemical contents (protein, oil, starch, multiple element contents) of waxy Corn genotypes and to determine the stable genotypes in terms of all traits using the Biplot (GGE and AMMI model) analysis. The mean grain yield of the genotypes ranged from 8560.6 to 17290.6 kg -1ha, the number of days to flowering from 71.3 to 77.5 days, plant height from 251.7 to 295.0 cm, the first ear height from 85.3 to 98.3 cm, crude protein ratio from 9.4 to 10.4%, crude oil ratio from 3.3% to 5.0% , total starch content from 57.5% to 60.0, 1000 grain weight from 317.7 to 402.2 g, hectoliter from 76.9% to 79.3, carbohydrate content from 69.6 to 71.6%, energy content from 383.8 to 393.7 kcal, grain/ear ratio from 81.3 to 85.5%, grain moisture content from 20.5 to 25.0, single ear weight from 145 to 286.3 g, and the number of ears per plant from 0.9 to 1.0 ear. Significant positive correlations were determined between the grain yield, 1000 grain weight and single ear weight of the genotypes. The results revealed that the chemical composition of wax Corn genotypes are within the acceptable limits and their energy values are low. The grain yields of Corn genotypes were 10 to 30% lower than the yields of dent corn varieties. Nevertheless, the enrichment of waxy corn gene pool with new half-way materials will increase the yield. In addition, investigating the fresh ear characteristics, macro and micro nutrient contents, vitamin values and amino acid contents is of great importance. The biplot (AMMI and GGE) analyzes evaluating the grain yield, yield components and chemical composition of waxy Corn together, revealed that ADAX11 and ADAX18 genotypes have the high stability.

Acknowledgments

I would like to thank the General Directorate of Agricultural Research and Policies (TAGEM) for the funding of this project and to the staff of the Corn Research Institute Directorate (MAEM) for the convenience in providing the materials used in the study.

References

1. Collins, G. N., A New Type of Indian Corn from China. Bureau of Plant Industry (Bulletin) 1909,161: 1-30.
2. Harakotr, B., et al., Anthocyanins and antioxidant activity in coloured waxy corn at different maturation stages. Journal of Functional Foods. 2014, 9: 109-118.
3. Fergason, V., Specialty Corn, High Amylose and Waxy Corns, Second Edition Ed: Halluer A.R. 2001, pp: 71-92.

4. Hung P, V., T, Maeda, and N. Morita, Study on physicochemical characteristics of waxy and high-amylose wheat starches in comparison with normal wheat starch. Starch/Staerke 2007, 59(3-4), p. 125-131. http://dx.doi.org/10.1002/star.200600577.
5. Alcázar-Alay S, C. and M. A, A, Meireles, Physicochemical properties, modifications and applications of starches from different botanical sources. Food Sci. Technol, Campinas 2015, 35(2): 215-236.
6. Cengiz, R., et al., Development of Waxy corn (Zea mays ceratina) varieties, 11th Field Crops Congress Proceedings Book-1 2015, 259-261.
7. Fergason, V., Specialty Corn, High Amylose and Waxy Corns, Second Edition Ed: Halluer A.R. 2001, pp: 71-92.
8. Zheng H, et al., Genetic diversity and molecular evolution of Chinese waxy corn germplasm. Plosone 2013, 8(6): e66606. Doi: 10.1371/journal.pone.0066606.
9. Mostafavi, K., et al., Heterotic grouping of Iranian corn inbred lines based on yield-specific combining ability in diallel crosses and GGE biplot. J. Res. Agric. Sci., 2012, 8: 113-125.
10. Badu-apraku, B., K., et al., Performance of extra-early corn cultivars based on GGE biplot and AMMI analysis. J. Agric. Sci., 2011, 150:1–11.
11. Badu-apraku, B., et al., Combining ability, heterotic patterns and genetic diversity of extra-early yellow inbreds under contrasting environments. Euphytica, 2013,192: 413-433.
12. Ruswandi, D, J., et al., Determination of combining ability and heterosis of grain yield components for Corn mutants based on line x tester analysis. Asian J. Crop Sci., 2015, 7: 19-33.
13. Stojaković, M, B., et al., Dodig grouping pattern of Corn test locations and its impact on hybrid zoning. Euphytica, 2015; 204, 2: 419-431.
14. Oyekunle, M., et al., Stability analysis of corn cultivars adapted to tropical environments using AMMI analysis. Cereal Res. Comm., 2017, 45, 2: 336–345.
15. Boţović, D., et al., Assessment stability of corn lines yield by GGE-Biplot analysis Genetıka, 2018, Vol. 50, No3, 755 -770, 2018. doi.org/10.2298/gensr1803755b
16. Anonymous,. Technical instruction for testing agricultural data. 2018 P.1-17, Ankara.
17. Lambert, H., High oil corn hybrids. Second Edition Halluer 2001, A.R. pp: 148-150.
18. Anonymous, Testing methodology and interpretation of results, 2013. Chapter 5, U.S. Grains Council, and U.S. America.
19. Sayaslan, A., et al., Wet-milling qualities of Dent corn (Zea mays indentata L.) hybrids grown as main crop in Adana and Sakarya. Journal of Agricultural Faculty of Gaziosmanpasa University (2016) 33 (3), 167-180. E-ISSN: 2147-8848. Doi: 10.13002/jafag1144
20. FAO., Food energy – methods of analysis and conversion factors. FAO food and nutrition paper. 2003ISSN 0254-4725 p.23
21. Yan, W. and N, A, Tinker, Biplot analysis of multi-environment trial data: principles and applications. Canadian journal of plant science, 2006, 86, 623-645. https://doi.org/10.4141/P05-169
22. Yan, W., et al., Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Science2000,40:597-605.
23. Yan, W., et al., GGE biplot vs. AMMI analysis of genotype-by-environment data. Crop science, 2007, 47, 643-653. https://doi.org/10.2135/cropsci2006.06.0374
24 Yan, W., and J. Bi Holland, A heritability-adjusted GGE biplot for test environment evaluation. Euphytica, 2010, 171, 355-369. https://doi.org/10.1007/s10681-009-0030-5
25. Yan, W., GGE biplot: a Windows application for graphical analysis of multi-environment trial data and other types of two-way data. Agron. J., 2001, 93: 1111–1118.

26. Yan, W., and N. A, Tinker, An integrated biplot analysis system for displaying, interpreting, and Exploring genotype X environment interaction. Crop Science 2005,45:1004-1016.https://doi.org/10.2135/cropsci2004.0076
27. Oliviera, T, R, A., et al., Hybrid Corn selection through GGE biplot analysis. Bragantia vol.78 no.2 Campinas Apr./June 2019 Epub Mar 14, 2019. https://doi.org/10.1590/1678-4499.20170438.
28. Fritsche-Neto, R., et al., Updating the ranking of the coefficients of variation from Corn experiments. Acta Scientarium, 2012, 34, 99-101. https://doi.org/10.4025/actasciagron.v34i1.13115
29. Solaimalai, A., P. Anantharaju, and S. Irulandi., Corn Crop: Improvement, Production, Protection and Post-Harvest Technology. Fırst Edition. 2020
30. Özata E., A, Öz, and H. Kapar, Determination of yield and quality characteristics of silage hybrid corn variety candidates. Agricultural Sciences Research Journal 2012, 5 (1): 37-41,
31. Özata, E., and H. Kapar, Determination of the quality and performance of some dent corn hybrid (Zea mays indentata Sturt) under Samsun conditions. Agricultural Sciences Research Journal 2014, 7 (2): 01-07, 2014 ISSN: 1308-3945, E-ISSN: 1308-027X,
32. Özata, E., et al., Determination of the Performances of Candidate Dent Corn Hybrids under the Main Product Conditions. Iğdır Univ. Journal of Science Institute, 2013, 3(1): 91-98
33. Özata, E., S. İkincikarakaya, Ünver, and A. Özturk, The Determination of silage yield and quality traits of candidate corn hybrids. Ekin Journal of Crop Breeding and Genetics. 2018, 4(1):31-40, 2018.
34. Öz, A., et al., A study on the development of corn varieties suitable for Samsun and Konya conditions. National Cereal Symposium, June 2-5 2008.
35. Acıbuca, A., Researches on Silage and Grain Yield and Yield Characteristics of Some Corn Varieties Grown as the Second Crop in Mardin Ecological Conditions. 2015, Ege University Institute of Science and Technology, Master Thesis (Unpublished), Izmir.
36. Sabancı, S., Determination of the yield, quality and antioxidant activities of some corn (Zea Mays L.) cultivars grown in the Aegean region. 2016, Ege University Institute of Science and Technology, Master Thesis (Unpublished), Izmir.
37. Creech, R., G., Genetic control of carbohydrate Synthesis in corn endosperm. Genetics 1965, 52: 1175-1186 December 1965.
38. Ma, Dongli Li, et al., Determination of the energy contents and nutrient digestibility of corn, waxy corn and steam-flaked corn fed to growing pigs. Asian-Australasian Journal Animal Science Vol. 32, No. 10:1573-1579 October 2019 https://doi.org/10.5713/ajas.18.0713 pISSN 1011-2367 eISSN 1976-5517.
39. Saygı, M., and F.Toklu, Evaluation of some grain Corn (Zea mays indentata sturt.) cultivars grown under the first crop conditions in Çukurova Region in terms of grain yield, some vegetative characteristics and inter-character relations. KSU Journal of Natural Sciences, 2017, 20 (Special Issue), 308-312, Kahramanmaraş.
40. Souvandouane, S., et al. Effects of Planting Dates and Mulch Types on the Growth, Yield and Chemical Properties of Waxy Corn Crosses Sonjajang×KNU-7 and Asan×KNU-7 Korean J. Crop SCI.), 55(2), 2010.
41. Thakur, S., N. Singh, and A. Kaur, Characteristics of normal and waxy corn: physicochemical, protein secondary structure, dough rheology and chapatti making properties. J Food Sci Technol (September 2017) 54(10):3285–3296 DOI 10.1007/s13197-017-2775-5
42. Storck, L., et al.,Sample size for single, double and triple hybrid corn ear traits. Scientia Agricola 2007, 64: 30-35
43. Akan, S., Determination of suitable Corn varieties for the ecological conditions of Muş province. 2017 Bingöl University, Institute of Science and Technology, Master's Thesis (Unpublished).

44. Sinay, H., et al., Proline content and yield components of local corn cultivars from Kisar Island, Maluku, Indonesia. International Journal of Plant Biology 2015; 6:6071.
45. Khan, A.K., et al., Estimation of protein, carbohydrate, starch and oil contents of indigenous corn (Zea mays L.). Germplasm European Academic Research Vol. II, Issue 4/ July 2014.
46. Edy, S, N., and I. Baktiar, Increased potential of protein pontent of Waxy corn. International Journal of Environment, Agriculture and Biotechnology (IJEAB) Vol-2, Issue-4, July-Aug- 2017. http://dx.doi.org/10.22161/ijeab/2.4.55 ISSN: 2456-1878
47. Klimek-Kopyra A, et al., Some aspects of cultıvatıon and utılızatıon of waxy maıze (Zea mays L. Ssp. Ceratina). ACTA Agrobotanica Vol. 65 (3): 3-12 2012.
48. Faria, S. V, et al.,Adaptability and stability in commercial Corn hybrids in the southeast of the State of Minas Gerais, Brazil. Revista Ciência Agronômica, 2017, 48, 347-357. https:// doi.org/10.5935/1806-6690.20170040.
49. Oliveira, T, R, A., et al., Correlation among adaptability and stability assessment models in Corn cultivars. Australian Journal of Crop Science, 2017, 11, 516-521. http://dx.doi.org/10.21475/ajcs.17.11.05.p304
50. Kendal, E. and Y. Dogan, Stability of a cveidate and cultivars (Hordeum vulgare L) by GGE Biplot analysis of Multi-environment Yield Trials in Spring Barley. Agriculture and Forestry. 2015; 61(4), 307-318.
51. Oral, E,, E. Kendal, and Y. Doğan, Evaluation of yield stability in some bread wheat varieties using biplot and AMMI analysis. Methods Journal of Agriculture, ADÜ, 2018;15(1):55-64 — doi: 10.25308/aduziraat.373685.
52. Mitrović B, et al., Evaluation of experimental Corn hybrids tested in multi-location trials using AMMI and GGE biplot analyses. Turkish Journal of Field Crops 2012:17(1): 35-40.
53. Yue, H, W., et al., Effects of genotype-by-environment interaction on the main agronomic traits of Corn hybrids. Applied Ecology and Environmental Research, 2020
54. Gauch, H, G., and R. W, Zobel, Predictive and postdictive success of statistical analysis of yield trials. Theoretical and Applied Genetics 1997, 76.1 1988: 1-10.
55. Gauch, H, G., Statistical Analysis of Yield Trials by AMMI and GCE. Crop Science 2006, 46: 1488-1500.
56. Oliveira, R.L., et al., Ferreira Evaluation of Corn hybrids and environmental Evaluation of Corn hybrids and environmental stratification by the methods AMMI and GGE biplot stratification by the methods AMMI and GGE biplot. Crop Breeding App. Biotech., 2010, 10: 247-253.

Research article

Achife, C. E., et al., Microbial Population of Soil and Water around Petroleum Depot Suleja, Nigeria, and their Hydrocarbon Utilization. International Journal of Life Sciences and Biotechnology, 2021. 4(1): p. 90-113. DOI: 10.38001/ijlsb.791853

Microbial Population of Soil and Water around Petroleum Depot Suleja, Nigeria, and their Hydrocarbon Utilization

Achife, C. E.[1*], Ijah, U.J.J[2], Bala J. D.[1], Oyeleke, S. B. [3]

ABSTRACT

Contamination by petroleum products is a common occurrence in various depots in Nigeria. Suleja depot in recent times has recorded several petroleum spillages and fire incidence attributed to petroleum products storage and distribution. This study was aimed at determining the microbiological quality of soil and water sources in communities around petroleum products depot in Suleja, Nigeria**.** Soil and water samples were collected from petroleum depot and the five communities around the petroleum products depot and a control site. Microorganisms in the soil and water samples were enumerated by spread inoculation on general purpose media and selective media. Bacterial and fungal isolates were tested for their potential to utilize petroleum products in a Bushnell Haas Broth containing 0.05 mL of petroleum products (diesel, kerosene, engine oil, crude oil) as a source of carbon and energy. The utilization rate was determined by spectrophotometry. The capacities of selected bacterial and fungal isolates to mineralize crude oil were further tested in minimal salt medium. The bacteria isolated were *Staphylococcus aureus, Streptococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia coli*. The microbial isolates were not evenly distributed in the six experimental and control plots. Soil samples had higher aerobic heterotrophic bacterial counts than the water samples. Crude oil was most utilized by the microbial isolates. Bacterial isolates from genera *Pseudomonas* and *Bacillus* had the highest capacity in utilizing the petroleum products. Among the fungal species, *Aspergillus niger* and *Penicillium notatum* exhibited greater capacity to utilize the petroleum products. Present study revealed isolates capable of utilizing the various petroleum products which can be useful in oil spill bioremediation in the tropical environments.

ARTICLE HISTORY
Received
10 September 2020
Accepted
20 October 2020

KEYWORDS
Bacteria,
fungi,
soil,
water,
hydrocarbons

Introduction

Crude oil pollution is currently considered to be a great threat to the health of living things in the environment including humans. Nigeria records an average of 300 oil spills in the oil producing States annually, making the Niger Delta regions the most polluted part of Nigeria, affecting the air, soil and water bodies [1]. Apart from the Niger Delta regions, other places that serve as depots for petroleum products such as diesel, premium motor

[1] Department of Microbiology, School of Life Sciences, Federal University of Technology Minna, Nigeria
[2] Department of Biological Sciences, Federal University of TechnoIogy, Minna, Nigeria
[3] Department of Crop Production. Federal University of Technology,Minna, Nigeria
* Corresponding Author**:** Achife, Chukwunonso Emperor. E-mail: achifeemperor@yahoo.com

spirit, and kerosene among others have in one way or the other encountered oil spills during transportation or storage either accidentally or due to human error contributing to soil and water pollution [2,1].

Crude oil also known as petroleum is composed of saturated alkanes and cycloalkanes, unsaturated alkynes and alkenes, as well as polycyclic aromatic hydrocarbons (PAHs) [3]. PAHs are difficult to degrade from the environment and their presence in the environment has been proven to have debilitating effects on living things [3]. Nwakanma *et al*. [2] reported that Nigerian crude oil could be hepatotoxic and hemotoxic which can cause cancer and infertility. Furthermore, crude oil has been reported to contain heavy metals and their accumulation in plant or animal tissues can cause mutation or even death depending on the dose and period of exposure [1, 4]. Oil spills have adverse effects on the environment, productivity of farmlands, water bodies and birds of the sky as well as microbial community and their distribution in the environment compared to a pristine environment [5].

Nigeria has 22 Nigerian National Petroleum Corporation/Pipeline and Products marketing Company (NNPC/PPMC) depots, saddled with the responsibility of transportation, storage and marketing of petroleum products such as; Premium Motor Spirit (PMS), Automotive Gas Oil (AGO) and Dual Purpose kerosene (DPK) in the domestic and efficient evacuation of refined petroleum products from the local refineries. The operation of the Nigerian Pipelines and Storage Company Ltd (NPSC) started in 1979 [6]. There are total of twelve storage tanks in Suleja depot in Niger State. Among these storage thanks, 4 tanks are for PMS, 4 storage tanks for AGO, and 4 storage tanks for DPK. Each storage tank of PMS, AGO, and DPK has a storage capacity of 12.6million, 7.6million and 7.6million liters respectively. These storage tanks are prone to leakages and washing which may lead to contamination of the soil and water in the surrounding communities.

Microorganisms are ubiquitous, and when faced with extreme conditions, some still find a way of surviving in the environment through various means of adaption such as alteration in membrane permeability, change of metabolic pathways, spore formation, mutation among others [5, 7]. Once faced with extreme conditions as is the case of crude oil pollutants, some microorganisms survive and eventually thrive in the environment utilizing

the hydrocarbons in the petroleum products as a source of carbon and energy. Others may assume a dormant form whereas those microorganisms that cannot withstand this extreme exposure die leaving the promising ones, which have been sought for in carrying out bioremediation process[2].

From the exploration, distribution and storage of crude oil and its products, several incidences of oil spillage have occurred, which caused huge adverse effects on health safety and the environment [8,9]. Several attempts have been tried to clean up oil spillage from the environment, but bioremediation is preferred [10]. Bioremediation is a process that involves the use of living microorganisms or their enzymes in detoxifying and degrading environmental pollutants, thus, restoring a polluted environment. The microbial population and activities in the affected environment such as microbial counts, respiration, biomass diversity, and enzyme activities can be used to evaluate the extent of bioremediation [1, 11]. Microbial biodegradation of petroleum contaminants is cheap and environmentally friendly and can be enhanced for better cleanup operations. Petroleum utilizing microorganisms are more in number in oil polluted environment than pristine environment. However, microorganisms that are indigenous to a petroleum contaminated site have been reported to best remediate the environment from oil spills and other pollutants than the non-indigenous microorganisms to that site [2, 10].

The bacterial genera that have been isolated from varying crude oil contaminated sites include *Nocardia*, *Pseudomonas*, *Gordonia*, *Micrococcus*, *Rhodococcus*, *Arthrobacter*, *Mycobacterium*, *Flavobacterium*, *Corynebacterium*, *Klebsiella*, *Alcaligenes* and *Bacillus* while the fungal genera include *Penicillium, Aspergillus, Trichoderma,* and *Fusarium* [12-15]. The aim of this study was to assess microbial population and their hydrocarbon utilizing potentials from soil and water sources around petroleum products depot in Suleja, Nigeria.

Materials and Methods

Experimental areas

[The sampling areas were five surrounding communities and depot premises (Table 1)] designated Plots 1-6. These communities include Dikko, Maje, Tunga Shanu, Dagweru,

and Gwatupe. Plots 1 and 5 are farthest to the petroleum products depot and are about 1500 meters away from the depot. They are Tunga Shanu and Tunga Koro communities respectively. Plot 4 and 6 are about 1000 meters each away from the depot while Plot 2 is 500 meters away and is closest to the petroleum products depot. They are Dagwe, Maje and Dikko communities respectively. Plot 3 hosts the petroleum product depot. [The control is Federal University of Technology (FUT) Bosso Campus, Minna, Niger State of Nigeria, which has no previous history of oil pollution]. The choice of control was selected to help obtain isolates in an unpolluted and uncontaminated habitat with similar soil characteristics as the plots.

Table 1 Distances of various plots to petroleum products depot and their coordinates

Plot	Distance (meters)	Community	Coordinates
1	1500	Tunga shanu	9.250958° N, 7.160060° E
2	500	Dikko	9.253754° N, 7.164931° E
3	(1500 to farthest communities)	Petroleum products depot	9.254262° N, 7.1668195° E
4	1000	Dagwe	9.2512229° N, 7.1687292° E
5	1500	Tunga koro	9.2559345° N, 7.1786521° E
6	1000	Maje	9.255818° N, 7.1788452° E
Control	93,000	FUT Minna (Bosso campus)	9.655311° N, 6.5267310° E

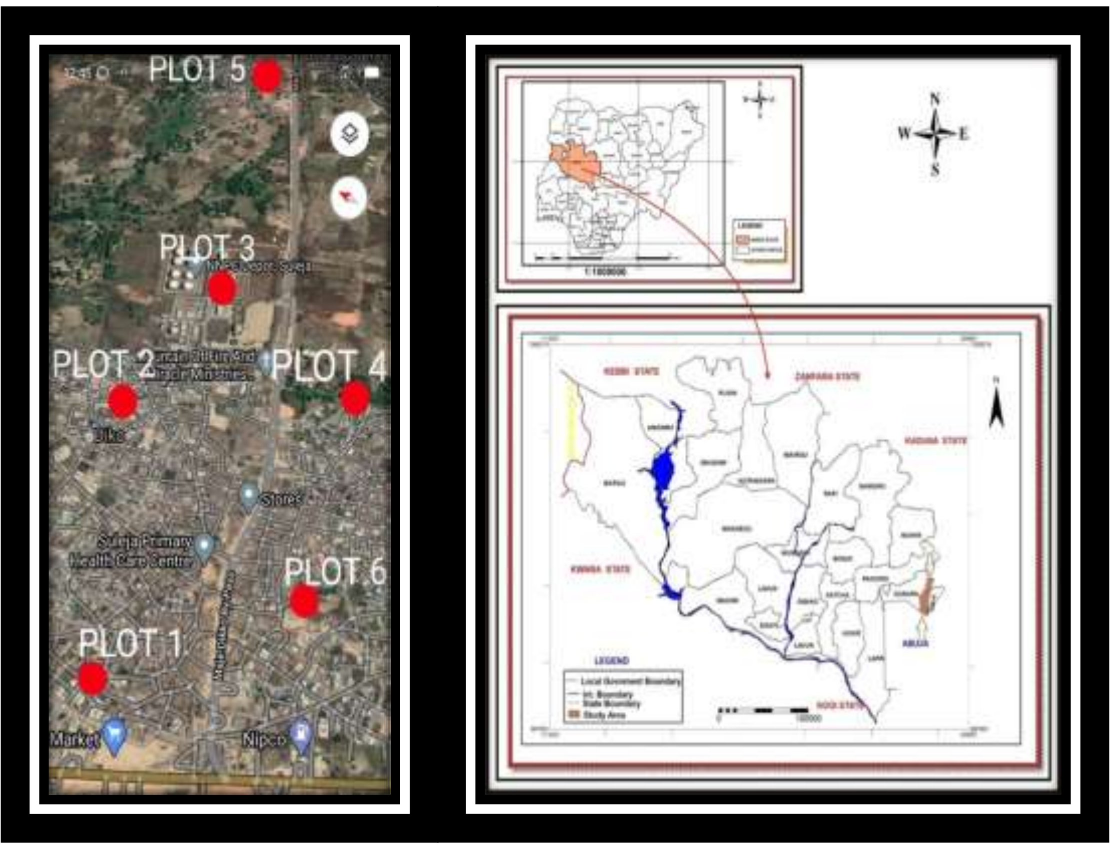


Fig 1 Map of study site showing various Communities (Plots)
Legend: Plot 1- Tunga shanu; Plot 2- Dikko; Plot 3- Petroleum products depot; Plot 4- Dagwe, Plot 5- Tunga Koro, Plot 6 Maje.

Collection of samples

Five soil samples were collected from each of the six plots into clean polyethylene bags for microbial analysis. Soil samples were collected with a hand auger after first stripping away litter to expose the first mineral based horizon (A-horizon). The samples were collected at depth of 0-15 cm, as described by Minnesota Pollution Control Agency, MCPA [16]. Soil samples were also collected from the control site. Samples were placed in WhirlPak bags and transported to the Microbiology Laboratory of the Federal University of Technology (FUT), Minna, Nigeria, for analysis.

Water samples were collected from wells, boreholes and stream of the study area and control. A total of 30 water samples were collected from the plots in sterile containers [16]. Water sample bottles were first rinsed with the water sample to be collected before

collection. Water samples were collected during rainy and dry seasons in icebox and transported to Microbiology Laboratory, FUT, Minna for analysis.

Collection of hydrocarbons

Bonny light crude oil and petroleum products (Diesel, Kerosene, and Engine oil) were collected from Kaduna Refinery and Petrochemical Company (KRPC), Kaduna, Nigeria, in clean bottles. The bottles were first rinsed with the product to be sampled before collection. The products were transported to the laboratory for biodegradation studies.

Enumeration and isolation of microorganisms in soil and water samples

Microorganisms in the soil and water samples were enumerated by spread inoculation. Ten grams (10 g) of soil samples were suspended in 90 mL of sterile distilled water and were serially diluted. One milliliter of the serially diluted soil was dispensed on the surface of Nutrient agar (NA) and Sabouraud dextrose agar (SDA) in petri dishes for the enumeration of total aerobic heterotrophic bacteria (TAHB) and fungi respectively. The NA plates were incubated for 24-48h at 30°C while the SDA plates were incubated for 3-5d at room temperature (28 ±2°C) [17]. Aliquot of the serially diluted water samples were also inoculated on MacConckey agar (MCA), Eosine methylene blue (EMB) agar, *Pseudomonas* base agar (PBA), thiosulfate-citrate-bile salt (TCBS) agar, *Salmonella*/*Shigella* agar (SSA) and spread evenly for the isolation of total coliform bacteria (TCB), *Pseudomonas, Vibrio, Salmonella* and *Shigella* respectively. Developed colonies on the surfaces of the nutrient medium were enumerated and recorded as colony forming units per gram of soil (cfu/g) and colony forming units per millilitre (cfu/mL) of water. The inoculated plates were incubated for 24 - 48h at 37°C after which colonies were counted. The isolates were sub-cultured repeatedly on fresh media to obtain pure cultures, and were maintained on agar slants for further characterization and identification.

Enumeration of total hydrocarbon utilizing bacteria and fungi

Total hydrocarbon utilizing bacteria (THUB) were enumerated using the method described by Hamamura *et al*. [18]. The method basically involves the dilution and plating out of 0.1 mL of sample suspensions on Bushnell Haas agar (Sigma-Aldrich, USA). Supply of hydrocarbon was carried out through the vapor phase to hydrocarbon utilizers by placing sterile Whatman filter papers (No.1) saturated with 3mL of hydrocarbons (crude oil, diesel,

kerosene, engine oil) on the lids of the inverted plates. The plates were incubated for 14 - 21d at 30°C. Colonies were counted and discreet colonies of different THUB were sub cultured on freshly prepared nutrient agar plates and incubated for 24-48h at 37°C while THUF were sub-cultured on freshly prepared SDA plates and incubated for 3- 5days at 28°C to obtain pure isolates.

Characterization and identification of microbial isolates

Bacterial isolates were characterized and identified on the basis of Gram's reaction and biochemical tests as described by Cheesbrough [19]. Some of the biochemical tests carried were: indole, sugar fermentation, catalase, coagulase, citrate utilisation, motility, spore formation, nitrate reduction, starch hydrolysis, urease and oxidase tests. The identities of the bacterial isolates were confirmed using Bergey's Manual of Determinative Bacteriology [20].

The fungal isolates were identified macroscopically and microscopically according to; aerial and substrate hyphae, type and shape of hyphae, spore formation, presence of specialized structures (foot cell, sporangiophores, conidiophores) and pigmentation. A small portion from each fungal isolate was placed in a drop of lactophenol cotton blue on a clean glass slide and then covered before viewing with 10X and 40X objective lenses of light microscope to confirm hyphae type. Fungal isolates were identified using Atlas of Clinical Fungi [21] and schemes of Domsch and Gams [22].

Screening for the bacterial utilization of petroleum products by bacterial isolates

Bacterial and fungal isolates were tested for their potential to utilize petroleum products under aerobic conditions by inoculating a calibrated loopful of 18-24h old culture of each isolate into 5 mL of Bushnell Haas Broth containing 0.05 mL of petroleum products (diesel, kerosene, engine oil, crude oil) which had been sterilized by autoclaving for 15 min at 121°C. Hydrocarbon utilization potential of the isolates was screened by determining the turbidity, total viable counts and emulsification of oil in the medium after 14 days' incubation at 30°C [23]. The optical density of the isolates was determined at a wavelength of 600 nm using PG T70 UV/VIS spectrophotometer.

Utilization efficiencies of petroleum products by selected bacterial and fungal isolates

The capacities of selected bacterial and fungal isolates to utilize crude oil were further tested in minimal salt medium (MSM). One hundred millilitres of MSM (10g NaCl, 0.29g KCl, 0.42g $MgSO_4$, 0.83g $KH2PO_4$, 0.42g $NaNO_3$, 1.25g $NaHPO_4$, 100ml distilled water, pH 7.2) was introduced into 250 ml conical flasks and 1.0% (v/v) of crude oil was introduced and sterilized using the method described by Okpokwasili and Okorie [24]. A 24 hours old broth culture of each selected organism was seeded into each flask and incubated in a rotatory shaker incubator (New Brunswick Scientific Incubator Shaker) at 150 rev/min and 30°C. The efficiency of utilization of other hydrocarbons was monitored at three days interval for 21d by monitoring bacterial and fungal growth measured by viable counts in the MSM. The optical density was also determined at a wavelength of 600 nm using PG T70 UV/VIS spectrophotometer.

Statistical analysis

Data obtained were subjected to statistical package for social science (SPSS 23) using one-way analysis of variance (ANOVA) and Duncan multiple range tests for the determination of the significant difference between values and comparison of various means respectively. P values less than 0.05 was considered significant while P values greater than 0.05 was considered as non-significant.

Result

Total aerobic heterotrophic bacteria (TAHB) in soil and water samples

The mean counts of total aerobic heterotrophic bacterial (AHB) in soil samples are presented in Table 2. The total mean counts of AHB observed in control were higher than the values obtained in the Plots. In the rainy season, observed mean counts of AHB were lower than the mean counts obtained in the control. The highest mean count (49×10^6 cfu/g) for the dry season was obtained in Plot 4 while the lowest count (12.0×10^6 cfu/g) was obtained in Plot 3. There were significant differences ($P<0.05$) among the counts in the Plots.

The results for water samples also revealed that AHB counts for both rainy and dry seasons were higher in the control samples when compared with the results from the Plots. The

mean counts in rainy season were higher than the counts in dry season (Table 2). The highest AHB mean counts in water samples (9.8×10^4 cfu/mL) were recorded in Plot 3 while the lowest counts (3.2×10^4 cfu/mL) were recorded in Plot 6. The bacterial counts in the various Plots were significantly differently ($P<0.05$).

Table 2 Mean counts of total aerobic heterotrophic bacteria (AHB) in soil and water samples

Plots	Soil samples ($\times 10^6$ cfu/g)		Water samples ($\times 10^4$ cfu/mL)	
	Rainy Season	Dry Season	Rainy Season	Dry
1	52.0^{d}	26.0^{b}	6.7^{f}	4.1^{f}
2	16.1^{f}	12.1^{f}	7.6^{d}	4.4^{d}
3	62.0^{b}	12.0^{g}	9.8^{b}	6.2^{c}
4	26.0^{e}	49.0^{a}	8.0^{c}	6.9^{b}
5	11.0^{g}	25.1^{c}	7.0^{e}	4.2^{e}
6	54.0^{c}	13.0^{e}	6.1^{g}	3.2^{g}
Ctr	84.0^{a}	18.0^{d}	11.5^{a}	7.9^{a}

Means with dissimilar superscript across the column differ significantly ($p<0.05$). Means with same superscript do not differ significantly ($P>0.05$). cfu/g: colony forming unit per gram, cfu/ml: colony forming units per milliliters, Ctr: control.

Mean counts of total fungi (TF) in soil and water samples

The values obtained for total fungi in soil were higher in the control than the plots in both rainy and dry seasons with the exception of Plot 2. The mean counts of total fungi in soil ranged from 2.20×10^3 cfu/g to 14.10×10^3 cfu/g for the rainy season and 1.00×10^3 cfu/g to 6.20×10^3 cfu/g for dry season. The control recorded mean counts of 12.00×10^3 and 6.10×10^3 cfu/g (Table 3) for rainy and dry seasons respectively.

The values obtained for total fungi in water were higher in the control than the plots in both rainy and dry seasons with the exception of Plot 2. The mean counts of total fungi in water samples ranged from 0.0 × 10^3 cfu/mL to 4.30 ×10^3 cfu/mL in the rainy season and 0.0 × 10^3 cfu/mL to 3.20 × 10^3 cfu/mL in dry season (Table 3).

Table 3 Mean counts of total fungi (TF) in soil and water samples

Plots	Soil samples (× 10^3 cfu/g)		Water samples (× 10^3 cfu/mL)	
	Wet season	Dry season	Wet season	Dry season
1	5.20 ^{d}	4.10 ^{b}	0.00 ^{d}	1.10 ^{a}
2	14.10 ^{a}	6.20 ^{a}	4.30 ^{a}	3.20 ^{a}
3	10.10 ^{c}	4.50 ^{b}	2.00 ^{b}	2.20 ^{b}
4	3.50 ^{e}	3.30 ^{c}	1.20 ^{c}	0.00 ^{c}
5	2.20 ^{f}	1.20 ^{d}	0.00 ^{d}	1.12 ^{c}
6	3.10 ^{e}	1.00 ^{d}	2.20 ^{b}	1.10 ^{c}
Ctr	12.00 ^{b}	6.10 ^{a}	4.10 ^{a}	2.00 ^{b}

Means with dissimilar superscripts across the column differ significantly ($p<0.05$). Means with same superscript do not differ significantly ($P>0.05$). cfu/g: colony forming unit per gram, Ctr: control

Mean counts of total coliforms and other bacteria (TCB) in water samples

The mean counts of total coliform bacteria ranged from 3.10 × 10^3cfu/g to 10.00 ×10^3cfu/mL and 2·03×10^3 cfu/mL to 10.01×10^3cfu/mL in rainy and dry seasons respectively. No count was found in the control water sample (Table 4).

Table 4 Mean counts of total coliforms bacteria (TCB) in water

Plots	Water samples (× 10^3 cfu/mL)	
	Rainy Season	Dry Season
1	15.00^{a}	7.10^{b}
2	8.41^{c}	6.00^{c}
3	10.00^{b}	10.01^{a}
4	4.01^{d}	5.31^{d}
5	3.10^{e}	4.03^{e}
6	4.23^{d}	2.03^{f}
Control	0.00^{f}	0.00^{g}

Means with dissimilar superscript across the column differ significantly ($p<0.05$). Means with same superscript do not differ significantly ($P>0.05$). cfu/mL: colony forming units per milliliter.

Hydrocarbon utilizing bacteria and fungi in soil and water samples

The mean counts of hydrocarbon utilizing bacteria (HUB) and fungi (HUF) are presented in Table 5. Generally, the results revealed that samples from the six plots have higher HUB counts than the control plot. The mean counts of HUB ranged from 1.28×10^7 cfu/g to 6.32×10^7 cfu/g in the various plots. The mean counts of HUB were highest in Plots 3 and 6 which recorded 6.32×10^7 - 5.05×10^7 cfu/g and 6.11×10^7 - 5.81×10^7 cfu/g in rainy and dry seasons respectively. Plot 5 recorded lowest mean counts of HUB of 1.21×10^7 cfu/g and 1.20×10^7 cfu/g in the rainy and dry seasons respectively.

The mean counts of hydrocarbon utilizing fungi (HUF) were highest in Plot 3 with 3.60×10^4 cfu/g and 3.05×10^4 cfu/g in rainy and dry seasons respectively. The lowest mean counts of HUF were obtained in Plot 5 (Table 5). There were significant differences ($P<0.05$) among mean HUB and HUF counts in the rainy and dry seasons, as well as the control.

The mean counts of HUB and HUF in water sample were presented in Table 6. Hydrocarbon utilizing bacteria recorded highest mean counts in Plot 6 of 2.58×10^7 cfu/mL and 2.18×10^7 cfu/mL) in rainy and dry seasons respectively. The mean counts of HUB were lowest in Plot 5 (Table 6) The mean counts of HUF were highest in Plot 3 with counts of 2.0×10^4 and 1.53×10^4 cfu/mL in rainy and dry seasons respectively. The results obtained showed that the mean counts of HUB and HUF were much lower in the control samples than in the six plots.

Table 5 Mean counts of hydrocarbon utilizing bacteria and fungi in soil samples

Plots	HUB ($\times 10^7$ cfu/g)		HUF ($\times 10^4$ cfu/g)	
	Rainy Season	Dry Season	Rainy Season	Dry Season
1	1.45^{d}	2.30^{e}	1.56^{e}	1.8^{g}
2	4.20^{c}	4.00^{c}	2.65^{c}	2.11^{c}
3	6.32^{a}	5.50^{b}	3.60^{b}	3.05^{b}
4	1.42^{d}	3.40^{d}	2.00^{d}	1.93^{d}
5	1.28^{e}	2.14^{f}	1.21^{b}	1.20^{f}
6	6.11^{c}	5.81^{a}	4.5^{a}	3.71^{a}
Control	1.23^{e}	1.24^{g}	0.10^{f}	0.30^{i}

Means with dissimilar superscript across the column differ significantly ($p<0.05$). Means with same superscript do not differ significantly ($P>0.05$). HUB/F= Hydrocarbon utilizing bacteria/fungi, cfu/g = colony forming units per gram.

Table 6 Mean counts of hydrocarbon utilizing bacteria and fungi in water samples

Plots	THUB (× 10^7 cfu/mL)		THUB (× 10^4 cfu/mL)	
	Rainy Season	Dry Season	Rainy Season	Dry Season
1	1.00^{c}	0.50^{f}	0.95^{d}	0.60^{c}
2	1.32^{b}	0.80^{c}	1.74^{b}	1.04^{b}
3	1.50^{b}	1.00^{b}	2.00^{a}	1.53^{a}
4	0.95^{c}	0.80^{b}	0.42^{e}	0.19^{d}
5	0.28^{e}	0.14^{d}	0.13^{f}	0.15^{d}
6	2.58^{a}	2.18^{a}	1.5^{c}	0.13^{d}
Control	0.40^{d}	0.1^{e}	0.05^{g}	0.0^{e}

Means with dissimilar superscript across the column differ significantly ($p<0.05$). Means with same superscript do not differ significantly ($P>0.05$). HUB/F= Hydrocarbon utilizing bacteria/fungi, cfu/mL = colony forming unit per milliliter.

Frequency of occurrences of microbial isolates in the various plots

The bacterial isolates were not evenly distributed in the six experimental Plots and control Plot. The control had the least frequency of occurrences (7.19 %) when compared to the plots. Plot 6 had the highest frequency of occurrence (20.92 %) of isolated bacteria (Table 7). Plots 1,2,3,4 and 5 had frequency of occurrence of 16.26 %, 19.57 %, 16.44 %, 10.02 %, and 9.55 % respectively.

The results showed that *Bacillus* had the highest frequency of occurrence (32.25%) while *Salmonella* had the lowest frequency of occurrence (2.2 %). Staphylococcus, *Pseudomonas* and *Streptococcus* had 18.15%, 13.57% and 13.16% respectively, frequency of occurrences (Table 7).

There were higher frequencies of occurrence of bacterial isolates in rainy seasons than in in dry season. The genera *Micrococcus*, *Enterobacter*, *Salmonella* and *Escherichia* were only observed in one season in some Plots. The results also showed that only the genus *Bacillus* was observed in both rainy and dry seasons in all the Plots (Table 7).

The frequency of occurrence of fungal isolates was presented in Table 8. The results revealed that fungal isolates were observed in all the plots but were not evenly spread. The control plots had the least frequency of occurrence of 5.27% with two fungal genera; *Penicillium* and *Aspergillus*. Plot 6 had the highest frequency of occurrence (28.57%) and had four fungal genera (Table 8). Plots 1 and 2 recorded equal number of frequencies of occurrence (15.80%).

The result showed that *Aspergillus* was the dominant fungal isolate with a total frequency of occurrence of 48.88%, while the *Rhizopus* had the lowest frequency of occurrence Table 8. There were more occurrences of fungal isolates in the rainy season than in the dry season.

Table 7 Frequency of occurrence of bacterial genera in the plots

Bacterial Genera	Plot 1 R/D	Plot 2 R/D	Plot 3 R/D	Plot 4 R/D	Plot 5 R/D	Plot 6 R/D	Ctr R/D	Total (%)
Bacillus	16/12 (4.38)	32/15 (7.35)	28/22 (6.26)	12/10 (3.45)	11/8 (2.98)	26/13 (6.10)	7/4 (1.72)	206 (32.25)
Staphylococcus	13/0 (2.03)	10/6 (2.50)	13/10 (3.60)	6/3 (1.41)	8/4 (1.88)	23/10 (5.17)	5/5 (1.56)	116 (18.15)
Streptococcus	13/6 (2.98)	14/0 (2.19)	12/8 (3.13)	6/0 (0.94)	5/3 (1.25)	5/4 (1.41)	10/0 (1.56)	86 (13.46)
Proteus	3/2 (0.78)	3/0 (0.47)	5/0 (0.78)	0/0 (0.00)	2/0 (0.32)	0/3 (0.47)	0/0 (0.00)	18 (2.82)
Pseudomonas	10/8 (2.82)	5/3 (1.26)	8/4 (1.88)	12/7 (2.97)	3/2 (0.78)	12/7 (2.92)	0/6 (0.94)	87 (13.57)
Escherichia	5/2 (1.09)	12/2 (2.19)	0/0 (0)	2/1 (0.47)	4/3 (1.09)	11/6 (2.66)	0/3 (0.47)	51 (7.97)
Klebsiella	0/6 (0.94)	5/2 (1.09)	1/0 (0.16)	3/1 (0.62)	3/0 (0.47)	4/0 (0.63)	0/0 (0.00)	25 (3.91)
Salmonella	3/0 (0.47)	4/2 (0.94)	0/0 0 (0)	0/0 (0.00)	1/0 (0.16)	3/0 (0.47)	1/0 (0.16)	14 (2.20)
Enterobacter	0/1 (0.16)	2/0 (0.31)	1/0 (0.16)	0/0 (0.00)	4/0 (0.62)	2/2 (0.62)	5/0 (0.78)	17 (2.65)
Micrococcus	3/1 (0.62)	0/8 (1.25)	3/0 (0.47)	1/0 (0.16)	0/0 (0.00)	3/0 (0.47)	0/0 (0.00)	19 (2.97)
Total	104 (16.26)	125 (19.57)	105 (16.44)	64 (10.02)	61 (9.55)	134 (20.92)	46 (7.19)	639 (100)

Ctr: Control, Percentage (%) in parenthesis, R/D: Rainy/Dry Seasons

Table 8 Frequency of occurrences of fungal isolates in the various plots

Fungal genera	Plot 1 R/D	Plot 2 R/D	Plot 3 R/D	Plot 4 R/D	Plot 5 R/D	Plot 6 R/D	Ctr	Total (%)
Aspergillus	6/3 (6.77)	6/2 (6.02)	8/3 (8.27)	3/2 (3.76)	6/5 (8.27)	11/6 (12.78)	2/2 (3.01)	65(48.88)
Penicillium	5/3 (6.02)	5/2 (5.26)	2/1 (2.25)	4/2 (4.51)	2/1 (2.26)	8/4 (9.02)	2/1 (2.26)	42(31.58)
Mucor	3/1 (3.01)	0/2 (1.50)	2/0 (1.50)	2/0 (1.50)	0/0 (0.0)	4/1 (3.76)	0/0 (0.00)	15(11.27)
Rhizopus	0/0 (0)	3/1 (3.01)	0/1 (0.75)	2/0 (1.50)	0/0 (0.0)	3/1 (3.01)	0/0 (0.00)	11(8.27)
Total (%)	21 (15.80)	21 (15.80)	17 (12.77)	15 (11.27)	14 (10.53)	38 (28.57)	7 (5.27)	133 (100)

Ctr: Control, (%): Percentage in parenthesis, R/D: Rainy/Dry Seasons

Utilization of petroleum products by microbial isolates

The ability of selected bacterial isolates from the various plots with potential to degrade hydrocarbons was tested on some petroleum products (Crude oil, kerosene, Diesel and engine oil), which served as sole source of carbon and energy in modified minimal salt nutrient broth. The bacterial isolates were; *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus thuringensis*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus epidemidis*, *Bacillus licheniformis* and *Pseudomonas putida***.** The bacterial isolates utilized the petroleum products to varying capacities after 21d of incubation (Table 9). Bacterial isolates from the genera *Pseudomonas* and *Bacillus* had higher absorbance of 0.663±0.55 and 0.601±0.14 respectively on crude oil than other isolates. The lowest absorbance on crude oil (0.081±0.41) was from *Proteus mirabilis* (Table 9). *Pseudomonas aeruginosa* recorded highest absorbance in kerosene (0.43± 0.002) while *B. thuringiensis* recorded highest absorbance in diesel (0.311±0.46) and engine oil (0.284±0.24) (Table 9), meaning that these organisms had efficient abilities in utilizing the hydrocarbons. The isolated fungi utilized the petroleum products to varying capacities within 21d of incubation. The organisms had highest percentage utilization in crude oil (0.211-0.713) and

lowest in engine oil (0.024-0.213). Kerosene and diesel recorded moderate rates of 0.124-0.453 and 0.053 – 0.325 respectively) by the fungal isolates (Table 10). Among the fungal species, *Aspergillus niger* and *Penicillium notatum* exhibited greater capacity to utilize the petroleum products when compared with other fungal isolates (Table 10).

Table 9 Utilization efficiencies of bacterial isolates in petroleum products after 21d of incubation

Bacterial Isolates	**Absorbance at 600 nm**			
	Crude oil	**Kerosene**	**Diesel**	**Engine oil**
Staphylococcus aureus	$0{\cdot}302{\pm}0.21^{e}$	$0{\cdot}291{\pm}0.31^{f}$	$0{\cdot}250{\pm}0.13^{d}$	$0{\cdot}180{\pm}0.15^{e}$
Bacillus subtilis	$0{\cdot}580{\pm}0.13^{c}$	$0{\cdot}391{\pm}0.13^{d}$	$0{\cdot}291{\pm}0.25^{b}$	$0{\cdot}264{\pm}0.26^{b}$
Bacillus thuringensis	$0{\cdot}601{\pm}0.15^{b}$	$0{\cdot}413{\pm}0.42^{b}$	$0{\cdot}311{\pm}0.46^{a}$	$0{\cdot}284{\pm}0.24^{a}$
Streptococcus faecalis	$0{\cdot}131{\pm}0.34^{g}$	$0{\cdot}049{\pm}0.38^{i}$	$0{\cdot}030{\pm}0.59^{i}$	$0{\cdot}062{\pm}0.33^{g}$
Pseudomonas aeruginosa	$0{\cdot}663{\pm}0.55^{a}$	$0{\cdot}431{\pm}0.02^{a}$	$0{\cdot}281{\pm}0.23^{c}$	$0{\cdot}250{\pm}0.16^{c}$
Proteus mirabilis	$0{\cdot}081{\pm}0.41^{h}$	$0{\cdot}103{\pm}0.35^{h}$	$0{\cdot}125{\pm}0.31^{h}$	$0{\cdot}031{\pm}0.11^{i}$
Staphylococcus epidemidis	$0{\cdot}180{\pm}0.30^{h}$	$0{\cdot}116{\pm}0.41^{g}$	$0{\cdot}135{\pm}0.21^{g}$	$0{\cdot}051{\pm}0.42^{h}$
Bacillus licheniformis	$0{\cdot}282{\pm}0.40^{f}$	$0{\cdot}381{\pm}0.16^{e}$	$0{\cdot}190{\pm}0.29^{f}$	$0{\cdot}152{\pm}0.13^{f}$
Pseudomonas putida	$0{\cdot}501{\pm}0.25^{d}$	$0{\cdot}410{\pm}0.44^{c}$	$0{\cdot}193{\pm}0.18^{e}$	$0{\cdot}183{\pm}0.43^{d}$

Means with dissimilar superscripts across the column differ significantly ($p<0.05$). Means with same superscripts do not differ significantly ($P>0.05$). Values are replicate mean determination ± SD

Table 10 Utilization efficiencies by fungal isolates of petroleum products after 21 days of incubation

Fungal Isolates	Absorbance at 600nm			
	Crude oil	Kerosene	Diesel	Engine oil
Aspergillus niger	0·713±0·41 [a]	0.453±0·31 [a]	0.325±0·43 [a]	0.213±0·14 [a]
Penicillium notatum	0.654±0·10 [c]	0.412±0·25 [ab]	0.250±0·15 [a]	0.213±0·23 [a]
Mucor plumeus	0.301±0·2 [e]	0.125±0·11 [d]	0.181±0·22 [b]	0.051±0·15 [d]
Rhizopus stolonifera	0.211±0·41 [f]	0.124±0·23 [d]	0.053±0·33 [c]	0.024±0·43 [e]
Aspergillus fumigatus	0.648±0·30 [b]	0.386±0·17 [b]	0.260±0·32 [a]	0.200±0·41 [b]
Aspergillus flavus	0.524±0·15 [d]	0.314±0·28 [c]	0.254±0·25 [a]	0.180±0·18 [c]

Means with dissimilar superscripts across the column differ significantly ($p<0.05$). Means with same superscripts do not differ significantly ($P>0.05$). Values are replicate mean determination ± SD

Counts of selected hydrocarbon utilizing bacteria and fungi in MSM amended with crude oil

There were variations in the viable cell counts of selected HUB and HUF in minimal salt broth amended with crude oil during 21d of incubation. The crude oil gradually emulsified as the degradation progressed. There were increases in the viable cell counts up to day 15 and 18 for *Bacillus* and *Pseudomonas* species respectively. *Pseudomonas aeruginosa* recorded the highest viable cell counts when compared with other isolates (Table 11). The viable cell counts increased from 4.32×10^6 to 12.15×10^6 cfu/mL and declined to 12.00×10^6 cfu/mL on day 21. *Penicillium notatum* and *Aspergillus niger* recorded a consistent increase in viable counts to 5.80×10^4 and 7.60×10^4 up to day 15 and 18 respectively, and decreased slightly after 21st day (Table 11). The results showed that there were significant differences ($p<0.05$) in the viable cell counts of microbial isolates within the period of incubation.

Table 11 Mean viable cell counts of selected hydrocarbon utilizing bacteria and fungi in MSM amended with crude oil

Time(days)	*Bacillus thuringiensis* (× 10^6 cfu/mL)	*Pseudomonas aeruginosa* (× 10^6 cfu/mL)	*Penicillium notatum* (× 10^4 cfu/mL)	*Aspergillus niger* (× 10^4 cfu/mL)
0	3.40^{h}	4.32^{h}	2.60^{h}	3.30^{g}
3	3.80^{g}	4.71^{g}	3.70^{g}	4.11^{f}
6	4.61^{e}	5.70^{f}	4.50^{f}	5.23^{e}
9	4.91^{d}	6.15^{e}	4.90^{d}	6.30^{c}
12	5.82^{c}	8.45^{d}	5.60^{b}	5.51^{d}
15	7.21^{a}	10.71^{c}	5.80^{a}	7.40^{a}
18	6.50^{b}	12.15^{a}	5.00^{c}	7.00^{b}
21	4.51^{f}	12.00^{b}	4.81^{e}	5.20^{e}

Means with dissimilar superscripts across the column differ significantly ($p<0.05$). Means with same superscripts do not differ significantly ($P>0.05$). cfu/mL: colony forming unit per milliliter

Discussion

The total mean counts of aerobic heterotrophic bacteria (AHB) in soil were greater than the counts obtained in water sample. This may be due to the fact that the soil is not mobile on its own and eventually leads to the deposition of organic substances that help in providing nutrients for microbial proliferation. The highest aerobic heterotrophic bacterial counts obtained during the rainy season is in concordance with the report of Olukunle [26] and Ikuesan [27] that moisture is one of the factors that influence microbial proliferation in soil. A dry soil can cause desiccation of microbial cells thereby reducing the overall microbial activities and causes some cells to be dormant. The soil can also hold substances in stationary mode allowing microorganisms to act on them conveniently without any disturbance unlike the liquid medium [28]. The low counts of AHB in water samples may be due to the fact that oxygen concentration in water was lower compared to soil sample, which may be due to presence of pore spaces that allowed diffusion of atmospheric oxygen into them, thus enhancing bacterial proliferation in the soil [27].

Among the fungi isolated in this study, *Penicillium notatum* was prominent across all plots sampled. Elemuo *et al.* [25] have reported the versatility of *P. notatum* in the environment

with ability to produce various enzymes, which helped the microorganisms degrade crude oil in contaminated environment.

The mean total fungi (TF) counts in the soil and water sample in this study was highest in Plot 2, which is closer to the petroleum products depot both in wet and dry seasons. This may be due to the ability of fungi to tolerate adverse environmental conditions, especially with the formation of resistant spores.

The low mean total coliform bacterial (TCB) counts generally observed in this study may be due to the inability of coliform bacteria to produce spores, which have been noted in the past to help microorganisms survive in environment that are not favorable for their existence and metabolisms. Moreover, there is paucity of information concerning the low proliferation of coliform bacteria on environment polluted by crude oil.

The total mean counts of hydrocarbon utilizing bacteria (HUB) and fungi (HUF) were lowest in the control soil both in wet and dry seasons. This indicated that the microbial population found in communities surrounding petroleum products depot had the ability to utilize hydrocarbon due to periodic exposure to petroleum products. Though, there was no established pattern of growth observed in the microbial community in this study, their counts were quite higher, than in the control. The total mean counts of HUB and HUF were generally low in the water sample in rainy and dry seasons compared to the soil sample. This is because, nutrient and oxygen availability in water is quite low compared to that of the soil sample. Different microbial genera use different pathways in the degradation of petroleum products, some of which take little time to complete a degradation process whereas others take longer time. Also, the types of enzymes utilized by microorganisms as well as the constituent of the petroleum products plays a great role in influencing biodegradation processes [29].

Microbial population and diversity are usually high in a pristine environment unlike an environment experiencing influx of contaminants from crude oil spills. The reduction and changes in the microbial communities occur as a result of the inability of the indigenous microorganisms to withstand such extreme conditions and those that are able to withstand and even strive often have machineries and pathways they utilize to achieve that [30, 29,

31]. The indigenous bacterial diversity obtained in this study was similar to the one obtained by Nkiru *et al.* [1]

This study reported some microorganisms that were peculiar to crude oil polluted environment, in which the genera *Bacillus* had the highest number of species, which include *B. subtilis, B. thurengiensis and B. licheniformis.* These bacteria in addition to *Pseudomonas aeruginosa* were all isolated from all the plots sampled. The dominant presence of *Bacillus* and *Pseudomonas* in this study supports the fact established by Osarumwense *et al.* [32] that these bacterial genera efficiently degrade hydrocarbons in the environment through the production of surfactants and enzymes such as lipase. Agu *et al.* [33] have reported that spore forming ability of *Bacillus* helped the organisms in resisting toxic effects exerted by the petroleum compounds.

All the microorganisms (bacteria and fungi) tested for biodegradation of petroleum products in this study were able to utilize hydrocarbons although at different rates. This observation was also made by Ikuesan (2017) where the test isolates utilized hydrocarbons at varying rates. Microbial genera such as *Bacillus* and *Pseudomonas*. *Penicillium* and *Aspergillus* had high degradation capabilities in this study. Ikuesan (2017) has suggested that these differences in degradation rates were associated with the natural ability of the different microorganisms. It may also be due to the presence of enzymes and biosurfactants, and petroleum products constituents [31, 34, 35].

The bacterial genus; *Bacillus* and *Pseudomonas* had high frequency of occurance and utilising efficiencies when compared with other bacterial genus. This may be connected with their high ability to breakdown complex organic and recalcitrant compounds due to the production of specialized enzymes [17].

The mean viable cell count (VCC) of bacteria and fungi in MSM amended with crude oil generally showed a progressive growth of microbial cells untill day 15 across all bacterial and fungal isolates. However, the microbial cells of *Bacillus thuringiensis*, *Penicillium notatum* and *Aspergillus niger* began to decrease after day 15, while the cells of *Pseudomonas aeruginosa* increased till day 18 before it declined. The general progressive increase of microbial cells observed at the initial and later stages as well as gradual decline of microbial cells depicts a typical microbial growth curve. The reduction in the number of

cells occurred as a result of cell aging as well as the exhaustion of nutrients in the mineral salt medium used. As microbial cells grow, they metabolize and release their products into the environment in which they live. Since the medium used in this study is typical of a batch culture, there was no renewal of nutrients and toxic metabolites, which eventually deterred the growth of the microorganisms, thus, a decline in the number of cells was observed in this study and is in concordance with the report of Nwakanma *et al.* [2].

Conclusion

The microorganisms isolated from soil and water close to petroleum products depot in this study were capable of utilizing crude oil, kerosene, diesel and engine oil in varying amount. *Pseudomonas aeruginosa* had the highest ability to utilize kerosene while *B. thuringiensis* was most efficient in utilizing diesel and engine oil. *Aspergillus niger* recorded a consistent increase in viable counts and also exhibited the highest capacity to utilize the petroleum products compared with other fungal isolates. The findings in this study therefore revealed that bacterial genera *Pseudomonas* and *Bacillus* as well fungal genera *Aspergillus* and *Penicillium* were effective in the bioremediation of petroleum products contaminated sites. It also showed that water sources from the plots were contaminated with coliform bacterial and poses great challenge to public health.

References

1. Nkiru, U.C., et al., Biodegradation of Crude Oil-Polluted Soil by Bacterial Isolates from Nigeria. Frontiers in Environmental Microbiology, 2019. 5(1): p. 14-28.
2. Nwakanma, C., E.C. Obih, and O. Onyia, Molecular Identification of Bacteria Involved in Degradation of Crude Oil. Nigerian Journal of Biotechnology, 2016. 31: p. 1 – 8.
3. Ding, A., et al., Characterizing Microbial Activity and Diversity of Hydrocarbon-Contaminated Sites. IntechOpen, 2013. p. 137-160. Doi: http://dx.doi.org/10.5772/50480.
4 Naowasarn, S. and S. Leungprasert, Abundance and diversity of hydrocarbon utilising bacteria in the oil-contaminated soils throughout a remedial scheme using compost amendment. Songklanakarin Journal of Science and Technology, 2019. 41(1): p. 12-20.
5. Patel, V., et al., Response and resilience of soil microbial communities inhabiting in edible oil stress/contamination from industrial estates. BMC Microbiology, 2016. 16, p- 50, doi: 10.1186/s12866-016-0669-8.
6. Nigerian National Petroleum Corporation (NNPC), Nigerian Pipelines and Storage Company Limited (NPSC), 2020. Retrieved from https://nnpcgroup.com/Downstream/pages/npsc.aspx 16/08/20.

7. Adeniji, A.O., O.O. Okoh, and A.I. Okoh, Analytical Methods for the Determination of the Distribution of Total Petroleum Hydrocarbons in the Water and Sediment of Aquatic Systems: A Review. Hindawi Journal of Chemistry, 2017. 2090-9063 Article ID 5178937. Doi: https://doi.org/10.1155/2017/5178937.
8. Iquatt, C.B., O.A. Oyewole, and O.P. Abioye, Bioremediation of petroleum polluted soil (a review). International Journal of Natural and Applied Science, 2005. 1(1): p. 21-25.
9. Osinowo, I., et al., Bioremediation of Diesel Contaminated Soil Using Bacterial Cocktail and Organic Nutrients. Journal of Microbiology Biotechnology and Food Sciences, 2020. 10(2): p. 1- 14.
10. Oyewole, O.A., et al., Isolation of bacteria from diesel contaminated soil for diesel remediation. Journal Bio-Sci, 2020. 28, p. 33-41.
11. Ubong, I.U. and J.D. Edwin, Assessment of petroleum products contamination of soil quality at selected truck parks in Calabar municipality, Nigeria. International Journal of Development and Sustainability, 2018. 7(4): p. 1336-1354.
12. Chikere, C. B., G.C. Okpokwasili, and B.O. Chikere, Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. African Journal of Biotechnology, 2009. 8(11): 2535-2540.
13. Ekhaise, F.O. and J. Nkwelle, Microbiological and Physicochemical Analyses of Oil Contaminated Soil from Major Motor Mechanic Workshops in Benin City Metropolis, Edo State, Nigeria. Journal of Applied Science and Environmental Management, 2011. 15(4): 597 - 600.
14. Kuang, S., et al., Soil Microbial Community Structure and Diversity around the Aging Oil Sludge in Yellow River Delta as Determined by High-Throughput Sequencing. Archaea*,* 2018, ID 7861805. Doi: https://doi.org/10.1155/2018/7861805.
15. Feng, X., et al., Distribution of Bacterial Communities in Petroleum-Contaminated Soils from the Dagang Oilfeld, China. Transactions of Tianjin University*,* 2020. 26, p. 22–32. Doi: https://doi.org/10.1007/s12209-019-00226-7.
16. Minnesota Pollution Control Agency, MPCA, *Soil* sample collection and analysis procedure, 2008. Guidance Document 4-04, Petroleum Remediation Program. 520 Lafayette Road, N., St. Paul, Minnesota. p. 1-10.
17. Cappuccino, J. and N. Sherman, Microbiology: A Laboratory Manual 8th Edition 1981. United States: Suny Rockland, 5-80.
18. Hamamura, N., et al., Microbial population dynamics associated with crude oil biodegradation in diverse soils. Applied Environmental Microbiology*,* 2006. 72, p. 6316 – 6324.
19. Cheesbrough, M., District Laboratory Practice in Tropical Countries Part 2, 2nd Edition, 2003. Cambridge University Presss, New York, USA, p 1-90.
20. Holt, J. G., et al., Bergey's Manual of Determinative Bacteriology. 9th Ed. 1994. Baltimore, USA, Williams and Wilkins Company, p. 71-561.
21. deHoog, G. S., J. Guarro, and M.J. Figueras, Atlas of Clinical Fungi 2nd Edition, Central Bureau voor Schimmel: 1995. Kluwer Accademic Publisher. p 23-1109.
22. Domsch, K. H. and W. Gams, Fungi in Agricultural Soils, 1970. Longman Group Limited, London.
23. Kostka, J. E., et al, Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the deep horizon oil spill. Applied Environmental Microbiology, 2011. 77, 7962-7974.
24. Okpokwasili, G. C. and B.B. Okorie, Biodeterioration potentials of microorganisms isolated from car engine lubricating oil. Tribology International, 1988. 21, p. 215-220.
25. Elemuo, N. G., et al., Production and Characterization of Lipase Enzyme Expressed by Crude Oil Contaminated Soil Isolates. Universal Journal of Microbiology Research, 2019. 7(1): p. 1-6.
26. Olukunle, F.O., Microbial Population and Physicochemical Properties of Oil-Polluted Sites in Selected Areas of Niger Delta, Nigeria. FUTA Journal of Research in Sciences, 2013. 2, p. 249-258.

27. Ikuesan, F.A., Evaluation of Crude Oil Biodegradation Potentials of Some Indigenous Soil Microorganisms. Journal of Scientific Research & Reports, 2017. 13(5): p. 1-9.
28. Teknikio, J.B., et al., Isolation and Identification of Bacteria in Petroleum Hydrocarbons Polluted Soils in North-West Bayelsa State. Covenant Journal of Physical & Life Sciences, 2018. 1(2): p. 1-13.
29. Das, N. and P. Chandran, Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnology Research International, 2011. 2011:941810. DOI: 10.4061/2011/941810.
30. Rampelotto, P.H., Resistance of Microorganisms to Extreme Environmental Conditions and Its Contribution to Astrobiology. Sustainability 2010. 2, p. 1602-1623; doi:10.3390/su2061602.
31. Jain, P.K. et al., Bioremediation of Petroleum oil Contaminated Soil and Water. Research Journal of Environmental Toxicology, 2011. 5: p. 1-26.
32. Osarumwense, J. O., F.A. Igiebor, and D.E. Idahosa, Isolation, Characterization and Identification of Bacterial isolates from Auto-mechanic Workshop contaminated with Hydrocarbon. The Pacific Journal of Science and Technology, 2019. 20(1): p. 349-355.
33. Agu, K. C., et al., Isolation and Characterization of Microorganisms from Oil Polluted Soil in Kwata, Awka South, Nigeria. American Journal of Current Microbiology, 2015. 3(1): p. 46-59.
34. Oyeleke S.B., et al., Production and Characterization of Biosurfactants by *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15 for crude oil recovery and cleaning of oil contaminated fabrics. International Journal of Life Sciences and Technology, 2017. 10 (15): 109-123.
35. Karlapudi, A.P., et al., Role of biosurfactants in bioremediation of oil pollution-a review. Petroleum, 2018. 4(3): p. 241-249.

Derleme makale / Review article

Elmas, S. and O. Elmas, Salvia fruticosa'nın (Anadolu Adaçayı) Terapötik Etkileri. International Journal of Life Sciences and Biotechnology, 2021. 4(1): p. 114-137. DOI: 10.38001/ijlsb.764602

Salvia fruticosa'nın (Anadolu Adaçayı) Terapötik Etkileri

Sinem Elmas[1]*, Onur Elmas[2]

ÖZET

Anadolu adaçayı (*Salvia fruticosa*), Lamiaceae familyasına ait bir bitki olup, Anadolu'da geleneksel halk hekimliğinde yaygın olarak kullanılmaktadır. Bitkinin yaprakları çay şeklinde tüketilir ve yapraklarından uçucu yağ elde edilir. *Salvia fruticosa* bitkisi farmasötik ajan olarak (antioksidan, antikanser, antimikrobiyal, antifungal, antihiperglisemik, antihipertansif vb.) kullanımı dışında aromaterapi, parfümeri ve kozmetik sektöründe; yiyeceklerde baharat, aroma verici ve koruyucu olarak pek çok alanda kullanıma sahiptir. Ancak bitkinin irrasyonel kullanımı ciddi yan etkilere ve komplikasyonlara yol açmaktadır. Bu derleme, *Salvia fruticosa*'nın botanik özelliklerini, uçucu yağ bileşenlerini, bu bileşenlerin potansiyel terapötik etkilerini ve neden olabileceği olası toksik etkileri değerlendirmeyi amaçlamaktadır.

MAKALE GEÇMİŞİ
Geliş
6 Temmuz 2020
Kabul
3 Eylül 2020

ANAHTAR KELİMELER
Anadolu adaçayı,
Salvia fruticosa,
terapötik etkiler,
toksik etkiler

Therapeutic Use of *Salvia fruticosa* (Anatolian Sage)

ABSTRACT

Anatolian sage (*Salvia fruticosa*) is a plant species belongs to Lamiaceae family and is widely used in traditional folk medicine in Anatolia. The leaves of the this plant species are consumed as tea and essential oil is obtained from the leaves. Besides utilizing as a pharmaceutical agent (antioxidant, anticancer, antimicrobial, antifungal, antihyperglycemic, antihypertensive etc.). *Salvia fruticosa* uses in the aromatherapy, perfumery and cosmetics industry, as a spice, flavoring and preservative in foods. However, irrational use of the this plant may causes serious side effects and complications. This review aims to evaluate the botanical properties, essential oil components, their potential therapeutic effects and possible toxic effects of *Salvia fruticosa*.

ARTICLE HISTORY
Received
6 July 2020
Accepted
3 September 2020

KEY WORDS
Anatolian sage,
Salvia fruticosa,
therapeutic effects,
toxic effects

Giriş

Antik çağlardan itibaren amprik uygulamalarla halk hekimliğinde kullanılan tıbbi ve aromatik bitkiler modern tıbbın temeli olarak kabul edilir [1]. Son yıllarda modern tıpta kullanılan sentetik kökenli ilaçların olası yan etkileri, antimikrobiyal olarak kullanılan bu ilaçlara karşı bazı mikroorganizmaların dirençli olması [2] ve gıda korumada kullanılan sentetik bileşiklerin yarattığı endişeler doğal bitkisel kaynaklara ilgiyi arttırmıştır [3]. Biyoteknolojinin gelişmesiyle birlikte yapılan çalışmalar, bitkilerdeki sekonder

[1] Muğla Sıtkı Koçman University, Faculty of Science, Department of Biology, Muğla / Turkey
[2] Muğla Sıtkı Koçman University, Faculty of Medicine, Department of Physiology, Muğla / Turkey
*Corresponding Author: Sinem Elmas, e-mail: sinemelmas@hotmail.com.tr

metabolitlerin çok çeşitli biyoaktif farmasötik bileşenler içerdiği ve çeşitli hastalıkların tedavisinde terapötik ajan olma potansiyelleri hakkında pek çok kanıt sunmaktadır [4].

Salvia (adaçayı) türleri eski zamanlardan itibaren geleneksel halk hekimliğinde, çay, merhem, tentür veya ekstrakt olarak hazırlanarak ağrı kesici, balgam söktürücü, gaz söktürücü, sakinleştirici, ter kesici, haricen yara iyileştirici olarak; soğuk algınlığı, bronşit, tüberküloz, menstrüel bozukluklar ve midevi rahatsızlıkların tedavisinde kullanılan şifalı bitkilerdir [5]. Bu nedenle *Salvia* cinsinin aktif bileşenlerini ve bunların biyolojik etkinliklerini belirlemek üzere çok sayıda araştırma projesi gerçekleştirilmiştir. *Salvia* türleri esas olarak fenolik asitler, flavonoidler, terpenler ve terpenoidler içermektedir. Araştırmalarda, *Salvia* türlerinden izole edilen sekonder metabolitlerin antimikrobiyal, antifungal, antiseptik, analjezik, antioksidan, antispazmodik, antidepresan, antimutajenik antikolinesteraz, halusinojenik, antidiyabetik, antikanser, antihipertansif, antienflamatuar, tüberkülostatik, vazodilatör, hipoglisemik ve insektisit aktiviteler gibi çeşitli biyolojik etkilere sahip olduğu gösterilmiştir [4, 6].

Salvia cinsinin Anadolu'da iyi bilinen, hem tıbbi açıdan hem de ekonomik açıdan önemli bir türü *Salvia fruticosa'*dır. Ülkemizde Anadolu adaçayı, elma çalbası, boz şalba, elma çalısı, almiya çalbası veya adaçayı olarak bilinen bu türün Kew kontrol listesine göre 19 eşanlamlısı vardır. Literatürde en çok *Salvia triloba*, *Salvia libanotica*, *Salvia cyrpia* ve *Salvia lobryana* olarak karşımıza çıkmaktadır [5, 7]. *Salvia fruticosa*'nın baş ağrısı, romatizma, epilepsi, hepatit, uykusuzluk, ishal, apse, öksürük, gastrit, gut, obezite, ses kısıklığı, herpes, solunum hastalıkları, akciğer hastalıkları, dalak hastalıkları, sinir hastalıkları, ağız hastalıkları, cilt hastalıkları, kas-iskelet hastalıkları, kardiyovasküler hastalıkların tedavisinde; kolesterol düşürücü, diüretik, karminatif, antispazmodik, iştah açıcı ve yara iyileştirici olarak kullanıldığı belirtilmiştir [6].

Salvia fruticosa bitkisi, tıbbi amaçlarla kullanımının dışında aromaterapi, parfümeri ve kozmetikte, doğal böcek kovucu olarak, balık, et, çorba, güveçlerde baharat olarak; turşu, peynir, içeceklerde aroma verici olarak ve işlenmiş gıdalarda antioksidan olarak kullanılmaktadır [8].

Geleneksel halk hekimliğinde yaygın olarak kullanılan *Salvia fruticosa*'nın ekstraktlarının aktif bileşenlerinin tanımlanması ve terapötik potansiyellerinin belirlenmesi bitkinin etno-farmasötik kullanımına destek vermektedir. Yapılan çalışmalar *Salvia fruticosa*'nın antioksidan [9] [10], antikanser [11], antimikrobiyal [12],

antianjiyogenik [13], antifungal [14], antikolinesteraz [10] hipoglisemik [15], antiproliferatif [16] gibi bir dizi terapötik özellik gösterdiğini ortaya koymuştur.
Bu derleme, *Salvia fruticosa*'nın botanik özelliklerini, uçucu yağ bileşenlerini, bu bileşenlerin potansiyel terapötik etkilerini, yanlış ve aşırı kullanımda neden olabileceği toksik etkileri açıklamayı amaçlamaktadır.

Salvia fruticosa Hakkında Genel Bilgiler

Salvia fruticosa çift çenekliler sınıfının (Magnoliopsida), ballıbabagiller (Lamiaceae) familyasından, *Salvia* cinsine ait çalı görünümlü çok yıllık bir türdür. Dünyada İtalya, Sicilya, Balkanlar, Sirenayka ve Suriye'nin batısında, ülkemizde ise Batı Ege, Kuzeybatı ve Batı Akdeniz bölgelerinde doğal yayılış göstermektedir [17]. *Salvia fruticosa* bitkisi 0-1350 m rakımlar arasında yetişebilen, maki veya frigana ekosistemlerinde ve adalarda yayılış gösteren, 160 cm yüksekliğe erişebilen, Mart-Mayıs aylarında çiçeklenen bir bitkidir. Yapraklar, genç gelişme devresinde beyazımsı griden, gümüş rengine kadar değişen renkte ve tüylüdür. Esas yaprakların yanında bir veya iki tarafı az veya çok gelişmiş yan yaprakçık bulunmaktadır [18, 19]. Bitkinin yapraklarından açık sarı renkte veya renksiz 'elma yağı' denilen yağ elde edilir. Elma yağı olarak adlandırılmasının nedeni bitkinin bazı dallarının ucunda elmaya benzeyen ve yaklaşık 2-3 mm uzunlukta, 2 mm genişlikte ve kalınlıkta olan mazıların (gal) bulunmasıdır [18, 20].
Uzun yıllardır floradan toplanarak kullanılan bu türün hem iç pazarda hem de dış pazarda önemli bir yeri bulunmaktadır. Türkiye İstatistik Kurumu'nun verilerine göre, 2018 yılında ülkemizden 7.696 milyon dolar karşılığı 1.843 ton kuru adaçayı yaprağı ihraç edilirken, 2019 yılında 9.488 milyon dolar karşılığı 2.261 ton kuru adaçayı yaprağı ihraç edilmiştir [21].

Salvia fruticosa'nın Uçucu Yağ Oranı ve Kimyasal Bileşenleri

Bitkilerden elde edilen uçucu yağ bileşikleri, monoterpenler, seskiterpenler ve bunların oksijenli türevleri olan alkoller, aldehitler, esterler, eterler, ketonlar, fenoller ve oksitlerin kompleks bir karışımıdır [12]. *Salvia fruticosa*'daki uçucu yağların başlıca kimyasal bileşimi mono-seskiterpen hidrokarbonlar (α-pinen, β-pinen, kamfen, mirsen, karyofilin) ve oksijenli monoterpenlerdir (1,8 sineol, kafur, borneol, α-terpinil asetat) [22]. Bitki uçucu yağının fenolik asitler, flavonoidler, kafeik asit ve özellikle rosmarinik asit açısından çok zengin olduğu bildirilmiştir [23].

Salvia fruticosa bitkisinin uçucu yağı genellikle hidrodistilasyon yöntemi ile yapraklarından elde edilir. Bitkinin yaprakları ortalama %1-3 oranında uçucu yağ taşır, Bornova lokasyonunda yetiştirilen *Salvia fruticosa* populasyonlarında bu oranın %5,5-6'ya kadar çıktığını belirtilmiştir [24]. Bitkide uçucu yağ veriminin, özellikle bitki gelişiminin farklı aşamalarının etkisi altında olduğunu gösterilmiştir. Farklı coğrafyalarda incelenen *Salvia fruticosa*'da en yüksek yağ verimini, bazı araştırmacılar çiçeklenmeden önce veya çiçeklenme başlangıcında olduğunu öne sürmüş [22, 25, 26, 27], bazıları ise en yüksek verimin çiçeklenme sonrası [28, 29] dönemde olduğunu belirlemişlerdir. Ayrıca öğle saatleri içeriklerindeki aktif maddelerin zirveye ulaştığı zaman olduğu bildirilmiştir [30].

Tıbbi ve aromatik bitkilerde, bitki materyalinin terapötik etkinliği fitokimyasal içeriğine ve bileşimine bağlıdır [27]. Farklı bölgelerde değişik araştırmacılar tarafından yapılan çalışmalarda *Salvia fruticosa'*nın uçucu yağ verimi ve bileşiminde geniş varyasyon olduğu göze çarpmaktadır. Bu varyasyonun nedeni fitokimyasal profili, toprak, sıcaklık, yağış, ışıklanma süresi ve şiddeti, rakım, bakı, kuraklık, tuzluluk, toprak besin maddeleri, toprak yapısı gibi pek çok farklı ekolojik, coğrafik ve iklimsel faktörlere [19, 29, 31] bağlı olabileceği gibi, genetik varyasyona [32, 33], bitkinin ontogenetik evresine [33], hasat zamanına ve farklı ekstraksiyon yöntemlerine [29] bağlı olarak değişebilmektedir. Bunların dışında azotlu gübre uygulamasının ve organik gübre uygulamasının bitkide uçucu yağ miktarını ve içeriğini arttırdığı bildirilmiştir [18, 34, 35].

Terapötik Etkileri

Antimikrobiyal etkileri

Günümüzde bazı patojenlerin antibiyotiklere karşı direnç geliştirmesi enfeksiyonlarla mücadelede zorluklar yaşanmasına neden olmaktadır. Mevcut antibiyotiklerin olası yan etkileri de göz önüne alındığında yeni antimikrobiyal ajanların araştırılmasını zorunlu hale getirmektedir. Araştırmalar, Lamiaceae familyasına ait tıbbi ve aromatik bitkilerin çoğunun uçucu yağlarının antimikrobiyal ajan olma potansiyellerine sahip olduklarını göstermektedir [36]. Bunun yanı sıra, bitkilerdeki aktif bileşenlerin gıda güvenliğini yüksek oranlarda korumayı başardığını belirlenmiş [37], özellikle *Salvia fruticosa* uçucu yağının gıdaların bozulmasında rol oynayan bakterilere karşı iyi bir antimikrobiyal aktivite gösterdiği, bu nedenle gıda güvenliğini ve raf ömrünü artırmaya katkıda bulunan geleneksel gıda koruyucularına alternatif olabileceğini gösterilmiştir [12].

Yapılan bir araştırmada *Salvia fruticosa*'dan izole edilen 1,8 sineol ve thujon bileşenlerinin *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Rhizobium leguminosarum* ve *Bacillus subtilis* suşlarına karşı antimikrobiyal aktivite sergilediği, ancak diğer bir bileşen kafurun ise test edilen bakterilere karşı neredeyse hiç aktif olmadığı tespit edilmiştir [38]. Başka bir çalışmada *Salvia fruticosa* uçucu yağının, *Bacillus cereus, Bacillus megatherium*, *Bacillus subtilis, Aeromonas hydrophila, Aeromonas sobria* ve *Klebsiella oxytoca*'ya karşı düşük konsantrasyonlarda bakteriyostatik, yüksek konsantrasyonlarda bakterisidal aktivite gösterdiği bildirilmiştir. Ayrıca uçucu yağının çok düşük konsantrasyonun bile *Staphylococcus aureus* ve *Aeromonas hydrophila*'nın büyümesini etkili bir şekilde inhibe ettiği belirtilmiştir [12]. Ülkemizde *Salvia fruticosa*'nın da bulunduğu 5 tıbbi bitkinin, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Enterobacter aerogenes* ve *Klebsiella pneumoniae*'ye karşı antibakteriyel aktivesinin araştırıldığı çalışmada, *S. typhimurium* ve *E. aerogenes* karşı en yüksek antibakteriyal aktiviteyi *Salvia fruticosa'*nın sergilediği bildirilmiştir [39]. Bir başka çalışmada, *Salvia fruticosa* uçucu yağının gram pozitif (*Staphylococcus aureus, Bacillus cereus, Micrococcus flavus, Sarcina lutea, Listeria monocytogenes*) ve gram negatif bakterilere (*Escherichia coli, Salmonella typhimurium, Salmonella enteritidis, Pseudomonas tolaasii, Pseudomonas aeruginosa, Proteus mirabilis*) karşı önemli derecede bakteriyostatik ve bakterisidal etki gösterdiği tespit edilmiştir [40]. *Salvia fruticosa* uçucu yağı ile 1,8 sineol, β-pinen ve kafur bileşenleri, gram-negatif (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Listeria monocytogenes, Proteus mirabilis*) ve gram-pozitif bakteriler (*Bacillus cereus, Micrococcus flavus* ve *Staphylococcus aureus*) üzerinde analiz edilmiş ve uçucu yağın test edilen tüm türlere antibakteriyel aktivite sergilediği, test edilen bileşenler arasındaki en iyi aktivitenin, ana bileşen olan 1,8 sineol (0.04-0.07 mg/ml'de bakteriyostatik aktivite ve 0.04-0.09 mg/ml'de bakterisidal aktivite) tarafından gösterildiği bildirilmiştir [41].

Uçucu yağlarda bulunan ana ve diğer bileşenlerin çeşitliliğinin sinerjistik etkileri, biyolojik aktivitelerini belirlemektedir [12]. Uçucu yağların farklı kombinasyonlarının, antimikrobiyal aktivite üzerindeki etkisini araştıran bir çalışmada, *Salvia fruticosa, Elettaria cardamomum* ve *Lavandula angustifolia* uçucu yağ kombinasyonlarından hazırlanan ağız gargaralarının insan patojenik bakterileri *Staphylococcus aureus*,

Escherichia coli, *Bacillus cereus* ve *Salmonella typhii*'ye karşı antimikrobiyal aktivitesi değerlendirilmiştir. *Salvia fruticosa* uçucu yağının tek başına *Staphylococcus aureus* ve *Bacillus cereus*'a karşı antimikrobiyal aktivite gösterdiğini ve bu üç bitkinin uçucu yağlarından hazırlanan kombinasyonun ise incelenen patojenlere karşı yüksek antimikrobiyal aktivite gösterdiği tespit edilmiştir [42].

Antifungal etkileri

Bitkilerin ürettiği sekonder metabolitler, mikrobiyal patojenlerden korunmayı sağlayan savunma mekanizmalarının bir parçasıdır. Bitki patojenik mantarları, taze ve işlenmiş ürünleri hem niceliksel hem de niteliksel olarak olumsuz etkiler. Hasat sonrası sentetik fungisit kullanımı, gıdalarda kimyasal kalıntı bırakması ve fungusit dirençli patojenlerin gelişmesi nedeniyle birçok ülkede giderek kısıtlanmaktadır [43, 44]. Gıdaların depolanmasında mantar oluşumunu kontrol etmede bazı bitkilerin uçucu yağlarının, fungusidal veya fungistatik olarak kullanım potansiyellerini olduğunu kanıtlanmıştır [45, 46]. Bitki sekonder metabolitlerine dayanan bu doğal fungisitler, sentetik fungusitlerin yerine, özellikle organik tarımda değerli alternatif bitki koruma ürünlerini temsil edebilir [47].

Lübnan'da doğal yetişen *Salvia fruticosa* bitkisinin *Verticillium dahliae*, *Botrytis cinerea, Fusarium oxysporum* ve *Alternaria solani* gibi bitkilerde ciddi hastalıklara neden olan mantarlar üzerindeki antifungal etkinliğinin araştırıldığı çalışmada bitkinin petrol eteri ekstraktlarının, bu bitki patojenik mantarlarına orta ila düşük seviyede antifungal etkisi olduğu gösterilmiştir [48]. Başka bir çalışmada, *Salvia fruticosa* bitkisinden izole edilen 1,8 sineol ve kafur bileşenlerinin her ikisinin de *Fusarium oxysporum* f. sp. *dianthi* ve *Fusarium proliferatum*'a karşı düşük seviyede antifungal etki gösterdiği, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* ve *Fusarium solani* sp. *cucurbitae*'ye karşı ise yüksek antifungal aktiviteler sergilediği bildirilmiştir [33]. Antifungal etkisine dair diğer bir çalışmada, Yunanistan'da doğal yetişen *Salvia fruticosa*'nın uçucu yağının *Penicillium expansum*, *Aspergillus terreus* ve *Verticillium dahliae* üzerinde doza bağlı olarak antifungal aktivite sergilediği, yüksek dozajda *Verticillium dahliae*'nin büyümesini güçlü bir şekilde inhibe ettiği rapor edilmiştir [47]. Yürütülen başka bir çalışmada *Salvia fruticosa*'nın etil asetat ekstraktının ve ana bileşenlerinden karnosik asit, karnosol ve hispidulinin, *Botrytis cinerea* ve *Penicillium digitatum*'a karşı antifungal aktivite sergilediği tespit edilmiştir [14]. Farklı araştırma sonuçları bitkinin farklı ekstraktlarının

ve uçucu yağının antifungal etkisini göstermektedir ancak *Salvia fruticosa* uçucu yağının toprak kaynaklı patojenler *Fusarium oxysporum* ve *Macrophomina phaseolina* ile yaprak patojenleri *Botrytis cinerea* ve *Exserohilum turcicum* fitopatojenik mantarların miselyum büyümesini inhibe etmediğini belirten bir çalışma literatür verilerinden sapma göstermiştir [49].

Bununla birlikte, literatürde, uçucu yağların insan mantar patojenlerine karşı antifungal aktivitesi hakkında sınırlı bilgi bulunmaktadır. *Salvia fruticosa*'nın insanlarda cilt enfeksiyonlarına neden olan üç patojenik mantar suşu *Malassezia furfur*, *Trichophyton rubrum* ve *Trichosporon beigelii*'ye karşı orta ila düşük seviyede antifungal aktivite sergilediği bildirilmiştir [50]. Diğer bir çalışmada, *Salvia fruticosa'*nın sulu ekstraksiyonun insanlarda dermatofitik enfeksiyonlara neden olan *Trichophyton mentagrophytes*, *Trichophyton violaceum* ve *Microsporum canis* üzerinde değişen oranlarda antifungal aktivite gösterdiği, en yüksek aktiviteyi ise *Trichophyton violaceum* üzerinde gösterdiği tespit edilmiştir [51].

Çalışmalarda, *Salvia fruticosa* uçucu yağının değişen kantitatif kompozisyonu ve test edilen mantar türlerinden dolayı çalışma sonuçları farklılık gösterse de bazı mantar türlerini kontrol etme yeteneği gıda, tarım ve sağlıkta potansiyel kullanımlarını göstermektedir. Bu nedenle doğal antifungal ajan olarak kullanılacak kaynağın tespit edilmesi, olası sonuçları ve toksik olmayan dozajını araştırmak için daha fazla çalışmaya gereksinim vardır.

Antioksidan etkileri

Sağlıklı aerobik organizmalarda, reaktif oksijen türlerinin (ROS) üretimi antioksidan savunma sistemleri ile dengelenir [52]. Antioksidanlar, vücut dokularındaki serbest radikallerin neden olduğu oksidatif hasara karşı koyabilen ve hücresel oksidatif stresin yükünü azaltmaya yardımcı maddelerdir. Antioksidan içeren besinlerin diyetle alınması vücut direncini arttırması, oksidatif stres seviyesini azaltması, kanser, diyabet gibi birçok dejeneratif hastalığın önlenmesi ve kronik hastalığın ilerlemesini geciktirebilmesi açısından önemlidir [53, 54]. Biyolojik sistemlerde serbest oksijen radikallerin oluşturduğu zararlı etkilerle baş edebilecekleri için bitkilerin ekstraktları ve spesifik bileşikleri koruyucu rol oynamaktadır [55].

Doğal antioksidanlar yüksek bitkilerde ve bitkinin yaprak, meyve, çiçek ve tohum gibi tüm kısımlarında görülür [52]. *Salvia* cinsine ait türlerin yaprak, çiçek ve odunsu

kısımlarında bulunan flavonoid ve fenolik asitlerin antioksidan aktiviteden sorumlu olduğu bilinmektedir. Fenolik bileşikler ve flavanoidler bu antioksidan etkilerini, hidroksil radikali, süperoksit anyon radikali, lipid peroksil radikalleri gibi serbest radikalleri temizleyerek, metal iyonlarıyla şelat oluşturarak, diğer antioksidanlarla etkileşime girerek ve lipid peroksidasyonunu engelleyerek gösterirler [56, 57]. Anadolu'da halk hekimliğinde yaygın olarak kullanılan *Salvia fruticosa* polifenolik bileşikler açısından çok zengindir. Rosmarinik asit, karnosik asit, kafeik asit, vanilik asit, gallik asit, karnosol, luteolin, metil karnosat, apigenin gibi fenolik bileşiklerin ve flavanoidlerin *Salvia fruticosa* bitkisinde antioksidan etkiden sorumlu olduğu belirtilmiştir [9].

Farklı adaçayı türlerinin (*Salvia officinalis* ve *Salvia fruticosa*) ve farklı kekik (*Origanum onites* ve *Origanum indercedens*) türlerinin antioksidan aktivitelerinin karşılaştırıldığı bir çalışmada, adaçayı türlerinin kekik türlerinden daha yüksek antioksidan aktiviteye sahip olduğu, ayrıca *Salvia fruticosa*'nın içeriğindeki karnosik asit ve metil karnosat miktarı daha fazla olduğu için *Salvia officinalis*'ten daha yüksek antioksidan aktiviteye sahip olduğu belirtilmiştir [58]. Çeşitli *Salvia* türlerinin (*Salvia fruticosa, Salvia chrysophylla, Salvia cilicica, Salvia tomentosa, Salvia halophila, Salvia crypthantha, Salvia sclarea* ve *Salvia palaestina*) antioksidan özelliklerinin araştırıldığı bir başka çalışmada *Salvia fruticosa*'nın diğer türlere kıyasla orta derecede antioksidan aktiviteye sahip olduğu belirlenmiştir [59].

Çalışmalarda *Salvia fruticosa* ekstraktlarının antioksidan aktiviteleri ile toplam fenolik/flavonoid içeriği ve belirli fenolik bileşenler arasında yüksek pozitif korelasyonlar olduğu sonucuna varılmıştır. Bu nedenle araştırmacılar, *Salvia fruticosa*'nın uçucu yağındaki fenolik bileşiklerin, anti-oksidatif aktiviteden sorumlu olduğunu bildirmişlerdir [14, 19, 58].

Salvia fruticosa'nın vejetatif döngüsü sırasında (çiçeklenme öncesi, çiçeklenme dönemi ve meyve dönemi) toplam fenoliklerin, flavonoidlerin ve antioksidan aktivitenin mevsimsel değişiminin incelendiği bir çalışmada flavonoid konsantrasyonu en yüksek çiçeklenme aşamasında (Mayıs), en düşük meyve aşamasında (Ağustos) tespit edilmiş olup; antioksidan aktivitenin en yüksek olduğu meyve safhası (Ağustos) yüksek fenolik asit içeriği ile karakterize edilmiştir. Antioksidan aktivite ile toplam fenolik konsantrasyonu arasında pozitif korelasyon belirlenmiştir. Araştırmacılar, sıcaklık, nem,

yağış vb. çevresel faktörlerin kombinasyonu bitkilerde bulunan fenoliklerin birikmesini hızlandırabilir veya yavaşlatabilir sonucuna varmışlardır [19]. İzmir'den toplanan *Salvia fruticosa*'nın yapraklarında ve çiçek ekstraktlarındaki toplam fenolik içeriklerin incelenen *Polygonum cognatum* Meissn, *Salvia officinalis*, *Thymus vulgaris*, *Rosmarinus officinalis* ve *Origanum vulgaris* türlerininkinden daha yüksek olduğu; yaprak ekstraktındaki hidroksil radikali süpürme kapasitelerinin (%) ise *Origanum vulgaris, Rosmarinus officinalis, Salvia officinalis* ve *Thymus vulgaris* ekstraktlarından daha yüksek olduğu belirlenmiştir. Ayrıca, *Salvia fruticosa*'nın yaprak ekstraktlarının toplam antioksidan kapasitesinin çiçek ekstraktlarından anlamlı derecede yüksek olduğu tespit edilmiştir [52]. Benzer bir çalışmada Yunanistan ve Girit'in farklı yerlerinden alınan *Salvia fruticosa*'nın yaprakların etanolik ekstraktlarının, çiçeklerinden daha yüksek antioksidan aktivite gösterdiği bildirilmiştir [60]. *Salvia fruticosa* kökünün ve toprak üstü kısımlarının antioksidan aktivitelerinin değerlendirildiği bir çalışmada, bitkinin kök ekstraktlarının en fazla toplam fenolik konsantrasyona sahip olduğu ve en yüksek antioksidan aktiviteyi sergilediği dolayısıyla da zengin bir antioksidan kaynağı olduğu bildirilmiştir [3]. Diğer bir çalışmada 28'i endemik olan 55 Türk *Salvia* taksonunun antioksidan aktivitelerini araştırılmış ve incelenen tüm türler arasında en yüksek antioksidan aktiviteyi (%89.23) *Salvia fruticosa*'nın diklorometan ekstraktlarının gösterdiği bildirilmiştir [10]. Antalya'da doğal yetişen ve kültürü yapılmış *Salvia fruticosa*'da fenolik içerik, kompozisyon ve antioksidan aktivitesi gibi ana kalite parametrelerinin iki yıl boyunca araştırıldığı bir çalışmada, kültürü yapılan örneklerde fenolik içeriğin daha yüksek olduğu, doğal yetişen örneklerde toplam flavonoidlerin ve toplam uçucu yağ oranının daha yüksek sahip olduğu belirtilmiştir. Numunelerin antioksidan aktivitesinin ikinci hasat yılı için daha yüksek olduğu, altı aylık saklama süresi boyunca doğal yetişen ve kültürü yapılan *Salvia fruticosa*'nın antioksidan aktiviteleri arasında anlamlı bir fark bulunmadığı saptanmıştır [9]. Libya'da doğal olarak yetişen ancak timol ve karvakrol gibi fenolik bileşiklerden yoksun *Salvia fruticosa*'dan izole edilen uçucu yağda düşük ise antioksidan aktivite belirlenmiştir [61]. Lipitlerin oksidasyonu, gıda ürünlerinin hem organoleptik özellikler hem de toksikolojik yönler açısından raf ömrünü sınırlayan ana faktörlerden biridir [58]. Bu gıdaların bozulmasını geciktirmek için sentetik katkı maddeleri yerine bitkilerden izole edilen antioksidan bileşikler kullanılabilir. Çalışmalar göstermiştir ki *Salvia fruticosa* antioksidanları, belirli gıda ve nutrasötik ürünlerin raf

ömrünü uzatmak ve korunmasını sağlamak için halihazırda kullanılan sentetik antioksidanlarına alternatif olarak kullanılabilir [58, 62].

Antikanser etkileri

Kanser, DNA hasarı sonucu doku veya organlarda hücrelerin kontrolsüz bir şekilde büyümesi ve çoğalmasıdır [63]. Dünya Sağlık Örgütü (DSÖ) ve Uluslararası Kanser Araştırmaları Ajansı, dünyada sadece 2018 yılında 18,1 milyon kişiye kanser teşhisi konulduğunu ve 9,6 milyon kişinin kanserden yaşamını yitirdiği belirtmiştir. Ayrıca raporda 2040 yılına kadar dünya genelinde kanser vakalarının %60 artış göstereceği öngürülmüştür [64]. Dünyada olduğu gibi ülkemizde de kanser vakaları her yıl katlanarak artmaya devam etmektedir. Türkiye'de kadınlarda en sık görülen kanser türleri, başta meme, tiroid, kolorektal, rahim ağzı ve akciğer kanserleri; erkeklerde en sık görülen kanser türleri sırasıyla akciğer, prostat, kolorektal, mesane ve mide kanserleri olduğu bildirilmiştir [65]. 100'den fazla çeşidi olan kanser, tedavi edilmezse ciddi sağlık sorunlarına, hatta ölüme neden olabilir.

Kanser tedavisinde kullanılan kemoterapötik ilaçlar, hücre siklus kontrol noktalarını hedefleyerek, hücre çoğalmasını durdurarak ve apoptoz indüksiyonu ile etki gösterir. Ancak klasik kemoterapi ilaçlarının kanserli olmayan dokularda sitotoksisiteye neden olmaları, ciddi yan etkileri ve belirli dozlar üzerinde ölümcül toksisitelere neden olabilmeleri gibi dezavantajları vardır. Tüm bu nedenler yüksek spesifikliğe sahip kanser hücrelerinin tedavisinde sentetik muadillerine göre yan etkileri daha az, etkili, doğal ve uygun fiyatlı yeni antikanser ajanları arayışını gerektirmektedir [66, 67].

Bitki kaynaklı bileşiklerin, serbest radikal süpürme, detoksifikasyon enzimlerini değiştirme ve transkripsiyon faktörünün tümör promotörleri tarafından indüklenmesini inhibe etme gibi çeşitli mekanizmalarla tümör oluşumunun çeşitli aşamalarını inhibe edebildiğine dair pek çok çalışma, kanser önleme ve tedavisinde doğal ürünlerin önemini belirtmektedir [68, 69]. Birçok *Salvia* türü ekstraktı, bir dizi farklı kanser hücresi tipinde *in vitro* ve *in vivo* koşullarda sitotoksik, anti-anjiyojenik ve apoptotik etkiler ile antikanser performansı sergilemiştir [1, 16, 70]. Özellikle *Salvia fruticosa*'nın farklı ekstraktlarının, çoğu insan ve memeli hücre hatlarında yapılmış çalışmalarında kemopreventif etkiler gösterdiği bildirilmiştir.

Deneysel bir çalışmada, DMBA / TPA cilt kanseri modeli Balbc farelerinin derisine topikal olarak uygulanan *Salvia fruticosa* uçucu yağının, tümör görünümünü 4 hafta

geciktirdiği ve tümör oluşumunu yaklaşık %78 oranında engellediği bildirilmiştir [71]. Potansiyel antineoplastik özelliklere sahip bitki ekstraktlarını belirlemek için hücre hatlarına karşı sergiledikleri sitotoksik aktiviteler önemli ön veriler sağlamaktadır, bu nedenle birçok çalışmada sitotoksik aktiviteler belirlenmiştir [72]. Yunanistan'ın farklı yerlerinden toplanan *Salvia* cinsine ait bazı türlerin ekstraktlarının *in vitro* koşullarda dört tür insan kanser hücre hattına (HCA, HepG2, MCF-7 ve HPC) karşı sitotoksik aktivitelerini test edilmiş, *Salvia fruticosa* örneklerinin test edilen insan kanseri hücre hatlarının en az bir veya daha fazlasına sitotoksik olduğunu belirlenmiştir [73]. Başka bir çalışmada, Filistin'den alınan *Salvia fruticosa*'nın da dahil olduğu 24 türün potansiyel anti-tümör ve anti-inflamatuar aktiviteleri, farelerin fibrosarkom hücreleri (L929sA) ile insan meme kanseri hücreleri MCF7 ve MDA-MB231 üzerinde test edilmiş, *Salvia fruticosa* diklorometan ve metanol ekstraktlarının incelenen tüm hücre hatlarına farklı derecelerde sitotoksik aktivite sergilediği bildirilmiştir [72].

Fitokimyasalların etkili bir antikanser terapötik seçenek olarak kabul edilebilmesi için sinyal yolaklarını ve hücre döngüsü kontrol noktalarını hedeflenmesi gerekir. *Salvia fruticosa*'da biyoaktif bileşikler polifenoller bakımından oldukça zengindir ve rosmarinik asitin antiproliferatif özellikleri iyi bilinmektedir [74, 75]. *Salvia fruticosa* ve *Salvia officinalis* sulu ekstraktları ile bunların ana fenolik bileşiği rosmarinik asitin, MAPK/ERK ve PI3K/Akt sinyal yolaklarında farklı mutasyonlara sahip insan kolon karsinom türevi hücre hatları (HCT15 ve CO115) üzerinde antiproliferatif ve pro-apoptotik etkilerinin değerlendirildiği bir çalışmada, *Salvia* ekstraktlarının ve rosmarinik asidin iki hücre dizisinde de konsantrasyona bağlı bir şekilde apoptozu indüklendiği, ancak sadece HCT15 hücre hattında her iki bitki ekstraktının proliferasyonu engellediği bildirilmiştir [16].

Benzer bir çalışmada, *Salvia fruticosa, Salvia officinalis* ve *Salvia lavandulifolia*'nın sulu ekstraktları, rosmarinik asit ile luteolin-7-glukozitin, oksidatif ajanlara maruz bırakılmış Caco-2 ve HeLa hücrelerinde DNA onarımı üzerindeki etkileri araştırılmıştır. Çalışma sonunda incelenen *Salvia* ekstraktlarının ve rosmarinik asit ile luteolin-7-glukozitin, hücreleri oksidatif DNA hasarlarına karşı koruduğu ve DNA onarımını uyardığı belirlenmiştir. *Salvia fruticosa'*nın kemoprotektif etkisini, DNA hasarını önlemesi, gelişmiş DNA onarımı, hücre çoğalması önlenmesi ve apoptoz indüksiyonu gibi çoklu mekanizmalarla gerçekleştirdiği öne sürülmüştür [76]. Benzer bir başka çalışmada ise

Salvia fruticosa sulu ekstraktının, insan embriyonik böbrek hücre hatlarını (HEK 293), hem intrinsik hem de H_2O_2 ile indüklenen DNA oksidasyonuna karşı koruduğu gösterilmiştir [54].

Tıbbi bitkilerin kemoprotektif özellikleri, hücre hattına bağlı olarak değişiklik göstermektedir. *Laurus nobilis, Origanum syriacum, Origanum vulgare* ve *Salvia fruticosa*'nın uçucu fraksiyonları, sulu ve etanol ekstraktlarının MCF7 meme kanseri hücre hattında kullanarak insan meme adenokarsinomu hücrelerine karşı antiproliferatif aktivitesinin incelendiği çalışmada antiproliferatif aktiviteyi sadece *Salvia fruticosa*'nın etanol ekstraktı göstermiştir [77]. Ürdün'de yürütülen bir çalışmada *Salvia fruticosa* etanol ekstraktının, normal insan periodontal fibroblastlarına karşı minimum toksisite ile östrojen reseptörü pozitif meme kanseri hücre hatlarına (MCF-7, T47D, ZR-75-1 ve BT 474) karşı seçici antiproliferatif aktivite sergilediği belirlenmiştir [78]. Aynı araştırmacı benzer başka bir çalışmasında, *Salvia fruticosa* ve *Salvia dominica* türlerinin etanol ekstraktlarının, MCF7 ve T47D meme kanseri hücre hatlarına karşı *in vitro* hücre proliferasyonu ve hücre döngüsü regülasyonu üzerindeki etkileri değerlendirmişler, her iki *Salvia* türünün etanolik ekstraktların MCF7 ve T47D hücre hatları üzerinde antiproliferatif aktivitelerinin hem apoptoz hem de nekroza bağlı olduğu sonucuna varmışlardır [67].

Farklı bir çalışmada, *Salvia fruticosa* ekstraktının metabolik aktivatör (S9mix) yokluğunda insan periferal lenfositlerinde, mitotik indeksi düşürerek sitotoksik etki yaptığı ancak proliferasyon indeksi ve nükleer bölünme indeksini düşürmediği, metabolik aktivatör varlığında ise sitotoksik olmadığı ancak yüksek dozlarda ise siklofosfamidinin sitotoksik etkiyi artırdığını saptamışlardır [79]. *Salvia fruticosa* metanolik ekstraktının prostat kanseri hücreleri üzerinde etkisini araştıran bir çalışmada, ekstraktın normal hücrelere sitotoksik etki göstermediği ancak kanser hücrelerinde konsantrasyona bağlı bir şekilde sitotoksisite gösterdiği ve apoptozu indüklediği belirtilmiştir [11]. *Salvia fruticosa* bitkisinin alt türü *Salvia fruticosa* Mill subsp. *thomasii* (Lacaita) Brullo, Guglielmo, Pavone & Terrasi'nin antitümör ve proapoptotik etkilerinin araştırıldığı çalışmada ise bitkinin metanol ekstraktının insan meme kanseri hücre hatlarında (MCF-7 ve MDA-MB-231) ve kolorektal hücre hatlarında (RKO ve Caco-2) apoptotik mekanizma ile kanser büyümesini inhibe ettiği gözlenmiş ayrıca tümöral olmayan 3T3-L1 hücreleri üzerinde düşük sitotoksik etkilere sahip bir apoptotik mekanizma ile ölüme yol açtığı belirlenmiştir

[1]. *Salvia fruticosa* ve *Salvia lanigera*'nın farklı türde ekstraktlarının insan kolon kanseri hücre hattı HCT-116 üzerindeki sitotoksik aktivitesini belirledikleri araştırmada ise sadece *Salvia fruticosa*'nın etanol ekstraktının aktivite gösterdiği bildirilmiştir [40]. *Salvia fruticosa* metanol ekstraktlarının, melanom hücre hattı WM 136.1A proliferasyonu üzerinde doza bağlı olarak anlamlı bir sitotoksik etki gösterdiği bildirilmiştir [80]. Antikanser etkisi ile ilgili yapılan diğer bir çalışmada, *Salvia fruticosa*'nın sulu ekstraktının kolorektal adenokarsinom hücre dizilerinde (Caco-2 ve HT-29) zamana ve doza bağlı anti-proliferatif aktivite sergilediği tespit edilmiştir. Bitki ekstraktının önemli bir Glutatyon S-transferaz (GST) inhibitörü olarak kabul edilebileceği ve kanser kemoterapisinde yardımcı madde olarak potansiyel bir kullanıma sahip olduğu sonucuna varılmıştır [23]. *Salvia fruticosa* ve *Salvia pomifera*'nın metanolik ekstraktlarının insan melanoma hücreleri üzerinde etkisinin değerlendirildiği çalışmada, *Salvia fruticosa* ekstresinin daha verimli bir şekilde, kanser hücrelerinin proliferasyonunu azalttığı bildirilmiştir. Çalışmada fenolik bileşenlerden karnosik asidin mikrotübül dinamiklerini etkilediği ve G2/M fazındaki hücre döngüsünü durdurduğu tespit edilmiştir. Bu nedenle karnosik asidin *Salvia fruticosa* ekstraktlarının sitostatik etkisinden sorumlu bileşik olabileceğini öne sürmüşlerdir [81]. Libya'da yetişen *Salvia fruticosa*'nın metanol ekstraktının üç meme kanseri hücre hattında (MCF-7, T47D ve MDA-MD-468) anti-proliferatif aktivitesinin değerlendirildiği bir çalışmada bitkinin, incelenen tüm meme kanseri hücre hatlarına karşı yüksek anti-proliferatif aktivite sergilediği bildirilmiştir [82].

Anti-Anjiyogenik etkisi

Anjiyogenez, var olan damarlardan yeni kan damarların oluşmasıdır [83]. Anjiyogenez, büyüme, gelişme, yara iyileşmesi gibi süreçlerde olması beklenen olağan bir durumdur ancak tümörlerin metastazında kritik bir role sahiptir. Çünkü, tümör boyutundaki herhangi bir artış, kan akışındaki artışla senkronize olmalıdır bu nedenle kanserin ilerlemesi anjiyogeneze bağlıdır. Ayrıca tümör hücreleri büyümek ve metastaz yapabilmek için yeni kan damarlarının taşıyacağı besin maddelerine ve oksijene ihtiyaç duyarlar. Bu nedenle kanser tedavisinde anjiyogenezin inhibisyonu önemli bir strateji olarak kabul edilmektedir [13].

Literatürde *Salvia* cinsine ait türlerinin anti-anjiyojenik aktivitesi hakkında bilgiler sınırlıdır, ancak farklı araştırmacılar tarafından çalışmalarda *Salvia miltiorrhiza* [84],

Salvia plebeia R. Brown [85], *Salvia officinalis* [86] ve *Salvia chinensis*'in [87] anti-anjiyojenik aktivitelerini bildirmişlerdir.

Bu türlerle yapılan umut verici sonuçlarından sonra Ürdün'de yetişen *Salvia fruticosa, Salvia dominica, Salvia syriaca* ve *Salvia hormium* türlerinin antianjiyojenik aktiviteleri sıçan aortik halka deneyi ile değerlendirilmiştir. İncelenen türler içinde *Salvia fruticosa*'nın hem doğrudan ve hem de dolaylı olarak antianjiyojenik aktivite gösterdiği ve bitkinin kemoterapötik ve/veya kemopreferans potansiyeline sahip güçlü bir aday olabileceği sonucuna varılmıştır [13]. Benzer başka bir çalışmada *Salvia fruticosa* metanolik ekstraktının, prostat kanseri hücrelerinde anjiyojenik sitokin salgılanmasında önemli değişiklikler oluşturarak anjiyogenezi inhibe ettiği bildirilmiştir [11].

Anti-kolinesteraz etkisi

Salvia türlerinin farklı ekstrakları ile -çoğunlukla yaşlı nüfusu etkileyen ilerleyici nörodejeneratif bir rahatsızlık olan- Alzheimer hastalığının tedavisi üzerine kapsamlı birtakım çalışmalar yapılmış ve hastalığın tedavisinde kolinerjik aktivite gösterdikleri belirlenmiştir [88, 89, 90]. *Salvia fruticosa*'daki bazı terpenoidlerin özellikle 1,8 sineol, kafur, α-pinen bileşenlerinin, hastalığın tedavisinde terapötik bir hedef olan asetilkolinesteraz (AChE) ile bütirilkolinesteraz (BChE) enzimlerinin aktivitesini baskıladığı gösterilmiştir.

Salvia fruticosa uçucu yağında düşük molekül ağırlıklı bileşenlerin ve yağdaki doğal kombinasyonların insanlarda BChE'nin inhibisyonuna katkısının *in vitro* olarak araştırıldığı bir çalışmada, *Salvia fruticosa* uçucu yağları zamana bağlı olarak BChE inhibisyonu göstermiş olup, bu etkinin uçucu yağ bileşenleri arasında sinerjik etkileşimden kaynaklanabileceği sonucuna varılmıştır, ayrıca düşük molekül ağırlıklı bileşiklerin AChE'yi kolayca inhibe ettiği tespit edilmiştir [89]. Diğer bir çalışmada araştırmacılar *Salvia fruticosa*, *Teucrium polium* ve *Melissa officinalis* etanol ekstraktlarının *in vivo* antiamnezik aktiviteleri ile *in vitro* antikolinesteraz aktivitelerini değerlendirmişler, antiamnezik deneyde en etkili olan bitkinin *Salvia fruticosa* olduğunu, antikolinesteraz analizinde ise asetilkolinesteraza karşı en yüksek inhibisyonu *Teucrium polium* bitkisinin ardından *Salvia fruticosa*'nın gösterdiğini belirtmişlerdir [91].

Antikolinesteraz etkinin araştırıldığı kapsamlı bir çalışmada, 55 Türk *Salvia* taksonunun AChE inhibe edici potansiyelleri değerlendirilmiş ve en aktif ekstraktın 100 µg/ml'de %51.08 inhibisyona sahip *Salvia fruticosa*'nın diklorometan ekstraktları olduğu, ardından

Salvia pomifera (%36.39) ve *Salvia fruticosa* (%34.27) etil asetat ekstraktlarının olduğu tespit edilmiştir [10]. Araştırmacılar diğer bir çalışmalarında, doğal olarak yetişen ve kültürü yapılmış *Salvia fruticosa* bitkisinin farklı ekstraktlarının, asetilkolinesteraz ve butirilkolinesteraz önleyici aktivitesini araştırmışlardır. Doğal yetişen bitkinin tüm ekstraktları, ekili türün ekstraktlarından daha düşük inhibitör etki gösterdiği belirtilmiştir. En yüksek anti-AChE aktiviteyi, kültürü yapılmış *Salvia fruticosa* türü sergilemiş olup, BChE üzerinde ise orta derecede bir inhibitör etki sergilediği bildirilmiştir [92]. Diğer bir çalışmada *Salvia fruticosa* gallerinin hekzan ekstraktında bulunan oleik asit, palmitoleik asit ve stearik asit sayesinde anti-BChE aktivite sergilediği; bitkinin toprak üstü kısımlarından elde edilen uçucu yağdaki ana bileşen 1,8 sineol sayesinde de yüksek AChE inhibe edici aktivite sergilediği bildirilmiştir [93]. Deneysel olarak Alzheimer hastalığı indüklenen bir sıçan modelinde, *Salvia fruticosa* ve *Piper nigrum* bitki ekstraktları ile tedavinin oksidatif stresi önemli ölçüde azalttığı ve bu hastalığın nörodejenerasyon karakteristiğini iyileştirdiği saptanmıştır. Alzheimer hastalığından korunmada iki bitki ekstraktının gücü kıyaslandığında ise, 750 mg.kg b.wt. dozunda *Salvia fruticosa* ile tedavinin, hem biyokimyasal hem de histopatolojik bulgularla *Piper nigrum*'ndan daha güçlü olduğu belirtilmiştir [94].

Alzheimer hastalığı progresif nörodejeneratif bir hastalıktır. Uçucu yağ bileşenleri, lipofilik yapıları ve küçük moleküler boyutları nedeniyle kan-beyin bariyerini geçebilir [89]. Yapılan çalışmalar, *Salvia fruticosa* uçucu yağ ekstraktının, Alzheimer hastalığına bağlı AChE ve BChE enzimlerini bloke ederek, beyindeki bir nörotransmitter olan asetilkolinin parçalanmasını engellediğini göstermektedir [71]. Ancak Alzheimer hastalığında nörodejeneratif süreci yavaşlatabilecek, AChE ve BChE inhibitörleri ile kombinasyon halinde daha etkili bir şekilde çalışabilecek farklı ajanların belirlenmesi için yeni çalışmalara ihtiyaç vardır [88, 89].

Anti-hiperglisemik etki

Farklı deneysel çalışmalar *Salvia fruticosa*'nın glikoz homeostazının terapötik düzenlenmesinde önemini belirtmiştir. Deneysel bir çalışmada, *Salvia fruticosa* ekstraktının alloksan-hiperglisemik tavşanlarda glikozun intestinal emilimini azaltarak, plazma insülin seviyelerini değiştirmeden kandaki glikoz seviyelerini azalttığını böylece hipoglisemiye neden olduğu bildirilmiştir [95]. Benzer bir çalışmada, *Salvia fruticosa* sulu ekstratlarının sıçanlarda açlık kan şekeri seviyelerini önemli ölçüde iyileştirdiğini

ancak plazma insülin düzeyi ve karaciğerdeki glikojen düzeyi üzerinde ise etkisinin olmadığını ve sadece diyabetik hayvanlarda enterosit fırça kenar membranında sodyum bağımlı glikoz transport proteni 1'i azalttığı belirlenmiştir [15]. Diğer bir çalışmada ise *Salvia fruticosa* metanol ekstraktının, sıçanlarda lipid sindirimi ve emiliminde rol oynayan önemli gastrointestinal enzimleri inhibe edebildiği, bu nedenle adaçayının obezite ile ilişkili hipertrigliseridemiyi kontrol etmek için potansiyel bir fitoterapötik/profilaktik strateji olabileceği sonucuna varılmıştır. Ayrıca adaçayının, vücut kilo alımını bastırmak için potansiyel olarak kullanılabileceğini gösteren çift hipotrigliseridemik ve antilipolitik özelliklere sahip olduğu belirtilmiştir [96]. Bu çalışmalara ek olarak, *Salvia fruticosa* yapraklarının metanol ekstresinin, streptozotosin/nikotinamid ile oluşturulan diyabetik sıçan modelinde hiperglisemi düzeylerini düşürdüğü, glikoz toleransını arttırdığı ve diyabetik sıçanlarda hiperinsülinemiyi önlediği bildirilmiştir [97].

Antifertilite etkisi

Literatürde *Salvia fruticosa* bitkisinin üremeye ve cinsel davranışlara etkisini değerlendiren çalışma sayısı oldukça sınırlıdır.

Deneysel bir çalışmada, *Salvia fruticosa* bitkisinin sulu ve etanolik ekstraktlarının antiimplantasyon, antifertilite ve üreme toksisite potansiyellerini erkek ve dişi sıçanlarda araştırılmıştır. *Salvia fruticosa*'nın sulu (800 mg/kg) veya etanolik ekstraktlarının (400 mg/kg) birbirini takip eden 30 gün boyunca yetişkin dişi fareler tarafından yutulmasının hamilelik oluşumu üzerinde hiçbir etkisinin olmadığı, ancak implantasyonların ve yaşayabilir fetusların sayısını azalttığı ve gebe sıçanlarda nefes alma sıklığını artırdığı saptanmıştır. Yetişkin erkek sıçanlar tarafından yine art arda 30 gün boyunca *Salvia fruticosa'*nın sulu ekstraktının (800 mg/kg) veya etanolik ekstraktın (400 mg/kg) yutulması ise bu erkekler tarafından döllenen dişi sıçanların sayısı üzerinde herhangi bir etkiye sahip olmadığı ancak, implantasyonların ve canlı fetüslerin sayısını azalttığı belirlenmiştir. Sonuç olarak, *Salvia fruticosa*'nın yutulmasının erkek ve dişi sıçanlarda doğurganlık üzerinde toksik etkiler oluşturduğu sonucuna varılmıştır. Ayrıca çalışmada, erkek ve dişi sıçan yavrularının *Salvia fruticosa'*nın 400 mg/kg etanolik ekstraktının yutulması prenatal maruziyetinin testiküler iniş ve vajinal açılma zamanlaması ile ölçülen cinsel olgunlaşma (ergenlik) parametreleri üzerinde hiçbir etkisi olmadığı bildirilmiştir [98]. Başka bir çalışmada ise *Salvia fruticosa* bitkisi toz halde, 30 gün boyunca günlük

800 mg kg^{-1} vücut ağırlığı dozunda cinsel olarak aktif erkek sıçanlara (Sprague-dawley sıçanları) oral olarak uygulanmış ve çiftleşme etkinlikleri üzerine etkileri değerlendirilmiştir. *Salvia fruticosa* verilen erkek sıçanlarda testosteron serum seviyesindeki önemli azalmaya bağlı olarak cinsel isteksizlik ve performans kaybı gözlenmiş bunun da bitkinin yapısında bulunan bileşen veya bileşenlerden kaynaklanabileceği belirtilmiştir [99].

Diğer etkiler

Salvia fruticosa ekstraktı ile kediler üzerinde yapılan bir çalışmada, ekstraktın kedilerdeki kan basıncını düşürdüğü, asetilkolin, histamin, serotonin ve $BaCl_2$ tarafından indüklenen düz kas kasılmalarını inhibe ederek hipotansif ve spazmolitik etkiler oluşturduğu bildirilmiş, ayrıca bitkinin içerdiği bileşiklerin heksobarbital uykuyu uzattığı tespit edilmiştir [100].

Salvia fruticosa etanolik ekstraktının, sıçanlardan izole edilmiş torasik aort halkaları üzerine etkilerinin araştırıldığı bir çalışmada, ekstraktın doza bağlı bir şekilde nitrik oksit üretimini ve gevşemiş endotelyum sağlam halkaları arttırdığı; endotel bağımlı vazorelaksasyonu indüklediği tespit edilmiştir. Bitkinin kardiyovasküler komplikasyonların yükünü azaltmak için antihipertansif bir ajan olarak işlev görebileceği bildirilmiştir [101].

Toksik etkileri

Salvia fruticosa bitkisinin terapötik etkinliği ile ilgili çok sayıda araştırma projesi olmasına rağmen, toksisitesi ile ilgili deneysel çalışmalar nadirdir. Adaçayı bitkisinin şifa verici özelliklerinin yanı sıra, yanlış veya aşırı kullanımı, uçucu yağının toksisitesinden dolayı çeşitli komplikasyonlara neden olabilmektedir [102,103]. Deneysel bir çalışmada tıbbi adaçayı (*Salvia officinalis*) uçucu yağının, sıçanlarda zehirlenmenin subklinik olduğu doz sınırının 0.3 g/kg olduğu tespit edilmiş, 3,2 g/kg yağın üzerinde kasılmaların ortaya çıktığı ve ölümcül olduğu belirtilmiştir [102]. Aynı araştırmacının bir başka deneysel çalışmasında *Salvia officinalis*'in bu defa insanlarda zehirlenmeye neden olduğunu ve bu toksisitelerin, *Salvia fruticosa*'da da bulunan ve konvülsan özellikleri iyi bilinen, thujon ve kafur bileşenlerinden kaynaklandığı belirtilmiştir [103]. Ayrıca adaçayı yağının diğer toksik etkileri arasında epileptik reaksiyonlar, denge kaybı ve taşikardi bildirilmiştir [71, 104].

Toksik etkileri ile ilgili diğer bir çalışmada, *Salvia fruticosa*'nın yağ kompozisyonundaki mevsimsel değişiklikleri toksisite ile ilişkilendirmek amacıyla yılın farklı zamanlarında toplanan bitkilerden elde edilen adaçayı yağının *in vivo* akut toksisitesi araştırılmıştır. Bu bitkinin yapraklarından elde edilen yağ ekstraktının bileşimi Ağustos (yaz), Ekim (sonbahar), Ocak (kış) ve Nisan (bahar) aylarında belirlemişler ve yağın bileşiminde önemli mevsimsel değişiklikler bulunduğunu rapor etmişlerdir. Her fraksiyonun toksisitesi, farelere intraperitoneal enjeksiyonun ardından araştırılmış buna göre kış mevsiminde (Ocak) toplanan bitkilerden ekstrakte edilen uçucu yağ, daha yüksek kafur, kamfen, α ve β-thujon içerdiğini dolayısıyla kış ekstraktının en toksik olduğu (LD50: 839 mg/kg vücut ağırlığı) ve farelerde güçlü konvülsan özellikler sergilediğini bildirmişlerdir. Bahar ekstraktının ise en az toksik (LD50: 1200 mg/kg vücut ağırlığı) olduğunu ve daha düşük seviyelerde kafur, kamfen, α ve β-thujon içerdiği bildirilmiştir. Araştırmacılar çalışmanın sonunda yağların bileşenleri ve toksisiteleri arasında güçlü bir korelasyon olduğu sonucuna varmışlardır. Ayrıca dört yağ ekstraktının her birinin enjeksiyonundan sonra farelerde doza bağlı farmakotoksik semptomlar kaydedilmiştir. Ölümden önce hayvanlarda, hareket koordinasyonunda bozukluk, kas kasılmaları ve konvülsiyonlar gibi epileptojenik semptomlar izlenmiş ve bunu motor aktivitede azalma takip etmiştir. Postmortem muayenede yüksek vaskülarize cilt ve periton tabakaları, kalpte koyu kan pıhtıları ve bağırsak iltihabı saptanmıştır [105]. Sulu ekstraktının toksik etkisi ile ilgili olarak, Yunanistan'ın ve Girit'in farklı yerlerinden alınan *Salvia fruticosa*'nın kolesterol metabolizmasında ve kalp hastalıklarında önemli rol oynayan Cr, Cu, Zn ve Mn başta olmak üzere toplam 18 element (V, Co, Se, Sr, Sn, Sb, Ba, Bi, Pb, Cd, As, Ni, Fe, Mg) içerdiği ancak otoyola daha yakın olan populasyonlarda *Salvia fruticosa* çiçeklerinin ve yapraklarının, infüzyonda dahi mevcut olan düşük konsantrasyonlarda bazı toksik ağır metaller (Pb ve Cd) içerdiği bildirilmiştir [60].

Salvia fruticosa toksisitesine ilişkin olarak, gaz giderici olarak topikal kullanımı önerilen bu bitki yağının çocuklarda oral ve yüksek dozda kullanımına bağlı gelişen ani solunum sıkıntısı ve kimyasal pnömonili bir olgu ile aynı şekilde yanlış kullanımına bağlı infantil kolik bebeklerde zehirlenme vakaları bildirilmiştir. Bitki yağının oral kullanımı sonucu ciddi sistemik yan etkiler gelişebildiği için topikal kullanımında da dikkatli olunması gerektiği bildirilmiştir [106, 107].

Sonuç ve Öneriler

Son yıllarda biyoteknolojinin gelişmesi ile beraber yapılan çalışmalar, tıbbi ve aromatik bitkilerin içerdiği sekonder metabolitlerin öneminin anlaşılmasında etkili olmuştur. Kullanımı insanlık tarihi kadar eski olan tıbbi ve aromatik bitkiler, içerdikleri biyoaktif maddeler sayesinde günümüzde hastalıkların önlenmesi, tedavisi ve yeni ilaçları keşfetmek için kullanılmaya devam etmektedir. Ancak bu bitkilerin biyoaktiviteleri, içsel ve dışsal parametrelerden etkilenen spesifik bileşenlerin varlığına ve oranına bağlı olduğundan, bitki materyalinin standardizasyonu gereklidir. Modern biyoteknolojik yöntemler istenilen nitelikte bitkinin standardize edilmesini mümkün kılmaktadır. Verimli bitkilerin genotip kombinasyonu ile kültüre alma işlemi, sürekli yüksek kalitede ve istenilen özelliklere uygun tıbbi ve aromatik bitki materyalinin elde edilmesine katkıda bulunabilir. Anadolu'nun geleneksel halk hekimliğinde kullanımı yaygın olan bitkilerden biri de *Salvia fruticosa*'dır. Farmakognozik prosedürlere göre bir bitkinin ilaç olarak kullanılması için, içeriğindeki etken maddeleri, bu etken maddelerin oranını, etki mekanizmalarını, olası yan etkilerini, başka ilaçlarla etkileşimlerini bilmek gerekir. *Salvia fruticosa* sekonder metabolitlerinin çok çeşitli ve yararlı biyoaktiviteleri farklı çalışmalarla gösterilmiştir bu nedenle bitki nutrasötik bir kokteyl olarak değerlendirilebilir. Ayrıca bitkiden elde edilecek bazı biyoaktif fitokimyasallar, birçok hastalığın tedavisine yardımcı olabilecek potansiyel hammadde kaynağı olarak düşünülebilir. Ancak bitkinin potansiyel toksik etkileri göz ardı edilmemeli, kontrollü ve doğru kullanımı sağlanmalıdır. *Salvia fruticosa* bileşenlerinin biyoaktiviteleri ile farmakolojik özelliklerinin doğrulanması ve irrasyonel kullanımda oluşabilecek ciddi yan etkiler ve komplikasyonların belirlenmesi için daha fazla çalışmaya ihtiyaç vardır.

Kaynaklar

1. Tundis, R., et al., Assessment of antioxidant, antitumor and pro-apoptotic effects of Salvia fruticosa Mill. subsp. thomasii (Lacaita) Brullo, Guglielmo, Pavone & Terrasi (Lamiaceae). Food and Chemical Toxicology, 2017. 106: p. 155-164.
2. Essawi, T. and M. Srour, Screening of some Palestinian medicinal plants for antibacterial activity. Journal of ethnopharmacology, 2000. 70(3): p. 343-349.
3. Boukhary, R., et al., Anti-inflammatory and antioxidant activities of Salvia fruticosa: An HPLC determination of phenolic contents. Evidence-Based Complementary and Alternative Medicine, 2016. 2016.
4. Lu, Y. and L.Y. Foo, Polyphenolics of Salvia—a review. Phytochemistry, 2002. 59(2): p. 117-140.
5. Topçu, G., Bioactive triterpenoids from Salvia species. Journal of natural products, 2006. 69(3):

p. 482-487.
6. Gürdal, B. and Ş. Kültür, An ethnobotanical study of medicinal plants in Marmaris (Muğla, Turkey). Journal of Ethnopharmacology, 2013. 146(1): p. 113-126.
7. WCSP, World checklist of selected plant families. Facilitated by the Royal Botanic Gardens, Kew, 2014.
8. Gali-Muhtasib, H., Anticancer and medicinal properties of essential oil and extracts of East Mediterranean sage (Salvia triloba). Advances in Phytomedicine, 2006. 2: p. 169-180.
9. Dincer, C., et al., A comparative study on phenolic composition, antioxidant activity and essential oil content of wild and cultivated sage (Salvia fruticosa Miller) as influenced by storage. Industrial Crops and Products, 2012. 39: p. 170-176.
10. Şenol, F.S., et al., Survey of 55 Turkish Salvia taxa for their acetylcholinesterase inhibitory and antioxidant activities. Food Chemistry, 2010. 120(1): p. 34-43.
11. Atmaca, H. and E. Bozkurt, Apoptotic and anti-angiogenic effects of Salvia triloba extract in prostate cancer cell lines. Tumor Biology, 2016. 37(3): p. 3639-3646.
12. Delamare, A.P.L., et al., Antibacterial activity of the essential oils of Salvia officinalis L. and Salvia triloba L. cultivated in South Brazil. Food chemistry, 2007. 100(2): p. 603-608.
13. Zihlif, M., et al., The antiangiogenic activities of ethanolic crude extracts of four Salvia species. BMC Complementary and Alternative Medicine, 2013. 13(1): p. 358.
14. Exarchou, V., et al., HPLC-SPE-NMR characterization of major metabolites in Salvia fruticosa Mill. Extract with antifungal potential: Relevance of carnosic acid, carnosol, and hispidulin. Journal of agricultural and food chemistry, 2015. 63(2): p. 457-463.
15. Azevedo, M.F., et al., Rosmarinic acid, major phenolic constituent of Greek sage herbal tea, modulates rat intestinal SGLT1 levels with effects on blood glucose. Molecular nutrition & food research, 2011. 55(S1): p. S15-S25.
16. Xavier, C.P., et al., Salvia fruticosa, Salvia officinalis, and rosmarinic acid induce apoptosis and inhibit proliferation of human colorectal cell lines: the role in MAPK/ERK pathway. Nutrition and cancer, 2009. 61(4): p. 564-571.
17. TUBIVES, Turkish Plants Data Service. 2004. Available from: http://www.tubives.com/ [Erişim Tarihi: 29.04.2020].
18. Bayram, E. and Ç. Sönmez, Adaçayı Yetiştiriciliği. EÜ Tar. Uyg. ve Araş. Merkezi Yayım Bülteni, 2006(48).
19. Papageorgiou, V., et al., Variation of the chemical profile and antioxidant behavior of Rosmarinus officinalis L. and Salvia fruticosa Miller grown in Greece. Journal of agricultural and food chemistry, 2008. 56(16): p. 7254-7264.
20. Ceylan A., Ege University Agriculture Faculty Press, Izmir, Turkey, p. 225 (1997).
21. TUIK, Dış Ticaret İstatistikleri.2020. Available from: https://biruni.tuik.gov.tr/disticaretapp/disticaret.zul?param1=25¶m2=0&sitcrev=0&isicrev=0&sayac=5802/ [Erişim Tarihi: 17.08.2020].
22. Putievsky, E., U. Ravid, and N. Dudai, The essential oil and yield components from various plant parts of Salvia fruticosa. Journal of natural products, 1986. 49(6): p. 1015-1017.
23. Altay, A., et al., Anatolian sage Salvia fruticosa inhibits cytosolic glutathione-s-transferase activity and colon cancer cell proliferation. Journal of Food Measurement and Characterization, 2019. 13(2): p. 1390-1399.
24. Bayram, E., A. Ceylan, and H. Geren, Anadolu adaçayı (Salvia fruticosa Mill.) ıslahında geliştirilen klonların agronomik ve kalite özellikleri üzerinde araştırma. Türkiye, 1999. 3: p. 212-217.
25. Zgheib, R., et al., Investigation of essential oil chemical polymorphism of Salvia fruticosa naturally growing in Lebanon. Journal of Essential Oil Bearing Plants, 2019. 22(2): p. 408-430.
26. Karioti, A., et al., Effect of nitrogen concentration of the nutrient solution on the volatile constituents of leaves of Salvia fruticosa Mill. in solution culture. Journal of agricultural and food

chemistry, 2003. 51(22): p. 6505-6508.
27. Sarrou, E., S. Martens, and P. Chatzopoulou, Metabolite profiling and antioxidative activity of Sage (Salvia fruticosa Mill.) under the influence of genotype and harvesting period. Industrial Crops and Products, 2016. 94: p. 240-250.
28. Bellomaria, B., et al., Contribution to the study of the essential oils from three species of Salvia growing wild in the eastern Mediterranean region. Journal of Essential Oil Research, 1992. 4(6): p. 60.
29. El-Wahab, A., et al., Effect of different planting locations in Egypt on Salvia fruticosa mill. Plants. Egyptian Journal of Desert Research, 2015. 65(2): p. 291-307.
30. Altindal, D., Determination of genetic diversity of natural sage populations in Muğla region of Turkey. International Journal of Environmental Science and Technology, 2019. 16(9): p. 5219-5226.
31. Karousou, R., D. Vokou, and S. Kokkini, Variation of Salvia fruticosa essential oils on the island of Crete (Greece). Botanica acta, 1998. 111(3): p. 250-254.
32. Skoula, M., J.E. Abbes, and C.B. Johnson, Genetic variation of volatiles and rosmarinic acid in populations of Salvia fruticosa mill growing in Crete. Biochemical systematics and ecology, 2000. 28(6): p. 551-561.
33. Pitarokili, D., et al., Volatile metabolites from Salvia fruticosa as antifungal agents in soilborne pathogens. Journal of agricultural and food chemistry, 2003. 51(11): p. 3294-3301.
34. Kaplan, M., et al. The effects of different organic manure applications on the dry weight and the essential oil quantity of sage (Salvia fruticosa Mill.). in I International Medicinal and Aromatic Plants Conference on Culinary Herbs 826. 2007.
35. Kocabas, I., et al., Effects of different organic manure applications on the essential oil components of Turkish sage (Salvia fruticosa Mill.). Asian Journal of chemistry, 2010. 22(2): p. 1599.
36. Uritu, C.M., et al., Medicinal plants of the family Lamiaceae in pain therapy: A review. Pain Research and Management, 2018. 2018.
37. Alzoreky, N. and K. Nakahara, Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International journal of food microbiology, 2003. 80(3): p. 223-230.
38. Sivropoulou, A., et al., Antimicrobial, cytotoxic, and antiviral activities of Salvia fructicosa essential oil. Journal of Agricultural and food Chemistry, 1997. 45(8): p. 3197-3201.
39. Aşkun, T., et al., Characterization of essential oils of some Salvia species and their antimycobacterial activities. Turkish Journal of Biology, 2010. 34(1): p. 89-95.
40. Duletić-Laušević, S., et al., Composition and biological activities of Libyan Salvia fruticosa Mill. and S. lanigera Poir. extracts. South African Journal of Botany, 2018. 117: p. 101-109.
41. Giweli, A., et al., Antimicrobial and antioxidant activities of essential oils of Satureja thymbra growing wild in Libya. Molecules, 2012. 17(5): p. 4836-4850.
42. Karadağ, A.E., et al., Antibacterial evaluation of Elettaria cardamomum (L.) Maton, Lavandula angustifolia Mill. and Salvia fruticosa Mill. essential oil combinations in mouthwash preparations. Natural Volatiles&Essential Oils, 2020. 7(1): p. 9-17.
43. Soković, M. and L.J. Van Griensven, Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, Agaricus bisporus. European Journal of plant pathology, 2006. 116(3): p. 211-224.
44. Ağırman, B., M.N. Akalın, and H. Erten, Meyve ve sebzelerde hasat sonrası fungal hastalıkların antagonistik mayalar ile biyokontrolü. Gıda, 2019. 44(1): p. 31-49.
45. Dwivedi, S.K. and N. Dubey, Potential use of the essential oil of Trachyspermum ammi against seed-borne fungi of Guar (Cyamopsis tetragonoloba L.(Taub.) Mycopathologia, 1993. 121(2): p. 101-104.
46. Mishra, A. and N. Dubey, Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. Applied and environmental microbiology, 1994. 60(4): p. 1101-1105.

47. Kadoglidou, K., et al., Inhibitory and stimulatory effects of essential oils and individual monoterpenoids on growth and sporulation of four soil-borne fungal isolates of Aspergillus terreus, Fusarium oxysporum, Penicillium expansum, and Verticillium dahliae. European journal of plant pathology, 2011. 130(3): p. 297-309.
48. Abou-Jawdah, Y., H. Sobh, and A. Salameh, Antimycotic activities of selected plant flora, growing wild in Lebanon, against phytopathogenic fungi. Journal of agricultural and food chemistry, 2002. 50(11): p. 3208-3213.
49. Shimoni, M., et al., Antifungal activity of volatile fractions of essential oils from four aromatic wild plants in Israel. Journal of chemical ecology, 1993. 19(6): p. 1129-1133.
50. Adam, K., et al., Antifungal activities of Origanum vulgare subsp. hirtum, Mentha spicata, Lavandula angustifolia, and Salvia fruticosa essential oils against human pathogenic fungi. Journal of Agricultural and Food Chemistry, 1998. 46(5): p. 1739-1745.
51. Ali-Shtayeh, M. and S.I. Abu Ghdeib, Antifungal activity of plant extracts against dermatophytes. mycoses, 1999. 42(11-12): p. 665-672.
52. Urek, R.O., et al., Comparison of antioxidant capacities of the leaves and flowers of Salvia fruticosa grown in Turkey. Asian Journal of Chemistry, 2008. 20(3): p. 2091.
53. Valko, M., et al., Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-biological interactions, 2006. 160(1): p. 1-40.
54. Hani, S.B. and M. Bayachou, Salvia fruticosa reduces intrinsic cellular and H2O2-induced DNA oxidation in HEK 293 cells; assessment using flow cytometry. Asian Pacific Journal of Tropical Biomedicine, 2014. 4(5): p. 399-403.
55. Garg, D., et al., In-vitro antioxidant activity and phytochemical analysis in extracts of Hibiscus rosa-sinensis stem and leaves. Free Radicals and Antioxidants, 2012. 2(3): p. 41-46.
56. Rice-Evans, C., N. Miller, and G. Paganga, Antioxidant properties of phenolic compounds. Trends in plant science, 1997. 2(4): p. 152-159.
57. Özyürek, M., Reaktif Oksijen Türleri Süpürücü Antioksidan Aktivitenin Ölçümünde Modifiye CUPRAC Yöntemlerinin Geliştirilmesi. İstanbul Üniversitesi, Doktora tezi, İstanbul, 2008.
58. Pizzale, L., et al., Antioxidant activity of sage (Salvia officinalis and S fruticosa) and oregano (Origanum onites and O indercedens) extracts related to their phenolic compound content. Journal of the Science of Food and Agriculture, 2002. 82(14): p. 1645-1651.
59. Bozan, B., et al., Antioxidant and free radical scavenging activities of eight Salvia species. Chemistry of Natural Compounds, 2002. 38(2): p. 198-200.
60. Pasias, I.N., et al., Elemental content and total antioxidant activity of Salvia fruticosa. Food analytical methods, 2010. 3(3): p. 195-204.
61. Giweli, A.A., et al., The chemical composition, antimicrobial and antioxidant activities of the essential oil of Salvia fruticosa growing wild in Libya. Archives of Biological Sciences, 2013. 65(1): p. 321-329.
62. Durling, N.E., et al., Extraction of phenolics and essential oil from dried sage (Salvia officinalis) using ethanol–water mixtures. Food chemistry, 2007. 101(4): p. 1417-1424.
63. Hoeijmakers, J.H., DNA damage, aging, and cancer. New England Journal of Medicine, 2009. 361(15): p. 1475-1485.
64. Wild, C., E. Weiderpass, and B. Stewart, World cancer report: cancer research for cancer prevention. Lyon: International Agency for Research on Cancer, 2020.
65. Yıllığı, S.İ., TC Sağlık Bakanlığı. Sağlık Araştırmaları Genel Müdürlüğü. Ankara, 2017.
66. Gali-Muhtasib, H. and N. Bakkar, Modulating cell cycle: current applications and prospects for future drug development. Current cancer drug targets, 2002. 2(4): p. 309-336.
67. Abu-Dahab, R., et al., Mechanistic studies of antiproliferative effects of Salvia triloba and Salvia dominica (Lamiaceae) on breast cancer cell lines (MCF7 and T47D). Zeitschrift für Naturforschung C, 2014. 69(11-12): p. 443-451.
68. Shih, H., G.V. Pickwell, and L.C. Quattrochi, Differential effects of flavonoid compounds on

tumor promoter-induced activation of the human CYP1A2 enhancer. Archives of Biochemistry and Biophysics, 2000. 373(1): p. 287-294.
69. Altay, A. and F. Bozoğlu, Salvia fruticosa modulates mRNA expressions and activity levels of xenobiotic metabolizing CYP1A2, CYP2E1, NQO1, GPx, and GST enzymes in human colorectal adenocarcinoma HT-29 cells. Nutrition and cancer, 2017. 69(6): p. 892-903.
70. Akaberi, M., S. Mehri, and M. Iranshahi, Multiple pro-apoptotic targets of abietane diterpenoids from Salvia species. Fitoterapia, 2015. 100: p. 118-132.
71. Gali-Muhtasib, H. and N. Affara, Chemopreventive effects of sage oil on skin papillomas in mice. Phytomedicine, 2000. 7(2): p. 129-136.
72. Kaileh, M., et al., Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity. Journal of ethnopharmacology, 2007. 113(3): p. 510-516.
73. Badisa, R., et al., Cytotoxic Activities of Salvia of the Labiatae Family. Pharmaceutical biology, 2005. 42(8): p. 640-645.
74. Agarwal, R., Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. Biochemical pharmacology, 2000. 60(8): p. 1051-1059.
75. Kan, S.F., et al., Anti-proliferative effects of evodiamine on human prostate cancer cell lines DU145 and PC3. Journal of cellular biochemistry, 2007. 101(1): p. 44-56.
76. Ramos, A.A., et al., Polyphenolic compounds from Salvia species protect cellular DNA from oxidation and stimulate DNA repair in cultured human cells. Journal of agricultural and food chemistry, 2010. 58(12): p. 7465-7471.
77. Al-Kalaldeh, J.Z., R. Abu-Dahab, and F.U. Afifi, Volatile oil composition and antiproliferative activity of Laurus nobilis, Origanum syriacum, Origanum vulgare, and Salvia triloba against human breast adenocarcinoma cells. Nutrition Research, 2010. 30(4): p. 271-278.
78. Abu-Dahab, R., et al., Comparison of the antiproliferative activity of crude ethanol extracts of nine salvia species grown in Jordan against breast cancer cell line models. Pharmacognosy magazine, 2012. 8(32): p. 319.
79. Sevindik, N. and E. Rencuzogullari, The genotoxic and antigenotoxic effects of Salvia fruticosa leaf extract in human blood lymphocytes. Drug and chemical toxicology, 2014. 37(3): p. 295-302.
80. Fraihat, A., et al., Inhibitory effects of methanol extracts of selected plants on the proliferation of two human melanoma cell lines. Tropical Journal of Pharmaceutical Research, 2018. 17(6): p. 1081-1086.
81. Koutsoulas, A., et al., Characterization of phenolic compounds and antiproliferative effects of Salvia pomifera and Salvia fruticosa extracts. Molecules, 2019. 24(16): p. 2921.
82. Eltawaty, S.I., et al., Anticancer effects of methanol extract of Libyan Salvia fruticosa Mill. on MCF7, T47D and (MDA-MB-468) breast cells lines. European Journal of Pharmaceutical and Medical Research, 7(2): p. 165-169.
83. Folkman, S. and R.S. Lazarus, Stress, appraisal, and coping. 1984: New York: Springer Publishing Company.
84. Hur, J.M., et al., Cryptotanshinone but not tanshinone IIA inhibits angiogenesis in vitro. Experimental & molecular medicine, 2005. 37(2): p. 133-137.
85. Jung, H.-J., et al., Anti-inflammatory, anti-angiogenic and anti-nociceptive activities of an ethanol extract of Salvia plebeia R. Brown. Journal of ethnopharmacology, 2009. 126(2): p. 355-360.
86. Keshavarz, M., et al., In vitro and ex vivo antiangiogenic activity of Salvia officinalis. Phytotherapy Research, 2010. 24(10): p. 1526-1531.
87. Liu, F., et al., Effect of Salvia chinensis extraction on angiogenesis of tumor. Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica, 2012. 37(9): p. 1285-1288.
88. Perry, N.S., et al., In-vitro inhibition of human erythrocyte acetylcholinesterase by Salvia lavandulaefolia essential oil and constituent terpenes. Journal of pharmacy and pharmacology, 2000. 52(7): p. 895-902.

89. Savelev, S.U., E.J. Okello, and E.K. Perry, Butyryl-and acetyl-cholinesterase inhibitory activities in essential oils of Salvia species and their constituents. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 2004. 18(4): p. 315-324.
90. Orhan, I.E., et al., Assessment of anticholinesterase and antioxidant properties of selected sage (Salvia) species with their total phenol and flavonoid contents. Industrial Crops and Products, 2013. 41: p. 21-30.
91. Orhan, I. and M. Aslan, Appraisal of scopolamine-induced antiamnesic effect in mice and in vitro antiacetylcholinesterase and antioxidant activities of some traditionally used Lamiaceae plants. Journal of Ethnopharmacology, 2009. 122(2): p. 327-332.
92. Şenol, F.S., et al., Evaluation of cholinesterase inhibitory and antioxidant activities of wild and cultivated samples of sage (Salvia fruticosa) by activity-guided fractionation. Journal of medicinal food, 2011. 14(11): p. 1476-1483.
93. Topçu, G., et al., Terpenoids, essential oil composition, fatty acid profile, and biological activities of Anatolian Salvia fruticosa Mill. Turkish Journal of Chemistry, 2013. 37(4): p. 619-632.
94. Mahdy, K., et al., Effect of some medicinal plant extracts on the oxidative stress status in Alzheimer’s disease induced in rats. Eur Rev Med Pharmacol Sci, 2012. 16(3): p. 31-42.
95. Perfumi, M., N. Arnold, and R. Tacconi, Hypoglycemic activity of Salvia fruticosa Mill. from Cyprus. Journal of ethnopharmacology, 1991. 34(2-3): p. 135-140.
96. Arabiyat, S., et al., Antilipolytic and hypotriglyceridemic effects of dietary Salvia triloba Lf (Lamiaceae) in experimental rats. Tropical Journal of Pharmaceutical Research, 2016. 15(4): p. 723-728.
97. Cam, M.E., et al., Antidiabetic effects of Salvia triloba and Thymus praecox subsp. skorpilii var. skorpilii in a rat model of streptozotocin/nicotinamide-induced diabetes. Marmara Pharmaceutical Journal, 2017. 21(4): p. 818-827.
98. Elbetieha, A., et al., Reproductive toxicity potentials of Salvia fruticosa (Labiatae) in rats. Journal of ethnopharmacology, 1998. 61(1): p. 67-74.
99. Daradka, H.M., The effect of Salvia fruticosa ingestion on male rat sexual behavior. Res J Biol Sci, 2009. 4(12): p. 1288-91.
100. Todorov, S., et al., Experimental pharmacological study of three species from genus Salvia. Acta physiologica et pharmacologica Bulgarica, 1984. 10(2): p. 13-20.
101. Anwar, M.A., et al., Salvia fruticosa induces vasorelaxation in rat isolated thoracic aorta: role of the PI3K/Akt/eNOS/NO/cGMP signaling pathway. Scientific reports, 2017. 7(1): p. 1-14.
102. Millet, Y., et al., Toxicity of some essential plant oils. Clinical and experimental study. Clinical toxicology, 1981. 18(12): p. 1485-1498.
103. Millet, Y., et al., Experimental study of the toxic convulsant properties of commercial preparations of essences of sage and hyssop (author's transl). Revue d'electroencephalographie et de neurophysiologie clinique, 1979. 9(1): p. 12-18.
104. Elisabetsky, E., J. Marschner, and D.O. Souza, Effects of linalool on glutamatergic system in the rat cerebral cortex. Neurochemical research, 1995. 20(4): p. 461-465.
105. Farhat, G., N. Affara, and H. Gali-Muhtasib, Seasonal changes in the composition of the essential oil extract of East Mediterranean sage (Salvia libanotica) and its toxicity in mice. Toxicon, 2001. 39(10): p. 1601-1605.
106. Harmancı, K., et al., Elma yağının yanlış kullanımına bağlı gelişen kimyasal pnömonili bir olgu. Asthma Allergy Immunol, 2011. 9: p. 101-104.
107. Gündüz, S., et al., Acı elma yağının yanlış kullanımına bağlı dört zehirlenme vakası. Dicle Tıp Dergisi, 2016. 43(2): p. 364-366.

Derleme makale/ Research article

Göre, M. and O. Kurt, Bitkisel Üretimde Yeni Bir Trend: Kenevir. International Journal of Life Sciences and Biotechnology, 2021. 4(1): p. 138-157. DOI: 10.38001/ijlsb.789970

Bitkisel Üretimde Yeni Bir Trend: Kenevir

Merve Göre[1*], Orhan Kurt[1]

ÖZET

Kenevir, Cannabaceae familyasında yer alan, tek yıllık, otsu ve çok yönlü kullanım potansiyeli olan bir endüstri bitkidir. Tarihin en eski bitkilerinden biri olan kenevir, ilaç, gıda, kâğıt, biyoyakıt, tekstil, kozmetik, inşaat ve otomotiv sektörüne kadar oldukça geniş yelpazede kullanım alanına sahiptir. Ekolojik ve topografı istekleri bakımından kanaatkâr bir bitki olan kenevirin adaptasyon kabiliyeti geniştir. Kenevir tohumu yaklaşık %35 yağ ve %25 protein içermesinin yanında Omega-3 kaynağı olarak da zengindir. Endüstriyel kenevir, kaliteli lif kaynağı özelliği sayesinde yakın zamanda geniş ekim alanlarında yer bularak endüstriye önemli ölçüde katkı sağlayacak potansiyele ulaşmıştır. Narkotik özelliklerinden dolayı birçok ülkede olduğu gibi ülkemizde de yasaklanan geleneksel kenevirin aksine son yıllarda geliştirilen endüstriyel amaçlı kenevir çeşitleri sayesinde kenevir tarımı birçok ülkede her geçen gün genişlemekte ve ülkelerin ekonomilerine önemli katkı sağlama potansiyeli arz etmektedir.

MAKALE GEÇMİŞİ
Geliş
3 Eylül 2020
Kabul
14 Eylül 2020

ANAHTAR KELİMELER
Kenevir,
Cannabis sativa,
bitkisel üretim,
kenevir tarımı

A New Trend in Crop Production: Hemp

ABSTRACT

Hemp is an industrial plant which has an annual, herbaceous and multi-use potential in the Cannabaceae family. Cannabis, one of the oldest plants in history, is an alternative plant with a wide range of uses ranging from pharmaceutical, food, paper, biofuel, textile, cosmetics, construction and automotive sectors. Cannabis is a satisfactory plant in terms of ecological and topographic requirements and has high adaptability. Hemp seed contains about 35% oil and 25% protein, also it is a rich source of Omega-3. Industrial hemp has recently reached the potential to contribute significantly to the industry by finding a place in large cultivation areas thanks to its quality fiber source. Unlike traditional cannabis, which is banned in our country as well as in many countries due to its narcotic properties, hemp cultivation is expanding in many countries day by day thanks to the industrial hemp varieties developed in recent years. Also, hemp has the potential to contribute significantly to the economies of countries.

ARTICLE HISTORY
Received
3 September 2020
Accepted
14 September 2020

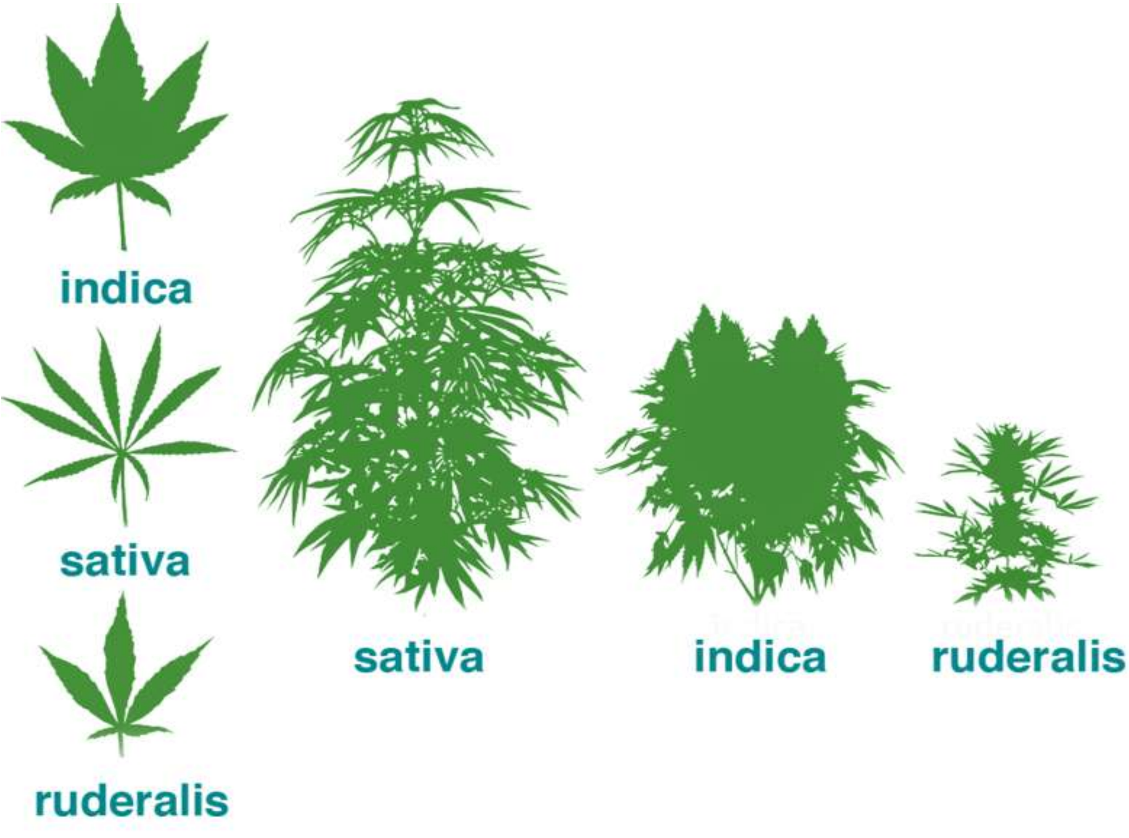
KEY WORDS
Hemp,
Cannabis sativa,
crop production,
hemp cultivation

Giriş

Kenevir (Cannabis), Cannabaceae familyasına ait tek yıllık, çok yönlü kullanım potansiyeli olan bir endüstri bitkisidir. Kenevir Türkiye'de kendir veya çedene olarak da adlandırılmaktadır. Lif, iplik, kumaş gibi dokuma, ilaç, kâğıt, biyoyakıt, kozmetik ve

[1] Ondokuz Mayıs Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, Samsun
* Sorumlu yazar: merve.gore@omu.edu.tr

otomotiv sektörü olmak üzere geniş bir kullanım potansiyeli bulunan kenevir, petrol ve petrokimyanın kullanıldığı her alanda da değerlendirilen endüstriyel bir bitkidir. Cannabis cinsi içerisindeki tür sayısı ve bu türlerin sınıflandırması tartışmalı olmakla birlikte, tanınan üç yaygın türü bulunmaktadır. Bu türler Cannabis sativa, Cannabis indica ve Cannabis ruderalis' tir [1]. Bazı kaynaklarda C. indica ve C. ruderalis, Cannabis sativa' nın bir alt türü olarak gösterilmektedir [2, 3, 4]. Üç tür arasında anatomik yapı, bitki habitusu, yetiştirilme şekli, çiçeklenme süresi ve ürettikleri kimyasal maddeler bakımından birçok farklılık bulunmaktadır (Şekil 1; [5]).



Şekil 1 Önemli kenevir türlerine ait morfolojik yapılar [5]

Kenevir kökeninin Güney Asya dahil olmak üzere Orta Asya ve Hindistan alt bölgesi olduğu kabul edilmektedir [6,7]. Bununla birlikte, kenevir bitkisinin ekim alanı ise Ekvator'dan Kutuplara kadar geniş bir coğrafik alana yayılmıştır [8].

Kültür bitkisi olarak kullanılmaya ve ıslah edilmeye başlanan ilk bitkilerden biri olan kenevir, tarih boyunca keyif verici bir madde ve birçok ürünün bitkisel hammadde kaynağı olarak kullanılmıştır [9]. Nitekim kenevirden psikolojik bir ilaç olarak Avrasya ve Afrika'da tarih öncesi toplumlarda kullanımına ilişkin bulgular

arkeolojik kalıntılarda da elde edilmiştir [10]. Ayrıca 16. yy'dan 18. yy'a kadar kenevir ve keten, Rusya, Avrupa ve Kuzey Amerika'nın başlıca lif bitkileri olmuştur [10, 11]. Yeni Dünya bitkilerinin keşfedilmesinden sonra, pamuk, jüt ve diğer tropikal lif bitkilerinin ekim alanlarının artması ile dünyadaki kenevir ekim alanları azalmıştır [12]. Sentetik liflerin ortaya çıkması ve birçok ülkede kenevir ekiminin yasal olarak sınırlandırılması ile kenevir ekim alanlarındaki azalma devam etmiştir [13]. İkinci dünya savaşından sonra lif amaçlı kenevir üretimi Çin, Sovyetler Birliği ve Doğu Avrupa ülkelerinde önem kazanmaya başlamıştır. 1960'lı yıllarda tekstil endüstrisinde kenevir lifi kullanımı azalmasına karşın kâğıt sanayiinde hammadde olarak kenevir kullanımı artmaya başlamıştır [14]. Son yıllarda ise sentetik liflerin yerine doğal liflere olan ilginin artması ve kullanım çeşitliliği sayesinde kenevire karşı ilgi yeniden artmıştır.

Ekim ve Üretim Durumu

Dünyada tohum üretim amacıyla kenevir ekim alanı son yıllarda sürekli olarak artmış ve 32.140 hektara ulaşmıştır. Dünya tohumluk kenevir üretimi de tıpkı ekim alanında olduğu gibi son yıllarda sürekli olarak artarak yaklaşık 142.883 tona ulaşmıştır. Kenevir tohumu üretiminin tamamına yakını (%99'u), ekim alanının %95'den fazla paya sahip olan 6 ülke (Fransa, Çin, Rusya, Şili, Macaristan ve Ukrayna) tarafından karşılanmaktadır (Tablo 1).

Tablo 1 Dünya tohum üretim amaçlı kenevir ekim alanı ve üretim miktarına (2014-2018) ilişkin veriler [15]

	2014	**2015**	**2016**	**2017**	**2018**
	Ekim Alanı (hektar)				
Dünya	**24.604**	**23.939**	**27.091**	**32.058**	**32.140**
Fransa	10.874	11.779	13.863	17.658	16.511
Çin	5.833	4.748	4.399	4.370	4.342
Rusya	1.969	1.261	2.333	3.600	4.691
Şili	2.282	2.518	2.735	2.555	2.660
Macaristan	1.369	1.387	1.441	1.518	1.606
Ukrayna	1.118	1.111	1.165	1.149	1.133
Toplam	**23.445**	**22.804**	**25.936**	**30.850**	**30.943**
Dünyadaki Payı (%)	**95,29**	**95,26**	**95,74**	**96,23**	**96,28**
	Üretim (ton)				
Dünya	103.097	78.195	99.842	146.223	142.883
Fransa	84.097	61.841	84.191	129.624	125.362
Çin	15.595	13.146	11.885	11.854	11.822
Rusya	520	275	589	1.078	2.117
Şili	1.466	1.484	1.496	1.515	1.533
Macaristan	409	402	402	396	390
Ukrayna	589	594	599	597	596
Toplam	**102.676**	**77.742**	**99.162**	**145.064**	**141.820**
Dünyadaki Payı (%)	**99,59**	**99,42**	**99,32**	**99,21**	**99,26**

Tohum dışı kenevir ekim alanı ve üretimine ilişkin veriler değerlendirildiğinde; ekim alanı ve üretim miktarının genel olarak dalgalanmalar gösterdiği görülmektedir. Bununla birlikte tohum dışı ekim alanının, tohum amaçlı ekim alanlarından daha fazla olmasına karşın, tohum amaçlı üretim miktarının tohum dışı üretim miktarından daha fazladır. Tohum dışı kenevir üretim alanının yaklaşık %95'inden fazlası ve üretim miktarının %85'inden fazlası 8 ülke (Kuzey Kore, Çin, Rusya, Şili, Romanya, Hollanda, Ukrayna ve Fransa) tarafından karşılanmaktadır (Tablo 2).

Tablo 2 Dünya tohum dışı kenevir ekim alanı ve üretim miktarına (2014-2018) ilişkin veriler [15]

	2014	**2015**	**2016**	**2017**	**2018**
	Ekim Alanı (ha)				
Dünya	**43.756**	**41.794**	**42.436**	**40.983**	**41.587**
Kuzey Kore	20.466	20.963	21.080	21.268	21.457
Çin	7.700	5.490	5.318	4.730	4.449
Rusya	3.651	3.121	3.344	3.303	3.262
Şili	4.411	4.468	4.430	4.403	4.386
Romanya	1.979	1.791	1.827	2.015	1.996
Hollanda	1.633	2.041	2.262	1.272	1.812
Ukrayna	1.554	1.467	1.480	1.480	1.480
Fransa	693	619	744	758	773
Toplam	**42.087**	**39.960**	**40.485**	**39.229**	**39.615**
Dünyadaki payı(%)	**96,19**	**95,61**	**95,40**	**95,72**	**95,26**
	Üretim (ton)				
Dünya	**76.707**	**62.475**	**66.796**	**55.826**	**60.657**
Kuzey Kore	14.250	14.610	14.623	14.757	14.891
Hollanda	13.065	14.596	17.417	9.539	13.851
Çin	32.000	15.521	15.178	13.391	12.623
Şili	4.162	4.236	4.194	4.166	4.146
Romanya	3.203	2.910	2.751	2.955	2.872
Fransa	1.155	1.041	1.229	1.256	1.283
Rusya	1.420	1.214	1.287	1.271	1.256
Ukrayna	740	698	713	708	703
Toplam	**69.995**	**54.826**	**57.392**	**48.043**	**51.625**
Dünyadaki Payı	**91,25**	**87,76**	**85,92**	**86,06**	**85,11**

Her iki grupta yer alan ülkeler değerlendirildiğinde; Çin, Şili, Fransa, Rusya ve Ukrayna'nın hem tohum amacıyla hem de tohum dışı üretim amacıyla kenevir ekim ve üretiminde önemli paya sahiptirler. Macaristan tohumluk amaçlı kenevir ekim ve üretiminde önemli paya sahip olmasına karşın, tohum dışı üretimde çok az bir paya sahiptir. Benzer biçimde Kuzey Kore, Romanya ve Hollanda tohum dışı ekim ve üretimde

önemli paya sahip olmalarına karşın tohumluk üretimi amacıyla ekim ve üretimde oldukça az paya sahiptirler (Tablo 1 ve Tablo 2).

Türkiye'de kenevir ekim alanı ve üretim miktarına ilişkin veriler değerlendirildiğinde; gerek tohum amaçlı gerek tohum dışı üretim amaçlı ekim alanı ve üretim miktarları artmaktadır. Nitekim 2019 yılında tohum amaçlı ekim alanı 536 dekar'a, üretim miktarı 20 ton'a çıkmış olup, tohum dışı üretim amaçlı ekim alanı 160 dekar'a ve üretim miktarı da 19 ton'a çıkmıştır (Tablo 3).

Tablo 3 Türkiye'de kenevir ekim alanı ve üretim miktarına (2015-2019) ilişkin veriler [16]

Yıl	Kenevir Tohum		Kenevir lif	
	Ekim Alanı (da)	**Üretim (ton)**	**Ekim alanı (da)**	**Üretim (ton)**
2015	10	1	10	1
2016	25	1	45	7
2017	24	1	46	7
2018	59	3	55	7
2019	536	20	160	19

29.09.2016 tarihli Resmi Gazetede yayınlanan yönetmelik kapsamında Türkiye'de 19 ilde (Amasya, Antalya, Bartın, Burdur, Çorum, İzmir, Karabük, Kastamonu, Kayseri, Kütahya, Malatya, Ordu, Rize, Samsun, Sinop, Tokat, Uşak, Yozgat ve Zonguldak) kenevir üretimine izin verilmiştir. Ayrıca kenevir tarımının geliştirilmesine yönelik olarak Samsun ilinde Ondokuz Mayıs Üniversitesi bünyesinde ve Yozgat Bozok Üniversitesi bünyesinde olmak üzere iki adet "Kenevir Araştırmaları Enstitüsü" kurulmuştur.

Türkiye'de kenevir ekim alanı ve üretim miktarları iller bazında değerlendirildiğinde; tohum amaçlı ekim ve üretimin Kastamonu, Kayseri, Kütahya, Samsun ve Yozgat illerinde olmak üzere 5 ilde yapıldığı, bu iller arasında Samsun ilinin diğer 4 ilin ekim ve üretim miktarından daha fazla paya sahip olduğu görülmektedir. Tohum dışı üretim amaçlı ekim ve üretimin sadece Samsun ilinde yapıldığı, Samsun ilinin ekim alanı 141 dekar ve üretim miktarı ise 18 ton'dur (Tablo 4).

Tablo 4 Türkiye'de il düzeyinde kenevir ekim alanı ve üretim miktarına (2015-2019) ilişkin veriler [16]

	İllere göre Kenevir Ekim Alanı (da)									
	Kastamonu		**Kayseri**		**Kütahya**		**Samsun**		**Yozgat**	
	Tohum	Tohum Dışı	Tohum	Tohum Dışı	Tohum	Tohum Dışı	Tohum	Tohum Dışı	Tohum	Tohum Dışı
2015	0	0	0	0	0	0	10	10	0	0
2016	0	0	0	0	0	0	25	45	0	0
2017	0	0	0	0	0	0	24	46	0	0
2018	0	0	0	0	0	0	59	55	0	0
2019	85	9	32	10	100	0	297	141	15	0
İllere göre Kenevir Üretim Miktarı (ton)										
	Kastamonu		**Kayseri**		**Kütahya**		**Samsun**		**Yozgat**	
	Tohum	Tohum Dışı	Tohum	Tohum Dışı	Tohum	Tohum Dışı	Tohum	Tohum Dışı	Tohum	Tohum Dışı
2015	0	0	0	0	0	0	1	1	0	0
2016	0	0	0	0	0	0	1	7	0	0
2017	0	0	0	0	0	0	1	7	0	0
2018	0	0	0	0	0	0	3	7	0	0
2019	1	0	2	1	1	0	15	18	1	0

Bitkisel Özellikleri

Kısa gün bitkisi olan kenevir, tek yıllık, otsu bir bitki olup, diploid (2n) kromozom sayısı 20'dır. Bitki boyu çeşit ve yetiştirme koşullarına bağlı olarak 50-300 cm arasında değişir. Kenevirin kök sistemi kazık olup, olgunlaşma sonunda kökler 1-5 m kadar derinlere inebilir. Kenevir fidelerinin iki sapsız kotiledon yaprağı vardır; sonraki tüm gerçek yaprakların bir sapı vardır. Birinci gerçek yaprak çiftinin her iki yaprağı tırtıklı kenarlara sahiptir [17]. İkinci gerçek yaprak çiftinden bir yaprak, yaprak sapının ucundan yayılan üç adet testere dişli yaprakçık içerir (Şekil 2; [18]). Bitkinin gövdesi çeşitli derecelerde oluklu olup, içi boştur. Bitki sıklığına bağlı olarak yüksek bitki yoğunluğunda gövdeler hemen hemen dalsızdır [19].

Şekil 2a. Kenevir bitkisi ve kısımları; A: erkek sürgün, B: dişi sürgün, C: Karpel, D: Stamen, E: Tapel, F: Tohum

Şekil 2b Kenevir tohumunun görünüşü

Şekil 2c Kenevir bitkisinin genel görünüşü

Şekil 2 Kenevir Morfolojik Yapısı ve Kısımları [18]

Kenevir iki evcikli bir bitki olup, genellikle rüzgârla tozlaşır. Çiçeklenme kısa günlerde hızlanır ve uzun günlerde gecikir. Genellikle dioik olan kenevirin monoik çeşitleri de bulunmaktadır. Kenevirde cinsiyet çiçeklenmeden önce morfolojik olarak ayırt edilememektedir. Çiçeklenmeye geçişin ilk işareti, farklılaşmamış çiçek taslaklarının oluşumudur. Erkekler, beş radyal bölüme sahip olan yuvarlak sivri uçlu çiçek tomurcuklarının ayrımı ile tanımlanmaktadır. Dişiler ise simetrik borumsu brakte veya çanak yaprak genişlemesi ile tanınmaktadır [20]. Dioik bir bitkide, erkek ve dişi bitkiler genellikle eşit sayıda bulunur ancak çeşitlere ve yetiştirme koşullarına bağlı olarak, erkek bitkilere göre %50 daha fazla dişi bitki bulunabilmektedir [21]. Erkek bitkilerin çiçek salkımları az ya da hiç yapraksız, kuvvetli bir şekilde dallanmıştır. Dişi bitkilerin çiçek salkımları yapraklı, kısa ve dalsızdır. Erkek bitki çiçekleri, çiçeklenmeden hemen sonra ölürken, dişi bitki çiçekleri tohumlar olgunlaşana kadar yaşamlarını devam ettirirler.

Dişi çiçeğin, iki stigmanın çıkıntı yaptığı yumurtalığı tamamen kaplayan küçük yeşil kılıfımsı bir organı vardır. Bu kılıf, ince kıllarla kaplanmış ve cannabinoid içeren reçine salgılayan dairesel salgı bezleri ile kaplanmıştır [17]. Ticarete konu olan tohum, kabuklu bir meyvedir ve içinde tek bir tohum bulunur. Tohum elips biçiminde, hafif sıkıştırılmış, pürüzsüz, 2-6 mm uzunluğunda, 2-4 mm çapındadır. Tohum rengi açık kahverengi ile koyu gri arasında değişir. Bazı durumlarda tohum benekli olabilmektedir.

Kimyasal İçeriği ve Besin Kompozisyonu

Kenevir tohumu yaklaşık %35 yağ içermektedir. Kenevir tohumunun doymuş yağ asitleri bakımından düşük bir profile sahip olması, tohumun kaliteli bir yağ içeriğine sahip olduğunu göstermektedir. Ancak, insan sağlığı için gerekli doymamış yağ asitlerinden biri olan omega-3 (linolenik asit) çok önemli bir yağ asidi kaynağıdır (Tablo 5). İnsan vücudu esansiyel yağ asitlerini üretemediği için bu yağ asitlerinin dışarıdan alınması gerekir. Genel olarak insanlar çok fazla omega-6 ve çok az omega-3 alma eğilimindedirler. Dolayısıyla diyete kenevir tohumunun eklenmesi dengenin sağlanmasını desteklemeye yardımcı olabilir. Nitekim yapılan bir araştırma sonucunda, tavuk yeminde kenevir tohumu ve kenevir tohumu yağı kullanılması, yumurta sarısındaki omega-3seviyesini artırmış ve daha sağlıklı bir omega-3/omega-6 oranına sahip yumurtaların elde edilmesini sağlamıştır [22].

Tablo 5 Kenevir tohumunun farklı kısımlarının besin değerleri

	Tüm tohum	Soyulmuş Tohum	Sıkılmış tohum
Yağ	%36	%44	%11
Protein	%25	%33	%34
Karbohidrat	%28	%12	%43
Nem	%6	%5	%5
Kül	%5	%6	%7
Toplam Diyet lifleri	%28	%7	%43
Enerji (KJ/100g)	2200	2093	1700
Sindirilebilir Lifler	6	6	16
Sindirilemeyen lifler	22	1	27

Kenevir tohumu %25 protein oranına sahiptir. Bu oran, soya fasulyesinin içerdiği protein oranına yakındır. Kenevir tohumu, insan vücudunun sentezleyemediği ve dışardan besin yoluyla alınması gereken dokuz temel aminoasitin tümüne sahiptir. Kenevir tohumu özellikle kalp sağlığı için yararları olan *arginin* aminoasidi bakımından zengindir.

Kenevir tohumunda *gluten* bulunmadığından Çölyak hastalığına sahip kişiler için ekmek yapımında kullanılabilecek bir besin kaynağıdır. Ayrıca, kenevir tohumu bünyesinde önemli vitaminleri barındıran önemli bir vitamin kaynağıdır ([23]; Tablo 6).

Tablo 6 Kenevir tohumlarının vitamin ve mineral olarak besleyici değeri

Kaynak	(mg/ 100 g)
Vitamin E (toplam)	90,00
alpha-tokoferol	5,00
gamma-tokoferol	85,00
Thiamine (B1)	0,40
Riboflavin (B2)	0,10
Fosfor (P)	1,16
Potasyum (K)	859.0
Magnezyum (Mg)	483,0
Kalsiyum(Ca)	145,00
Demir (Fe)	14,00
Sodyum (Na)	12,00
Manganez (Mn)	7,00
Çinko (Zn)	7,00
Bakır (Cu)	2,00

Kenevir bitkisi *Cannabinoid* adı verilen kimyasal bileşikleri içerir. Doğal olarak oluşan bu bileşenler kenevirin hem olumlu hem olumsuz etkilerinin çoğunun üretilmesinden sorumludurlar. Tüm *cannabinoid'*lerin içeriği tam olarak bugüne kadar tespit edilmese de iki ana bileşenin içeriği belirlenmiştir. Bu iki bileşikler *cannabidiol* (CBD) ve *tetrahidrocannabinol* (THC)'dur. Bu iki kimyasal bileşik aynı moleküler yapıya sahiptir ve 21 C atomu, 30 H atomu ve 2 O_2 atomu içerirler. İki bileşik de insan beyninde bulunan *canabinoid* reseptörleri ile etkileşime girerler. Ancak atomların düzenlenme şeklindeki küçük bir farklılık, farklı etkilerin ortaya çıkmasını sağlamaktadır. *Cannabidiol* (CBD) psikoaktif değildir. Bu özelliği nedeniyle, *cannabidiol* doğal takviyelerde ve diyette *tetrahidrokanabinol* (THC)'den daha sık görülmektedir [24]. Ayrıca, *Cannabidiol*, hem endüstriyel kenevirde hem de narkotik kenevirde bulunmaktadır. *Tetrahidrocannabinol* (THC) ise kenevir bitkisinin ana psikoaktif bileşeni olup, kenevir bitkisinin narkotik kısmıyla doğrudan ilişkili olan bir maddedir. Kenevir tarımının geliştirilmesi bakımından *tetrahidrocannabinol* oranı düşük, lif ve tohum yönünden üstün verimli kenevir çeşitlerinin ıslah çalışmaları devam etmektedir. Endüstriyel tip kenevirlerde *tetrahidrocannabinol* oranının üst sınırı Kanada için %0,3, Avrupa Birliği ülkeleri için %0,2 olarak belirlenmiştir [25, 26].

Kenevir Tarımı

Birçok ülkede olduğu gibi Türkiye'de de kenevir tohumu üretimi uzun süre geri planda kalmıştır. Bunda yapraklarının esrar yapımında kullanılması sebebiyle çiftçinin bu bitkiye önyargı ile yaklaşılmasının etkisi fazla olmuştur. Ayrıca kanun, yönetmelik ve mevzuatlar da üretimin sınırlı kalmasında rol oynamışlardır. Nitekim Kenevir ekimi yapacak çiftçilerin il veya ilçe müdürlüklerinden yasal izin almak durumu ve üretim sürecinde sürekli olarak kontrol yapılması zorunluluğu bulunmaktadır. İzinsiz yetiştirilen kenevir bitkisi hangi amaca yönelik olursa olsun kanun hükümlerine göre imha edilmekte ve yetiştirici hakkında cezai işlemler uygulanmakta ve yasal kovuşturma yapılmaktadır. İzinli kenevir ekilen alanlar ise il ve ilçe müdürlükleri teknik elemanları tarafından ekimden hasat zamanına kadar düzenli olarak kontrol edilmektedirler [27]. Tarım Orman Bakanlığı tarafından yapılan değerlendirmeler sonucunda Türkiye'de 2019 yılı itibariyle endüstriyel amaçlı kenevir ekiminin 19 ilde serbest bırakılmasına karar verilmiştir. Bu karar sonrası Türkiye'de lif amaçlı kenevir üretiminin artması beklenmektedir [28].

Kenevir bitkisinin adaptasyon kabiliyeti oldukça geniştir. Bu avantajından dolayı kenevir her türlü ekolojik koşullarda yetiştirilebilir. Vejetasyon süresi; lif elde etmek için yaklaşık 120 gün, tohumluk elde etmek için ise yaklaşık 150 gün civarındadır [29].

Toprak

Kenevir her yerde yetiştirilebilmesine karşın yetiştiriciliği için toprağın derin ve tavında sürülerek iyi bir toprak hazırlığı yapılması gerekir. Dolayısıyla gevşek, iyi havalandırılmış killi, organik madde açısından zengin, besim maddesi içeriği iyi olan pH değeri 6,0-7,5 aralığında, gevşek yapıda alüvyal topraklarda kenevir iyi yetişir Asitli, kumlu, tınlı, ağır killi topraklar ve drenaj özelliği bulunmayan topraklar kenevir tarımı için uygun değildir [29].

Drenajı iyi yapılmış ve eşit kil yoğunluğuna sahip topraklar kullanılabilir fakat drenajı yetersiz veya yanlış yoğunluktaki topraklara yapılan ekimlerde yaygın olarak fide çökelmesi durumuna maruz kalınmakta ve başarısız olunmaktadır. Bunu yanında kumlu topraklar da yeterli sulama ve gübreleme ile verimli bir üretim için hazırlanabilmektedir. Fakat bunun getirdiği ekstra maliyetler üretimi ekonomik olmaktan çıkartabilmektedir [30].

Lif tipi kenevir, yüksek nispi nem, uygun sıcaklık ve en az 700 mm yağış alan bölgelerde sulamaksızın yetiştirilebilir. İlkbahar geç donlarına karşı toleransı az olan kenevir, -5

°C'den düşük sıcaklıklara maruz kaldığında zarar görür [27]. Fide döneminde su ihtiyacı yeterince karşılanırsa iyi bir gelişme gösterir. Toprakta fazla nemin bulunması durumunda, toprak asitliğinin artmasıyla birlikte bitkinin topraktaki besin maddelerini alması güçleşir ve yapraklarda kloroz görülmeye başlar. Bu nedenle kenevir tarımında düzenli yağış rejimi arzu edilir [31].

Ekim derinliği

Tohum yataklarının hazırlanması, kenevir üretiminin önemli çaba harcanması gereken kısımlarındandır. Sonbaharda pullukla sürme işlemi tavsiye edilmekte ve sonrasında ilkbahara doğru tohum yataklarının hazırlığına başlanmalıdır. Sıkı ve 19 - 31 mm derinliğinde yataklar kullanıldığında en iyi sonuçların alındığı görülmüştür. Daha derin ekimlere karşı da tolerans vardır fakat bu durumda fide çökelmesi ile daha sık karşılaşılmaktadır [32].

Ekim zamanı

Kenevir bir kısa gün bitkisidir, yani günlerin 12 saatten az olduğu zamanlarda çiçeklenmektedir. Dolayısıyla bu bilgi göz önünde bulundurularak yapılan erken ekimler sonucu hem vejetatif büyüme daha yoğun olacak hem de tohum üretimi için daha dirençli, lif üretimi içinse daha uzun ve dayanıklı yapıda bitkiler elde edilecektir. Yüksek verim için erken ekim yapılması önerilir. Tohumların donma derecesinin biraz üzerindeki sıcaklıklara kadar çimlenebilmesine rağmen 12-15°C derece sıcaklığındaki toprak koşulları en uygun olarak kabul edilmektedir. Toprak nemliliği çimlenme için çok önemli bir değişkendir ve özellikle ilk 6 hafta boyunca yeterli yağış gereklidir. Yıllık 62-76 cm yağış, ortalama olarak ideal kabul edilmektedir. Lif için üretim yapılacaksa ilkbaharın son don zamanı geçtikten sonra, toprak sıcaklığının 8-10 °C olduğunda ekim yapılması uygundur. Kuzey yarımküredeki ülkeler göz önünde bulunduğunda 21 Haziran tarihini takip eden 4-5 hafta içinde vejetatif büyüme yavaşlamaya başlar ve çiçeklenme süreci tetiklenir. Türkiye'de bölgelere göre ekim zamanı değişmekle birlikte genellikle Mart-Nisan döneminde ekim yapılır [33].

Ekim

Serpme ekim yöntemi hala bazı bölgelerde uygulansa da genellikle mibzerle sıraya ekim yöntemi uygulanmaktadır. Sıra aralığı belirlenirken bölgenin topoğrafik koşulları ve yetiştirilecek olan çeşidin özelliği dikkate alınması gerekir. Sıra aralığı; tohumluk üretimi için genellikle 30-40 cm ve lif için 20-25 cm arasında değişir. Sıra aralığının sık olması

lif verimini ve lif kalitesini, seyrek olması ise tohum verimini ve tohum kalitesini artırır. Tıbbi Tohum olarak yetiştirilen kenevir *cannabinoid* üretimini maksimumda tutabilmek için genellikle 70-100 cm aralığında sıklıklarla ekilmektedir [29, 32].

Tohum miktarı

Dekara atılacak tohum miktarı, tohumluğun çimlenme ve çıkış kabiliyetini dikkate alınarak belirlenir. Lif üretiminde teknik sap uzunluğunun ince ve uzun olması istendiği için sık ekimi sağlamak için dekara 6-9 kg tohumun atılması önerilir. Yüksek kaliteli sak lifi üretiminin kor lif üretimine göre daha yüksek tutulabilmesi için ekimin yoğun yapılması gerekir. Sak lifi yoğunluğu vejetasyonun yoğunluğu ile birlikte artmaktadır fakat optimal yoğunluk bölgeden bölgeye farklılıklar göstermektedir. Neticede hedeflenen yoğunluk ise ortalama olarak m2 başına 60-100 bitkidir.

Tohumluk üretiminde fazla dallanma sağlayabilmek için dekara 4-5 kg tohum atılması yeterli olur [30]. 1kg'a yaklaşık 54.000 tohum düşmektedir. Neticede ulaşılmak istenen ise m^2 de yaklaşık 40-70 adet bitkidir [30].

Bakım

Kenevir özellikle vejetatif aksamını oluşturma döneminde çapalama ve sulama gibi bakım işlerine ihtiyaç duyar. Yağışı yeterli olmayan kurak bölgelerde iyi bir verim için gelişme periyodu boyunca 2-4 kez sulama yapılması önerilir. Tohumluk yetiştiriciliğinde uygulanan fazla su, generatif dönemi ve olgunlaşmayı geciktirdiğinden tavsiye edilmez [30].

Gübreleme

Sıklıkla karşılaşılan bir düşünce kenevir bitkisinin azot ve potas bazlı besin maddelere ihtiyacı olmadığıdır. Kenevir üretiminde dekar başına 8-14 kg azot ve 5-8 kg arası fosfor ile 3,5-8,0 kg arası potas önerilir. Kenevir, özellikle tohum üretimi hedeflendiğinde iyi bir azot gübrelemesine ihtiyaç duymaktadır. Fosfor seviyeleri toprakta orta - yüksek arası tutulmalıdır (ortalama >40ppm). Kükürt 5000 ppm'in bir miktar üzerinde ve kalsiyumun 6000 ppm' i geçmeyecek şekilde tutulduğunda doğru bir üretim koşulu sağlamaktadır. İyi havalandırılmış killi toprağın yanı sıra organik madde miktarının %3,5'dan yüksek olduğu koşullarda üretimdeki performansın arttığı gözlenmiştir. Kıyaslamak gerekirse kenevir, buğday ve mısıra uygulanan gübreleme ile benzer bir uygulamaya ihtiyaç duymaktadır. Koşullara bağlı olarak dekara 2-4 ton çiftlik gübresi uygulanması da önerilmektedir [34].

Münavebe

Kenevir bitkisi uzun yıllar aynı arazide ekilebilir. Ancak haşere üremesi, özellikle kök solucanları, delici kurtlar, çürütücülerin varlığı bu durumu riske etmektedir. Kendisinden sonra rotasyona girecek olan kültür bitkisi için temiz bir tarla bırakması münavebe açısından kenevir bitkisini tercih önceliği olan bitkiler arasına sokar. İyi bir ön bitki olan kenevir, baklagillerle ve buğdaygiller ile ekim nöbetine girebilir. Nitekim kenevir, buğday ve fasulye ile münavebeye girmektedir. Ancak kanola, soya fasulyesi ve ayçiçeği, kenevir bitkisinde beyaz küf ve bazı pestisit risklerini arttırdıkları gerekçesiyle üretiminde dönüşümlü olarak kullanılmaması gereken bitkiler olarak gösterilmiştir [35].

Hastalık ve zararlılar

Endüstriyel kenevir üretiminde en sık rastlanan mantar bazlı hastalıklar; gri küf (*Botrytis cinerea*) ve beyaz küf (*Sclerotinia sclerotiorum*)' tür. Ayrıca *Pythium* enfeksiyonları, kök çürümesi, pirinç yanığı, yaprak bakterileri ve bazı viral enfeksiyonlar karşılaşılmış hastalıklardandır [29].

Birçok bitki türüne göre böceklere çok daha dayanıklı olmasına karşın mevsimsel olarak problemlerle karşılaşılmaktadır. Mısır biti, kök kurtları, çekirgeler sıklıkla karşılaşılan böcek türleridir. Özel olarak kenevir için kayıt altına alınmış pestisitler bulunmamaktadır. Genel olarak 4 yıllık rotasyonlar yapılarak böcek kontrolü sağlanmaya çalışılır [32].

Hasat ve harman

Kenevir bitkisi dioik (iki evcikli) bir yapıya sahiptir. Dolayısıyla bitkiler erkek veya dişi olurlar (Şekil 3a ve Şekil 3b). Erkek ve dişi bitkilerin büyüme hızı ve gelişimi farklılık gösterir [26]. Erkek bitkiler erken çiçeklenmeye ve erken olgunlaşmaya daha meyilli olurlar. Erkek bitkilerin olgunlaşması, dişi bitkilerin olgunlaşmasından yaklaşık 4 hafta erkendir. Olgunlaşmada dişi bitkiler ile erkek bitkiler aralarındaki farkı en aza indirmede populasyondaki bitkilerin çoğunluğunun dişi olması ve tozlama için az sayıda erkek bitkinin olması yeterli olur [33].

Şekil 3a Çiçeklenmiş dişi kenevir bitkisi (orijinal)

Şekil 3b Çiçeklenmiş erkek kenevir bitkisi (orijinal)

Hasat zamanı lif verimi ve lif kalitesini etkiler. Erken hasat, lif veriminin düşmesine neden olurken, geç hasat ise sapların havuzlamasın güçleştirerek kaliteye etki eder. Hasadın çok geç dönemde yapılması durumunda ise lif elde edilmesi mümkün olmaz [35].

Türkiye'de Ünye-Fatsa, Gümüşhaciköy ve Kastamonu yöntemi olmak üzere 3 farklı hasat yöntemi uygulanmaktadır. Ünye-Fatsa Hasat Yönteminde; erkek ve dişi bitkiler olgunlaşma dönemlerine göre ayrı ayrı hasat edilirler. Gümüşhacıköy Hasat Yönteminde; erkek bitkilerin hasadı dişi bitkilerin olgunlaşmasına kadar bekletilir ve erkek ve dişi bitkiler birlikte hasat edilirler. Kastamonu Hasat Yönteminde ise erkek bitkiler olgunlaşınca dişi bitkilerle birlikte hasat edilirler [27].

Tohum elde etmek amacıyla yetiştirilen kenevirlerden dekara 600-1000 kg arasında sap elde edilir. Tohum verimi lif tipi kenevirlerde dekara 80-100 kg, yağ tipi kenevirlerde ise 80-300 kg arasındadır [35, 36].

Kullanım Alanları

Kenevir, çok yönlü kullanım alanına sahip bir bitkidir. Kenevir bitkisinden elde edilen yağ biyodizel ve alkol üretiminde, kozmetik sanayiinde, ilaç, makina ve boya sanayisinde, tohumundan protein elde edilmesiyle insan ve hayvan beslenmesinde, saplar

tekstil sanayisi, kâğıt sanayisi, otomotiv sanayisi ve inşaat malzemesi yapımında değerlendirilmektedir.

Kenevir lifi yüksek mukavemet, yüksek nem çekme özelliği, nefes alabilirlik kabiliyeti, anti bakteriyel özelliği, UV koruma, anti-alerjik özelliği ve iyi elektrostatik özellikler göstermesi ile katma değeri yüksek ürünlerin üretiminde tercih edilmektedir [37, 38, 39]. Bu üstün özellikleri ile iç ve dış giyim ürünleri, ev tekstil ürünleri ve aksesuar ürünleri %100 kenevir lifinden veya kenevir lifi ile farklı oranlarda çeşitli liflerin karışımları ile üretilmektedir.

Kenevir lifi ve sapları, çevre dostu malzeme sınıfında yer alırlar. Bu çevre dostu özelliği ile kenevir yalıtım malzemeleri, jeo-tekstil ürünleri veya kâğıt gibi endüstriyel kullanımlar için tamamen yenilenebilir bir kaynaktır [37]. Kenevir lifi, yüksek oranlarda kullanıldığında düşük yoğunluklu kompozit üretimine imkân sağladığı, yüksek sağlamlık ve sertlik performansı verdiği bilinmektedir. Ayrıca esnek özelliği sayesinde işleme sırasında kırılmaya karşı da dirençlidir [40, 41, 42]. Daha uzun kullanım süresine olanak sağlayan biyolojik bozunabilirlik ve düşük ısı iletim katsayısına sahip olması sayesinde diğer bitkisel liflere oranla kenevir lifi ısı yalıtım malzemesi olarak yüksek performans gösterir [43]. Nitekim kenevir lifi bu üstün özelliğinden dolayı inşaat sektöründe izolasyon malzemesi olarak tercih edilmektedir.

Kuzey Amerika, diğer ülkelere kıyasla otomotiv sektöründe kenevir lifi kullanımı bakımından lider durumdadır. Amerika'daki otomotiv şirketlerinde üretilen araçlarda kenevir gibi doğal liflerle güçlendirilmiş termoplastik ve termoset kompozitlerini kullanmaktadırlar [44, 45]. Brezilya'da bulunan Mercedes Benz otomotiv şirketi 1992 yılından itibaren doğal lifleri, ürünlerinde kullanmaya başlamıştır [45, 46]. Bu doğal lifler; araç gövde altı panelleri, koltuk arkalığı, tavan döşemesi, kaput altındaki radyatör deposunun altı, kapı pervazı paneli, arka panel rafları, bagaj pervaz kaplaması, çamurluk parçaları, bagaj bölmesi, hoparlör, motor ve vites kutusu kapağı, yedek lastik bölümlerinin kapağı, motor kapağı pervazı, kapı paneli, zemin üst levhası, halı döşemesi, koltuk altlıkları, alet kutusu bölmesi gibi otomobil içi kısımlarda kullanılmaktadır [47, 48, 49].

Kâğıt-karton üretiminde selüloz hammaddesi olarak kenevir gibi tek yıllık bitkiler değerlendirilmektedir. Kenevir selülozlarının elyaf boyları, genellikle diğer ağaçlardan elde edilen selülozların elyaf boylarından kısa olduğu için kâğıda daha düzgün görünüm

vermektedirler. Türkiye'de üretilen kenevirin büyük bir kısmı sigara kâğıdı üretiminde hammadde olarak kullanılmaktadır. Kenevir selülozlarının elyafları yüksek mukavemet, opasite, beyazlık ve sigara kâğıdına daha iyi bir tat vermesi bakımından diğer ağaçlara göre daha avantajlıdır [50].

Kenevir tohumu ve yağı yüksek besin değerine sahip olmasından dolayı Avrupa'nın birçok ülkesinde insan tüketimi için kenevir bazlı işlenmiş gıda piyasası bulunmaktadır: İçeceklerden fermente edilmiş özel ürünlere, tatlı, peynir, ekmek, salata ve çikolata gibi birçok gıda ürününün hazırlanmasında kenevirin besinsel özelliklerinden yararlanılmaktadır [51]. Kenevir tohumu ayrıca sabun üretmek amacıyla da işlenmektedir.

Besin değerine ek olarak kenevir, binlerce yıldır tıbbi amaçla kullanılmıştır. Nitekim Antik çağlardan beri, birçok anti-enflamatuar, anti-atopik, anti-radikal ve cilt iyileştirici olarak kenevir kullanılmaktadır. Günümüzde, kenevirin tıbbi kullanımını yasallaştıran yasalar Dünya çapında giderek artan sayıda çıkarılmaktadır. Kenevir tohumunun kolesterolün dengelenmesi ve yüksek tansiyon dâhil olmak üzere olumlu sağlık yararları vardır [52]. Kenevirin yağı, nörodermatoz ve sedef hastalığının tıbbi tedavisinde potansiyel olarak önemli bir kaynaktır [53, 54, 55]. ABD Gıda ve İlaç İdaresi (FDA), 2018'de ciddi epilepsi formuyla ilişkili nöbetlerin tedavisi için *cannabidiol* içeren oral solüsyonu (Epidiolex) onaylamıştır. FDA, kanser hastalarında kemoterapiye bağlı olarak ortaya çıkan bulantı ve kusmayı tedavi etmek için sentetik *cannabinoid'*ler olan *dronabinol* ve *nabilon*'u onaylamıştır. Dronabinol aynı zamanda AIDS hastalarının iştahsızlık ve kilo kaybı tedavilerinde kullanılmak üzere onaylanmıştır [56]. Ayrıca, ıslahı edilen kenevir çeşitlerinden elde edilen özütlerden, son yıllarda kenevir özlü şaraplar üretilmeye başlanmıştır [57].

Organik olarak yetiştirilen kenevir tohumu yağına dayalı olarak üretilen cilt bakım ürünleri potansiyel bir Pazar olarak önem arz etmektedir [58]. Omega-3, omega-6 ve tokoferoller (E Vitamini) bakımından zengin olan bu yağ, nemlendirici faydaları ve cildin geçirgenlik bariyerini güçlendirme kapasitesi ile bilinmektedir. Kenevir tohumu yağı, cilde hızla nüfuz eden oldukça kuru bir yağdır.

Kümes hayvanlarının beslenmesinde karışım olarak kenevir tohumu kullanımının yumurta ağırlığını artırdığı ve yumurtalarda Omega-3 ve Omega-6 yağ asitlerinin içeriklerini artırdığı belirlenmiştir [59].

Kenevir samanı yalıtım özelliği ve su emme yeteneğinden dolayı hayvan çiftliklerinde altlık olarak kullanılmaktadır. Ayrıca bahçelerde kaplama malzemesi olarak kullanılan kenevir samanı sayesinde, yabancı otların büyümesi engellenmekte ve toprak nemi korunmaktadır [60].

Kenevir biyodizel yapımı için enerji değerleri üzerinden en uygun, en doğa dostu, en hızlı üretimi olan, en bol kaynaktır. Ek olarak, kenevirin bir yakıt kaynağı olarak bir diğer avantajı, biyoetanol veya biyobutanol gibi düşük karbonlu yakıtlar oluşturmak için fermente edilebilecek yüksek bir biyokütle içeriğine sahip olmasıdır [61]. Nitekim kenevir; damıtma yoluyla kömür, metanol, metan veya benzine işlendiği gibi yakılabilir veya işlenebilir ve selülozik bazlı etanol elde etmek için kullanılmaktadır [62, 63].

Kenevir türleri bütün bitki ve/veya metabolitleri hammadde olarak değişik amaçlarla kullanılmaktadır. Bitki bileşenlerinin içeriği ve bileşimi genotip, çevre, yetiştirme tekniği paketi uygulamalarına bağlı olarak değişkenlik gösterir. Geleneksel üretim yöntemlerinin yanında özellikle standart ürün elde edilmesinde biyoteknolojik yöntemler üzerinde durulmaktadır. Son yıllarda yapılan çalışmalarda özellikle üretim yöntemlerinin ve tekniklerinin geliştirilmesi amacına yönelik yoğun çaba sarf edilmektedir [64].

Sonuç

Kenevir, adaptasyon kabiliyeti ve yabancı ot rekabeti bakımından üstün performanslı, bitki besin elementi bakımından kanaatkâr olmasından dolayı çevre dostu bir bitkidir. Besin kompozisyonu bakımından hem insan hem de hayvan beslenmesi bakımından yüksek bir potansiyele sahiptir. İlaç, gıda, kâğıt, biyoyakıt, tekstil, kozmetik, inşaat ve otomotiv sektörüne kadar oldukça geniş kullanım alanına sahip olan kenevir, petrol ve petrokimyanın kullanıldığı her alanda da kullanılabilecek alternatif bir bitkidir. Narkotik özelliğinden dolayı 19. asrın ortalarında birçok ülkede üretimi yasaklanan kenevir, son yıllarda özellikle endüstriyel kenevir olarak üretilmek için yeniden önem kazanmaya başlamıştır. Türkiye'de 2019 yılında endüstriyel kenevir üretim amacıyla 19 ilde kenevir tarımına izin verilmiştir. Bu illerde kenevir üretiminin artırılmasına yönelik olarak yasal ve teknik altyapının tamamlanması yönünde önemli çalışmalar yapılmaktadır.

Birçok kaynakta geleceğin bitkileri arasında gösterilen kenevir bilhassa endüstriyel kenevir olarak tarımsal üretimde mutlaka hak ettiği yeri alacak ve geniş kullanım alanı sayesinde ulusların ekonomilerine katkı sağlayacak önemli bir kaynak haline gelecektir.

Kaynaklar

1. Kew Science: The International Plant Names Index and World Checklist of Selected Plant Families 2019, Cannabis sativa L". Plants of the World Online, Royal Botanic Gardens, Kew, 2019. Available from: http://www.plantsoftheworldonline.org/taxon/ [Accesed 27 September 2019].
2. Whittle, B.A., G.W. Guy and P. Robson, Prospects for cannabis-based prescripton Medicines. Journal of Cannabis Therapeutics, 2001. 1: 3-4.
3. Colbert, M.,"Indica, Sativa, Ruderalis – Did We Get It All Wrong?", 2015 Available from: www. http://theleafonline.com [Accessed: 29 September 2019].
4. USDA,United States Department of Agriculture, Classification Report, 2019. Available from: https://plants.usda.gov/java/nameSearch. [Accessed27 September 2019].
5. Anonymous, 2020, https://craftsense.co/cannabis/cannabis-levelup/cannabis-ruderalis-having-moment (Erişim 14.09.2020).
6. ElSohly, M. Marijuana and the Cannabinoids, 2007. Humana Press,ISBN 978-1-58829-456-2.
7. Lambert, D.M. Cannabinoids in Nature and Medicine, 2009. Wiley-VCH Publisher, ISBN 978-3906390567.
8. Vavilov, N.I. Centers of origin of cultivated plants,in Origin and geography of cultivated plants, V.F. Dorofeyev, Editor, 1926,Cambridge, Cambridge University Press, pp.22-135. ISBN 0-521-40427-4.
9. Schultes, R.E. Random thoughts and queries on the botany of Cannabis. In: The botany and chemistry of Cannabis, C.R.B.Joyce and S.H. Curry, Editors, 1970, J & A Churchill, London, pp.11-38. ISBN: 0700014799.
10. Abel, E. The First 12,000 Years Marijuana, 1980. Plenum Press. New York. ISBN 978-1-4899-2189-5.
11. Pounds, N.J.G. An historical geography of Europe, 1979. Cambridge University Press. ISBN: 9780511572265.
12. Conrad, C. Hemp, lifeline to the future, 1993. Creative Xpressions Publishing, Los Angeles, California. ISBN-10: 0963975404
13. Herer, J. Hemp & the marijuana conspiracy: The emperor wears no clothes, 1992. Van Nuys: Hemp Publications, California.
14. Mathieu, J.P. Chanvre, Techniques Agricoles, 1980. 5: 1-10.
15. FAO, Food and Agriculture Organization of Hemp Cultivation and Production Statistical Data, FAOSTAT, 2019. http://www.fao.org/faostat/ [Erişim tarihi: 21 Mart 2020].
16. TÜİK, Bitkisel Üretim İstatistikleri, Türkiye İstatistik Kurumu, 2020. https://biruni.tuik.gov.tr/medas/, [Erişim Tarihi: 21 Mayıs 2020].
17. Stearn, WT. The Cannabis Plant: Botanical Characteristics, in The botany and chemistry of Cannabis. C.R.B. Joyce and S.H. Curry, Editors, 1970.J & A Churchill, London, pp.1-10. ISBN: 0700014799.
18. Wikipedia, 2020. https://tr.wikipedia.org/wiki/Kenevir. [Erişim tarihi: 14 Ağustos 8.2020]
19. Heslop-Harrison, J. and Y. Heslop-Harrison, Studies on flowering-plant growth and organogenesis III. Leaf shape changes associated with flowering and sex differentiation in Cannabis sativa. Proceedings of the Royal Irish Academy,1958. 59: 257-283.
20. Clarke, R.C. Marijuana Botany, 1980. Berkeley: Ronin publishing, California.
21. Hoffmann, W., Hanf, Cannabis sativa, 1957. Handbuch der Pflanzenzüchtung, 5: 204-263.
22. Neijat, M, et al., Hempseed products fed to hens effectively ıncreased n-3 polyunsaturated fatty acids in total lipids, triacylglycerol and phospholipid of egg yolk. Lipids, 2016. 51:601–614.
23. Karakaş, Ş. Sanayi kenevir yağının elde edilmesi ve besleyici özellikleri, 2018. Available from:https://arastirma.tarimorman.gov.tr/ktae/Belgeler/End%C3%BCstriyel%20Kenevir%20Ger%C3%A7e%C4%9Fi%20Paneli%20Sunular%C4%B1/ekg_7.pdf. [Accessed: 10 Ekim 2019].

24. Atakan, Z. Cannabis, a complex plant: different compounds and different effects on individuals, The Advanced Psychopharmacol,2012. 2(6): 241–254.
25. Holoborodko, P., et al., Results of efforts by french and ukranian breeders to reduce cannabinoid levels in industrial hemp (Cannabis sativa L.), 2014. Available from: www.interchanvre.com/docs/article-Laiko.pdf. [Accessed 13 October 2019].
26. Sawler, J., et al., The genetic structure of marijuana and hemp, Plos One,2015. 10:8.
27. Aksoy, D., S. Aytaç ve R. Paslı, Endüstriyel kenevir gerçeği, in 2.Uluslararası 19 Mayıs Yenilikçi Bilimsel Yaklaşımlar Kongresi, M. Abdullayev, Editor, 2019.Samsun, Türkiye, 27-29 Aralık 2019, İktisadi Kalkınma ve Sosyal Araştırmalar Enstitüsü, pp.850-858.
28. ORAN, Orta Anadolu Kalkınma Ajansı, Kenevir Yetiştiriciliği, 2019. Available from: http://www.oran.org.tr/images/dosyalar/20190318134910_0.pdf.[Accessed 08 Kasım 2019].
29. İncekara, F. Endüstri Bitkileri ve Islahı: Keyif Bitkileri ve Islahı, 1964.Ege Üniversitesi Ziraat Fakültesi, İzmir.
30. García-Tejero, I.F., et al., Impact of plant density and irrigation on yield of hemp (Cannabis sativa L.) in a Mediterranean semi-arid environment, Journal of Agriculture Science Technology,2014. 16: 887-895.
31. Monteith, J.L. Climate and efficiency of crop production in Britain, 1997. Philosophical Transactions of the Royal Society of London, 281: 277-294.
32. Megep, Tarım Teknolojileri: Lif Bitkileri Yetiştiriciliği Modülü,2012. Ankara. Available from:http://www.megep.meb.gov.tr/mte_program_modul/moduller_pdf/Lif%20Bitkileri%20Yeti%C5%9Ftiricili%C4%9Fi.pdf. [Accessed 12 November 2019].
33. Cosentino, S.L., et al., Sowing Time and prediction of flowering of different hemp (Cannabis sativa L.) genotypes in Southern Europe, Industrial Crops and Products,2012. 37: 20-33.
34. Van der Werf H.M.G. and W. Van den Berg, Nitrogen fertilization and sex expression affect size variability of fibre hemp (Cannabis sativa L.). Oecologia, 1995. 103: 462–470.
35. Amaducci, S., et al., Influence of agronomic factors on yield and quality of hemp (Cannabis sativa L.) fibre and ımplication for an ınnovative production system. Field Crop Research, 2008. 107: 161-169.
36. Schumacher, A.G.D., S. Pequito and J. Pazour, Industrial hemp fiber: A sustainable and economical alternative to cotton. Journal of Cleaner Production, 2020. 268. DOI: https://doi.org/10.1016/j.jclepro.2020.122180.
37. Ebskamp, M.J.M., Engineering flax and hemp for an alternative to cotton. Trends in Biotechnology,2002. 20 (6): 229-230.
38. Gedik, G., O.O Avinç ve A. Yavaş, Kenevir lifinin özellikleri ve tekstil endüstrisinde kullanımıyla sağladığı avantajlar. Tekstil Teknolojisi ve Elektronik Dergisi,2010. 4 (3): 39-48.
39. Şahinbaşkan, YB. Kenevir Dokuma Kumaşa Enzimatik Ön İşlemlerin Etkisi. International Journal of Advanced Engineer Pure Science, 2019. 3: 208-213.
40. Vantreese, V.L. Hemp support: evolution in EU regulation. Journal of International Hemp Association, 2002. 7 (12): 1-23.
41. Santos, P.A., et al., Natural fibers plastic composites in automotive applications, 2008. SPE Automotive Composites Conference & Exhibition, Troy, MI, USA.
42. Spoljaric, S., A. Genovese and R.A. Shanks, Polypropylene-microcrystalline cellulose composites with enhanced compatibility and properties. Composite Part A:Applied Science and Manufacturing, 2009. 40: 791-799.
43. Kymäläinen, H.R. and A.M. Sjöberg, Flax and hemp fibres as raw materials for thermal insulations. Building and Environment, 2008. 43: 1261–1269.
44. Faruk, O. Cars from jute and other bio-fibers. Macromoleculer Materials and Engineer, 2009.291: 449–457.
45. Çavdar, D.A. ve S. Boran, Doğal Liflerin Otomotiv Sanayinde Kullanımı. Kastamonu Üniversitesi Orman Fakültesi Dergisi, 2016. 16 (1): 253-263.

46. Heitzmann, L.F, et al., Aplicaçã de materiais de fontes renová veis na indú stria automobilí. Daimler Chrysler of Brazil, 2001. SIMEA -Simpó sio De Engenharia Automotiva, Sã Paulo.
47. Ashori, A., Wood–plastic composites as promising green-composites for automotive industries. Bioresource Technology, 2008. 99: 4661- 4667.
48. Hill, K., B. Swiecki, and J. Cregger, The bio-based materials automotive value chain, 2012. 20585: Center for Automotive Research, Washington, DC.
49. Biron, M., The plastics industry: economic overview,in Termosets and composites, M. Biron, Editor, 2014. William Andrew Press, Oxford.pp. 25–104. ISBN:1856174115.
50. Sekizinci Beş Yıllık Kalkınma Planı, Basım Sanayii Özel İhtisas Komisyonu Raporu, 2000. Available from: http://www.sbb.gov.tr/wp-content/uploads/2018/11/08_BasimSanayii.pdf. [Accessed: 21 Kasım 2019].
51. Vogl, C.R., et al., Hemp (Cannabis sativa L.) as a resource for green cosmetics.Journal of Industrial Hemp,2004. 9 (1): 51-68 DOI: 10.1300/J237v09n01_06.
52. Jones, K. Nutritional and medicinal guide to hemp seed, 1995. Rainforest Botanical Laboratory, Gibsons, BC, Canada.
53. Anstey, A., M. Quigley and J.D. Wilkinson, Topical evening primrose oil as treatment for atropic eczema, Journal of Dermatological Treatment, , 1990. 1: 199-221.
54. Fiocchi, A., et al., The efficiency and safety of gamma-linolenic acid in the treatment of infantile atopic dermatitis. Journal of International Medicine Research,1994. 22: 24-32.
55. Pate, D.W., Hemp Seed: A valuable food source, in: Advances in Hemp Research. Food Products Press New York, P. Ranalli, Editor, 1999. pp. 243-255. ISBN:1560228725.
56. U.S National Institutes of Health, 2019. Available from: https://nccih.nih.gov/health/marijuana. [Accessed 03 October 2019].
57. Herb, Introducing Alcohol-Free, Cannabis-Infused Wine for the World’s Classiest High, 2019. Availeble from: https://herb.co/read/[Accessed5 October.2019].
58. Maxwell, B.A., Effects of herbicides on ındustrial hemp (cannabis sativa) phytotoxicity, biomass, and seed yield, 2016.Msc Thesis, Department of Agriculture, Western Kentucky University, Kentucky, USA.
59. Gakhar, N., et al., Effect of feeding hemp seed and hemp seed oil on laying hen performance and egg yolk fatty acid content: Evidence of their safety and efficacy for laying hen diets. Poultry Science,2012. 91: 701–711.
60. Carus, M. et al., The European Hemp Industry: Cultivation, processing and applications for fibres, shivs and seeds, European Industrial Hemp Association (EIHA) Report, 2012.
61. Moxley, G., Z. Zhu and Y.P. Zhang, Efficient sugar release by the cellulosesolvent-based lignocellulose fractionation technology and enzymatic cellulosehydrolysis. Journal. Agriculture Food Chemistry,2008. 56: 7885–7890.
62. Biewinga E.E. and G. van der Bijl, Sustainability of energy crops in Europe: A Methodology Developed and Applied, No. 234,1996. Centre for Agriculture and Environment: Utrecht, The Netherlands.
63. Kuglarz, M., et al., Integrated production of cellulosic bioethanol and succinic acid from industrial hemp in a biorefinery concept. Bioresource Technology, 2016. 200: 639–647.
64. Çalışkan, K. U. Her Yönüyle Cannabis. Mised, Türk Eczacılar Birliği Yayını- Meslek İçi Sürekli Eğitim Dergisi. 2019. 43-44: 37-43.

Derleme makale / Review article

Kırkıncı, S.F., et al., Antarktika: Yaşam Bilimleri ve Biyoteknoloji Araştırmalarının Gözden Geçirilmesi. International Journal of Life Sciences and Biotechnology, 2021. 4(1): p. 158- 177.
DOI: 10.38001/ijlsb.853472

Antarktika: Yaşam Bilimleri ve Biyoteknoloji Araştırmalarının Gözden Geçirilmesi

Süleyman Faruk Kırkıncı[1], Sevgi Marakli[2,3], Hasan Murat Aksoy[4], Didem Ozcimen[5], Yilmaz Kaya[1, 6]

ÖZET

Yeryüzünde insanlar tarafından en son keşfedilen, en yüksek, en soğuk, en kurak ve nüfus yoğunluğu en az olan kıta Antarktika'dır. Aynı zamanda yeryüzünün kullanılabilen tatlı su kaynaklarının yaklaşık % 70'i buz halinde bu kıtada bulunmaktadır. Bu özellikleriyle geçmişten günümüze insan yaşamı olmadığı halde canlı yaşama doğal seleksiyon ile devam etmiştir. Antarktika, üzerinde barındırdığı doğal yaşam habitatlarıyla, bilim insanları için sınırları tüm kıta olan eşsiz bir laboratuvar gibidir. Antarktika'da az sayıda olmakla birlikte kıtaya özgü olan hayvan ve bitki türleri ile çeşitli alg, liken ve mikroorganizma türleri bulunmaktadır. Bilim insanları bu canlı formları üzerinde araştırmalar yaparak, küresel ısınma ve çevre problemleri gibi güncel sorunlara bir çözüm aramaktadırlar. Kıtadan izole edilen bazı türler, enzimler ve genler kullanılarak başta biyolojik kontrol olmak üzere biyoteknoloji, biyoremidasyon gibi farklı alanlarda çalışmalar devam etmektedir. Bu çalışmada, Antarktikada gerçekleştirilen yaşam bilimleri ve biyoteknoloji araştırmaları gözden geçirilmiştir.

MAKALE GEÇMİŞİ
Geliş
4 Ocak 2021
Kabul
24 Şubat 2021

ANAHTAR KELİMELER
Antarktika,
antarktika biyoçeşitliliği,
psikrotolerant bakteri,
kutup algleri

[1] Tarımsal Biyoteknoloji Bölümü, Ziraat Fakültesi, Ondokuz Mayıs Üniversitesi, Samsun, Türkiye
[2] Merkezi Araştırma Uygulama Laboratuvarı Uygulama ve Araştırma Merkezi, Amasya Üniversitesi, Amasya, Türkiye
[3] Tıbbi Hizmetler ve Teknikler Bölümü, Sabuncuoğlu Şerefeddin Sağlık Hizmetleri Meslek Yüksek Okulu, Amasya, Türkiye
[4] Bitki Koruma Bölümü, Ziraat Fakültesi, Ondokuz Mayıs Üniversitesi, Samsun, Türkiye
[5] Biyomühendislik Bölümü, Kimya-Metalurji Fakültesi, Yıldız Teknik Üniversitesi, İstanbul, Türkiye
[6] Biyoloji Bölümü, Fen Fakültesi, Kırgısiztan Türkiye Manas Üniversitesi, Bişkek, Kırgısiztan
*Corresponding Author: Yılmaz Kaya, e-mail: yilmaz.kaya@manas.edu.kg

Antarctica: A review of Life Sciences and Biotechnology Researches

ABSTRACT
Antarctica is the last discovered by humans on earth with the highest, the coldest, the driest and the lowest population density. At the same time, approximately 70 % of the usable fresh water reserves of the earth are found in this continent as a ice form. It has continued to live with natural selection with these features even though there is no human life from the past to the present. Antarctica with this natural habitat is like a unique laboratory for scientists whose borders are the entire continent. In Antarctica, there are a small number of animal and plant species specific to the continent as well as various algae, lichen and microorganism species. Scientists are searching for a solution about current problems such as global warming and environmental problems by performing investigations on these living forms. Studies in different areas such as biotechnology, bioremediation especially biological control are continue by using some species, enzymes and genes isolated from the continent. Life sciences and biotechnology researches carried out in Antarctica have been reviewed in this study.

ARTICLE HISTORY
Received
4 January 2021
Accepted
24 February 2021

KEY WORDS
Antarctic,
Antarctic biodiversity,
psychrotolerant
bacteria,
polar algae

Giriş

Arktik (Kuzey Kutbu), ismini Arktos'tan yani Kuzey Kutbundaki Büyük Ayı takımyıldızından almıştır [1,2]. Antarktika kelimesi ise etimolojik olarak Arktik bölgesinin karşısı anlamına gelmektedir. Kıtalar arasında Antarktika, büyüklük bakımından beşinci sırada yer almaktadır [3]. Bu kıtanın % 98'lik kısmı buzlarla kaplı olup toplamda 13.209.000 km^2 ölçümü ile yaklaşık olarak Avrupa'dan % 30, Avustralya'dan ise % 50 daha büyük alana sahiptir [4]. Bölge olarak yeryüzünün en güneyinde yer almaktadır. Bu kıta; yeryüzünün en kurak, en rüzgarlı ve en soğuk kıtasıdır. Kıta, Doğu ve Batı Antarktika olmak üzere iki bölgeden oluşmaktadır. Doğu Antarktika büyük ölçüde yüksek buz kaplı bir platodan oluşurken, Batı Antarktika büyük ölçüde dağlık adaları ve takımadalarını kapsayan bir buz tabakasından meydana gelmiştir. Doğu boylamlarında bulunan Doğu Antarktika, batı boylamlarında bulunan Batı Antarktika'dan daha büyüktür. Doğu ve Batı Antarktika yaklaşık 3.400 km uzunluğunda Transantarktik Dağlarıyla birbirinden ayrılır [3].
Ortalama kalınlığı yaklaşık 1.800 metre olan buz kütlesi, küresel tatlı su rezervleri açısından çok önemlidir. Kıtaya hakim olan buz tabakası, dünya buzunun yaklaşık % 90'ını ve dünya tatlı suyunun % 70'ini temsil eden yaklaşık 29 kilometreküp hacmindedir [5]. Kıtanın bazı bölgeleri ise kullanılabilir suya erişimin zor olduğu soğuk ve kuru bir çöldür. Kıtada hakim olan karasal ekosistemde, bilinen binlerce organizma türü bulunmaktadır ve bu organizmaların çoğunu ekstrem koşullara kolaylıkla uyum

sağlayabilen mikroorganizmalar oluşturur. Kıtanın sahil ve deniz bölgelerindeki yaşam formları farklılaşarak zenginleşmektedir [3].

Tarihin erken döneminden beri birçok denizci, kâşif, gezgin ve araştırıcı Antarktika ile ilgili çalışmalar yapmıştır. Antarktika, 1800'lü yıllarda batılı devletler tarafından kıta olarak keşfedilmesine karşın denizcimiz Piri Reis tarafından 1510'lu yıllarda Antarktika'ya en yakın nokta olan Tierra del Fuego çoktan haritalandırılmıştı. Bunun yanı sıra Dünya'nın Antarktika kısmına ve çevresine ait tek haritayı çizen Piri Reis'in 1528 tarihli dünya haritasında ise Atlantik Okyanusu'nun kuzeyinde yer alan Grönland'ın, Kanada'nın kuzey doğu kıyılarını gösterdiği bilinmektedir [6]. Piri Reis'in haritasından sonra batılılara ait kıta ile ilgili bilgiler ortaya çıkmıştır. James Cook denizcilik kariyerine Fransa ve Birleşik Krallık arasında meydan gelen "Yedi Yıl Savaşları" sırasında başlamıştır. Gösterdiği başarılar sonrasında İngiliz Deniz kuvvetlerinde görevli olan James Cook ve ekibi 1773'te Antarktika yakınlarındaki adaları keşfetmelerine karşın Antarktika'yı görememişlerdir [7]. Antarktika'nın 1800'lü yıllarda yine denizci olan asker kökenli Amudsen, Scott ve Shackelton tarafından keşfedilmesiyle birlikte sonraki yıllarda batılılar tarafından kıta olarak keşfi yapılmış ve bununla birlikte 1910'lu yıllardan sonra Antarktika üzerinde toprak kazanım istekleri başlamıştır [8]. Hiçbir ülkeye ait olmayan bu kıta "bilim ve barış" için 53 ülkenin imzası ile korunmakta ve bu ülkelerden 29'unun kararları ile merkezi Buenos Aires (Arjantin)'de bulunan bir sekretarya tarafından yönetilmektedir. Kararlar, her yıl danışman devletlerin alfabetik sırayla ev sahipliğinde ve oy birliğiyle alınmaktadır [9].

Antarktika Yarımadası, Güney Kutbu'nun tabanında bulunan Antarktika anakarasının en kuzey bölümüdür. Onu örten buz örtüsünün altında bir dizi kayalık adalardan oluşur. Bu kaya adaları toprak görevi gören bir buz tabakasıyla birbirlerine bağlanmıştır. Kıtanın tek uzantısı olan Antarktika Yarımadası aynı zamanda Antarktika'nın Güney Amerika'ya en yakın kısmıdır. Kıtanın en yumuşak iklimine sahip yer olan Antarktika Yarımadası, biyolojik çeşitlilik bakımından da kıtanın en zengin bölgesidir. Aynı zamanda bu yarımada; ABD'nin Palmer, İngiltere'nin Rothera, Avustralya'nın Casey, Güney Afrika'nın Sanea gibi birçok ülkenin araştırma üssüne ev sahipliği yapmaktadır (Şekil 1) [10].

Şekil 1 Antarktika kıtasının genel görüntüsü (www.geology.com/world/antarctica-satellite-image.shtml adresinden alınarak modifiye edilmiştir)

Antarktika'yı ziyaret eden ilk Türk bilim insanı Atok Karaali'dir ve bu bölge 1968 yılında Antarktika Adları Danışma Kurulu tarafından "Karaali Kayalıkları" olarak isimlendirilmiştir. Antarktika'da bulunan "İnan Tepesi" de kıtada bilimsel araştırmalar yürütmüş Türk Bilim İnsanı Prof. Dr. Umran İnan'ın adını taşımaktadır. 2019 yılı itibariyla ülkemizin, 'Horseshoe' isimli yarımadada geçici üssü de bulunmaktadır.
Bu derleme çalışması kapsamında Antarktika'da bulunan bitki, mikroorganizma ve alg biyoçeşitliliği ile ilgili biyoteknoloji araştırmaları incelenmiştir.

Antarktika Biyoçeşitliliği

Atlas, Hint ve Pasifik okyanusları ile çevrili olan Antarktika ekosisteminin oldukça karmaşık bir yapıya sahip olduğu görülebilmektedir. Antarktika, yeryüzünde bulunan en belirgin ekstrem şartları gösterebilen yeryüzündeki sayılı bölgelerden biridir. Antarktika; kıtadaki buzun kendisi, tatlı su, tuzlu su gölleri ve buz örtüleri de dahil olmak üzere kutup çöllerinden yemyeşil otlaklara ve ötrofik göllere kadar birbirinden

farklı ekosistemleri bünyesinde barındırır. Yani ekosisteminde hem ekstrem tuzlu ortamları hem de hiç tuzlu olmayan ortamları içerebilmektedir [11, 12].

Yapılan son çalışmalar, Antarktika orijinli canlıların kökeninin çok eski olduğunu ve milyonlarca yıldır izole bir şekilde canlılıklarını koruduklarını göstermiştir. Buradaki canlıların, 30 milyon yıldan fazladır yaşamlarını sürdürdüğü ama bazılarının da 12-1.8 milyon yıl önce soylarının tükenme noktasına geldiği düşünülmektedir. Günümüzde fosiller, buz tabakalarının arasında varlığını sürdürebilmiş tundra vejetasyonu ile karasal ve tatlı su faunalarının tanımlanmasına izin vermektedir. Bugün gördüğümüz karasal biyota, LGM (Last Glacial Maximum - Son Maksimum Buzul)'den bu yana yerleşik düzene geçen türlerden oluşmaktadır. 1960'lı yılların başlarında Antarktika'da gerçekleştirilen araştırmalar sayesinde özellikle Transantarktika Dağları ve Antarktika Yarımadası'ndaki makro düzeyde biyolojik çeşitliliğin çoğu tanımlanmıştır [13]. Daha yakın tarihli biyolojik çalışmalar karasal biyotanın çoğunun, bu kıtada izole bir şekilde süregelen uzun bir geçmişe sahip olduğuna işaret etmektedir. Biyocoğrafik analizler ise Antarktika'nın LGM ve Gondwana parçalanmasının son evreleri arasında (40 ile 60 milyon yıl önce) Güney Amerika ve Avustralya'dan izole hale geldiğini ve tatlı su kopepodları ile yaşayan akarların, Antarktika'daki evrimsel sürekliliğe uygun dağılıma sahip olduklarını belirlemiştir [14]. Çalışmalar kitlesel olarak, Antarktika Yarımadası ve Doğu Antarktika'da karasal biyotanın halen yaşamını sürdürdüğünü kanıtlamaktadır. Yapılan çalışmalara göre kanatsız Chironomidler, 49 ile 68 milyon yıldan beri Güney Georgia, Güney Shetland Adaları ve Antarktika Yarımadası'nda bulunmaktadır. Buzul barınaklarında, nematod faunası ve toprak ile ilişkili mikroorganizma çeşitliliği yoğun olarak bulunurken Cladosera, rotiferler ve diatomlar ise en az 130.000 yıldan beri Antarktik göllerde yaşamaktadır [14]. Ayrıca toplu olarak nototeniyoidler olarak adlandırılan türler de Antarktika'yı çevreleyen kıtasal buz sahanlıklarındaki balık faunası biyokütlesinin yaklaşık % 90'ını oluşturur. Bu canlıların, bir antifriz görevi gören glikoproteine sahip oldukları için Güney Okyanusu'nun donma noktasının altındaki buzlu sularında hayatta kalabildiği bildirilmiştir [15].

Kıta ekosisteminde yaşayabilen kuşlar ve su canlılarının başında penguenler ve fok balıkları gelmektedir. Güney Okyanusu'nun çeşitlilik içeren su yaşamı; balina ve diğer memeli deniz canlılarını kapsamaktadır.

Antarktik florası incelendiğinde ise kıtada hüküm süren aşırı soğuklar, birçok liken ve yalnızca birkaç yosun türü ile birlikte çiçek açan iki bitki türünün yaşamasına izin verir. Deniz ekosistemleri ise sığ kıyı bölgelerinden açık okyanusun derinliklerine ve buz ile kaplı bölgelerden buzsuz sıvı ortamlara kadar birbirinden çeşitli habitatlara sahiptir [13].

Bitki Çeşitliliği

Arktikte 400'e yakın bitki türü sayılabilmektedir. Buna karşın Antarktika sadece çiçekli iki bitki türüne sahiptir [24]. Bu kıta ayrıca özellikle düşük sıcaklıklara ve kuraklığa tolerans gösteren alt bitki gruplarına da (yosunlar, ciğer otları, likenler ve funguslar) ev sahipliği yapmaktadır. Bu ilkel yaşam formlarıyla beraber, bitki ve hayvanlar da kıtadaki yerlerini almışlardır [16] .

Deschampsia antarctica Desv. (Antarctic hairgrass; Antarktika çayırsaçı)

Poaceae ailesinin bir üyesi olan ve 2n=26 kromozoma sahip olan *Deschampsia antarctica* Desv. Antarktika'da bulunan iki çiçekli bitkiden monokotil olanıdır [17]. *Deschampsia antarctica* en zor çevre koşullarına (aşırı düşük sıcaklık, kuraklık, yüksek tuzluluk, sel, yüksek UV radyasyonu ve düşük yağış) başarıyla adapte olmuştur. Doku kültürü, genetik, morfolojik, fizyolojik, biyokimyasal ve moleküler düzeylerde olmak üzere farklı biyoteknolojik çalışmalarda yaygın olarak kullanılan bir bitkidir [18,19,20,24,26,33,34,35].

Romero ve ark. (1999), yaptıkları çalışmada Antarktika'da yetişen *D. antarctica* örneklerinin *in vitro* koşullarında yetişenlere kıyasla (2 °C ve 13 °C'de) küçük epidermal hücre boyutu, yüksek hücre yoğunluğu, karmaşık hücre formu, kalın kütikül yapısı, yüksek stoma yoğunluğu, fazla yaprak kalınlığı ve küçük lümen damarları gibi anatomik özelliklerinin farklı olduğunu göstermiştir [21]. Bu bitki, ayrıca ekstrem soğuk ve kuru koşullara tolerans göstererek donma noktasında fotosentez yapabilir. Maksimum fotosentetik aktivitenin 13 °C'de gerçekleştiği ve 0 °C'de maksimal fotosentezin % 30'unun korunduğu bildirilmiştir [22].

Deschampsia antarctica donma toleransı mekanizmasının bir parçası olarak büyüme döneminde antifriz proteinleri üretmekte ve yapısal olmayan karbonhidratları biriktirmektedir [21]. RI (Recrystallisation Inhibition - Buz Kristalizasyonunu Engelleme) aktivitesi; bitkiyi, buz kristallerinin zararlı etkilerine karşı korumakta, Antarktika çimlerinin hayatta kalmasını ve donma toleransını sürdürmesini

sağlamaktadır. Bahsi geçen özellikler bu bitkinin stres toleransı ile ilgili genler açısından önemli ve değerli bir genetik kaynak olduğunu göstermektedir [23]. Çeşitli araştırmalar, *D. antarctica*'nın abiyotik etmenlere (özellikle soğuğa karşı) geliştirdiği savunma mekanizmalarının bazılarına ışık tutmuştur. Byun ve ark. (2015) yaptıkları çalışmada, *D. antarctica* C repeat binding factor 7'yi (DaCBF7), monokot grubu V CBF homologlarının bir üyesi olarak tanımlamıştır. Bitkilerde donmaya karşı adaptasyondan sorumlu mekanizmaların araştırılması için *D. antarctica* bitkisinin, model bir organizma olarak kullanılabileceği belirtilmiştir [23]. Ayrıca bu bitkinin; değerli tarımsal mahsullerde üreme stratejilerinin geliştirilmesine izin veren, stres toleransı ve çevresel adaptasyonla ilişkili olan bir gen kaynağı olarak kullanılabileceği de öne sürülmektedir [24]. Bu amaçla, John ve ark. (2009), bir antifriz geni olan *DaIRIP4* genini *Arabidopsis thaliana* bitkisine aktarmış ve bu genin soğuk iklimli yaşam alanlarında buz kristalizasyonunu engelleyici aktiviteyi meydana getirmek için yeterli olduğunu rapor etmişlerdir [25]. Ayrıca *D. antarctica*'nın farmasötik amaçlı kullanılan ekstraktlarının, UV radyasyonuna karşı koruyucu etkiler gösterdiği de bilinmektedir [24,26].

Canlılar âleminde bitkiler, çoğu zaman zengin bir mikroorganizma çeşitliliği ile iletişim ve etkileşim halindedir. Bakteriler, bitkilerin toprak üstü ve toprak altı organlarının yüzeylerinde olabildiği gibi doku içinde de görülebilir. Bu bakterilerden olan ve bitkilerin iç dokularında yer alan mikroorganizmalara endofit bakteriler adı verilir [27,28]. Podolich ve ark. (2019), *D. antarctica* bitkisini, Antarktika'nın birbirinden uzak bölgelerinden toplayarak bakteriyel etkileşimleri incelemişlerdir. Endofik bakterilerden olan *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Firmicutes, Cytophaga-Flavobacteria* ve *Actinobacteria* bakterilerini tespit etmiş ve bunlar arasında en fazla bulunanın ise *Pseudomonas* olduğunu rapor etmişlerdir [29,30,31,32].

Colobanthus quitensis (Antarktika pearlwort; Antarktika karanfil otu)

Colobanthus quitensis karanfiller ailesinden Antarktika'da doğal olarak yetişen dikotil bir bitkidir. Ekstrem şartlarda yaşayan model bir bitki olan *C. quitensis*, 2n=80 kromozoma sahiptir ve genom büyüklüğü 1.95 pg'dir [36,37].

Bu bitki, Antarktika biyomu içinde sadece Antarktika yarımadasında ve Antarktika sınırları içinde yer alan adalarda bulunmaktadır [40]. Bununla birlikte bu bitkinin

dağılım coğrafyası ise Andes bölgesinin bir kısmını ve Falkland adalarını içine alan Sub-Antarktika'yı kapsamaktadır. Bu bitki, kendi kendine döllenen ve kendine tozlanan bir bitkidir [41,42,44,45]. Antarktika'nın koşullarına karşın bu bitki hemen hemen her yıl bolca çiçek açmakta, tozlaşma ve döllenmeden sonra tohum üretmektedir. Tohumlarda çevre koşullarına bağlı olarak kayıplar yaşanmaktadır [38,39,46, 53,54,55].
Deschampsia antarctica bitkisinde olduğu gibi *C. quitensis* bitkisinde de farklı alanlarda çalışmalar yapılmaktadır. Cuba Díaz ve ark. [43], *C. quitensis* ile ilgili populasyonlar arasında potansiyel farklılıklar göz önüne alınarak çimlenme ve çimlenme öncesi çalışmalar yapmış ve asitle muamele etmenin çimlenme üzerindeki olumlu etkilerini bildirmişlerdir [49,50,51,52]. Elde edilen bulgular ile tüm *C. quitensis* populasyonlarına uygulanabilen etkili protokoller oluşturulmuştur. Hughes [47] ise küresel ısınma ile ilgili araştırmalarında kutup bölgeleri ve benzeri bitki örtüsünü çalışmıştır. Antarktika kıtasına özgü *D. antarctica* ve *C. quitensis* bitkilerini ve küresel ısınma etkileşimlerini incelemiştir. Yaptığı çalışmanın sonucunda isimleri geçen iki damarlı bitkide 0 °C'nin, bu bitkilerin fiziksel büyümesini etkilediğini ve sınırlandırdığını rapor etmiştir. Ayrıca bu bitkilerde, özellikle soğuk direnci yaygın olarak çalışılmıştır ve ticari olarak uygulamaları değerlendirilmiştir [48,56]. Zuniga ve ark. (2009) ise *C. quitensis* bitkisi ile ilgili *in vitro* şartlarda yaptığı mikroçoğaltım çalışmasında temel doku kültürü parametrelerini belirlemiştir. Bu çalışma ile iki ay içinde bitki sayısının yaklaşık dört ile beş kat arttığı rapor edilmiştir. Bu metot, Antarktik gen kaynaklarının *in vitro* koşullarda çoğaltılmasını, muhafaza edilmesini ve gelecek çalışmalar için erişilebilirliğini sağlayabilir [59].
Yukarıda belirtilen farklı araştırmalar olmak üzere birçok çalışmada Antarktika kaynaklı bitkilerin genetik materyalleri, ekstem koşullara toleransta rol oynayan adaptasyon mekanizmaları ve bu mekanizmaların ürünü olan metabolik bileşikler yaygın olarak incelenmektedir. Elde edilen sonuçların tarım ve sanayi sektörünün gelişmesine katkıda bulunabileceği ve ilgili konularda yeni ufuklar açabileceği vurgulanmaktadır.

Mikroorganizma Çeşitliliği

Kutup toprakları; doğrudan kuvvetli rüzgarlara, ekstrem düşük sıcaklıklara, kışın düşük yazın yoğun UV radyasyonuna bununla birlikte düşük seviyeli yağışlara maruz kalmaktadır [60]. Bu ekstrem çevre koşullarına karşın Antarktik tundra topraklarının

diğer biyomlara benzer hatta daha fazla mikrobiyal çeşitlilik barındırdığı bilinmektedir [61]. Bu durum, ekosistemdeki biyolojik aktivitelerin çoğunun mikroorganizmalar tarafından kontrol edildiğini göstermektedir [62]. Her ne kadar kutupsal mikroorganizmalar iklim koşullarından dolayı düşük mikrobiyal aktivite gösterselerde besin döngüsündeki rolleri yine de önemlidir [63]. Bu mikroorganizmalar besin ağının temelini oluştururlar ve biyojeokimyasal döngülerde biyoçözünürlük gibi önemli görevleri vardır [64, 65]. Bununla birlikte ekolojik önemlerine karşın Antarktika mikrobiyal çeşitliliği ve bu çeşitliliğin coğrafik dağılımı hakkında yeterince bilgi bulunmamaktadır [66, 67]. Sistematik örnekleme bulunmaması, coğrafik izolasyon ve özellikle Antarktika'ya ulaşabilirlik gibi problemlerden dolayı mikrobiyal biyoçeşitlilik hakkında yapılan çalışmalar, teknolojinin gelişmesiyle beraber artmıştır [68,69,70,71,72] .

Bu biyolojik çeşitliliğe verilecek örneklerden biri bazı göllerin organik karbon kaynağının çok az olması veya çok fazla olması ya da fosforca zengin olması gibi özelliklerinden dolayı, metanol, azot ve fosfor parçalayan bakterileri bünyelerinde barındırmasıdır [73,74]. Farklı olarak Yergeau ve ark. (2007) tarafından elde edilen bulgular, büyük ölçekli biyocoğrafik bölgelerde azalan mikrobiyal çeşitliliği göstermektedir [75]. Ancak, Kara Antarktikası dışında kalan bazı habitatlarda hiçbir azalma durumu yoktur, tam tersine bu habitatlarda bulunan fungus çeşitliliği üzerine yapılan birçok olumlu çalışma bulunmaktadır [76, 77]. Ayrıca, Antarktika'daki stabil deniz ortamlarındaki habitatlarda da mikroorganizma çeşitliliğinde hiçbir azalma durumu söz konusu değildir. Antarktika topraklarındaki bakteri çeşitliliği ile ilgili daha fazla çalışma yapıldığı için diğer canlılara kıyasla bakterilerin çok daha çeşitli ve sayıca fazla olduğu bildirilmiştir [78, 79, 80, 81]. Bununla beraber, Antarktika'da mikroorganizmaların biyocoğrafik çeşitliliği üzerine yapılan çalışmalar hala başlangıç seviyesindedir.

Gloeocapsa cinsi, kuru vadilerin kayalıklarındaki ekstrem koşullara yüksek adaptasyon gösteren az sayıdaki kriptoendolitik taksondan biridir. *Arthrobacter* spp., *Brevibacterium* spp. ve *Corynebacterium* spp. gibi aktinobakteriler, Antarktika'nın kuru vadilerinde öne çıkmaktadır [81]. Termofilik bakteriler ise 'Kuzey Victoria Land''daki 'Mt. Rittman' ve 'Mt. Melbourne' yakınlarındaki termal olarak ısıtılmış sıcak topraklardan izole edilmiştir [82]. Antarktika; *Staphylococcus*, *Bacillus*,

Corynebacterium, *Micrococcus*, *Streptococcus*, *Neisseria* ve *Pseudomonas* cinsine ait bakterileri de bünyesinde barındırmaktadır [83]. Yürütülen araştırmalarda tanımlanmış bakteri türleri şu şekildedir; *Acinetobacter* spp., *Alicyclobacillus acidocaldarius*, *Aquaspirillum* spp., *Arthrobacter* spp., *Azospirillum* spp., *Bacillus* spp., *Bacillus fumarioli*, *Bacillus thermoantarcticus*, *Bizionia argentinensis*, *Brevibacterium* spp., *Brevibacterium antarcticum*, *Brevundimonas* spp., *Chryseobacterium* spp., *Corynebacterium* spp., *Flavobacterium* spp., *Gloeocapsa* spp., *Hymenobacter roseosalivarius*, *Leptolyngbya frigida*, *Massila* spp., *Micrococcus* spp., *Modestobacter multiseptatus*, *Neisseria* spp., *Nocardia* spp., *Nostoc commune*, *Paenibacillus* spp., *Planococcus* spp., *Pseudonocardia antarctica*, *Pseudomonas* spp., *Psychrobacter* spp., *Sphingobacterium* spp., *Staphylococcus* spp., *Stenotrophomonas* spp., *Streptococcus* spp., ve *Streptomyces* spp. [82]. Bu mikroorganizmaların birbirinden farklı işlevleri bulunmaktadır. Azot döngüsünde önemli rolleri olan *Planctomycetes* mikroorganizması, azot üretimine yol açan amonyumun anaerobik oksidasyonuna katılır. Deniz ve tatlı su ortamlarında genellikle daha fazla bulunurlar. Bununla birlikte kutup toprakları da dahil olmak üzere karasal ortamlarda değişken miktarlarda tespit edilmiştir [84].

Bunların yanı sıra III. Ulusal Antarktik bilimseferi kapsamında, Galindez adasından alınan su örneklerinden literatürde ilk defa pestisitleri parçalayan bakteriler tanımlanmıştır. Bu bakteriler; *Psychrobacter* sp. strain *TaeBurcu001* (Aksesyon numarası MN061637.1) ve *Psychrobacter* sp. strain *TaeBurcu002* olarak isimlendirilerek NCBI’a (Aksesyon numarası MN960390.1) yüklenmiş ve literatüre kazandırılmıştır [85]. Ayrıca bu bakteri türlerinin dışında, III. Ulusal Antartik bilimsefer kapsamında, ‘Horseshoe’ Adası’ndan alınan buz örneklerinden literatürde ilk defa Antarktika’dan izole edilmiş *Blastomonas sp.* strain YTU.POLAR.001 (Aksesyon numarası MN384971) ve *Achromobacter sp.* strain YTU.KUTUP.001 (Aksesyon numarası MN396385) isimli bakteriler de NCBI’a yüklenerek literatüre kazandırılmıştır [86,87].

Arkea ve fungal toplulukları da kutup mikrobiyal topluluklarının önemli parçalarıdır. Fungal toplulukların kutuplarda varyasyon göstermesine karşın genellikle *Ascomycota* ve *Basidiomycota* diğer varyetelere kıyasla daha baskın olan karasal ayrıştırıcılardır. Bununla birlikte bölgede bollukları, çeşitlilikleri ve dağılımları çalışılmaya devam edilmektedir [88].

Kutuplarda sürekli donmuş olan topraklar (permafrost), Dünya'nın kara yüzeyinin önemli bir alanını kapsamaktadır. Kuzey Kutbu ve Antarktika bölgelerindeki permafrost çökeltilerinde bulunan bazı mikroorganizmaların, uzun süreler boyunca hayatta kalabildiği gösterilmiştir [89]. Permafrost özellikli bölgelerden çok sayıda soğuğa dirençli mikroorganizmanın bulunduğu bilinmektedir [90]. Permafrost alanlar sadece bir bakteri deposu değildir, aynı zamanda arkeler [91], maya [92], aktinomiset ve mikromisetler de [93] içerir. Bunlarla beraber farklı yaş ve kökene sahip permafrost yüzeylerden alınan örneklerde canlı protozoanlar bulunmuştur [94].
Ayrıca bu mikroorganizma türlerinin yanısıra III. Ulusal Antarktik bilim seferi kapsamında toplanan örneklerde yeni mikroorganizma ırkları da izole edilmiştir. Bu mikroorganizmalar üzerinde genomik DNA izolasyonundan sonra 18S rRNA analizleri yapılmıştır. Her bir izolat için elde edilen dizi analiz sonuçları, NCBI veri tabanında bulunan suşlara ait diziler ile karşılaştırılmıştır. Elde edilen bu ırklara ait diziler, NCBI veri tabanına kayıtı yapılarak aksesyon numarası alınmıştır. Özellikle kutuplardan alınan örneklerden literatürde ilk defa Antarktika'dan izole edilmiş *Paracercomonas* sp. strain TAE3-YTU.004 (Aksesyon numarası MW485507.1) (95), *Flamella arnhemensis* strain TAE3-YTU.007 (Aksesyon numarası MW485950.1) (96), *Flamella balnearia* strain TAE3-YTU.005 (Aksesyon numarası MW487484.1) (97), *Flamella arnhemensis* strain TAE3-YTU.006 (Aksesyon numarası MW513457.1) (98), *Paracercomonas* sp. strain TAE3-YTU.008 (Aksesyon numarası MW521096.1) (99) olarak NCBI'a yüklenmiş ve literatüre kazandırılmıştır.

Mikrobiyal Çeşitliliğin Potansiyel Kullanım Alanları

Kutupsal çevre koşullarına karşın Güney Kutbu topraklarında yaşayan mikroorganizmaların hepsi ekstremofilik değildir. Psikrofiller soğuk ortamlarda yaşarlar ve bu organizmaların çoğu 0 °C'nin altındaki düşük sıcaklıklarda hayatta kalabilirler ancak daha yüksek sıcaklıklarda da optimum şekilde büyüyebilirler [100]. *Pseudomonas*'ın *B17* ve *B18* suşları, optimum büyüme sıcaklığı 25 °C olmasına karşın 0 °C ile 30 °C arasında da büyüyebilmektedir. Ayrıca, 5 °C'de metabolik olarak aktiftirler ve hem alkanları hem de naftaleni petrol hidrokarbonundan bozabilirler [101]. Soğuğa adapte olan bazı organizmalar gıda endüstrisinde mikrobiyal kontaminasyonu önlemek, hücre dokularının kriyoprezervasyonunu artırmak ve dondurulmuş gıdaların dokusunu ve lezzetini korumak için yaygın olarak kullanılan antifriz proteinleri

üretebilmektedir [102]. Bu proteinler, fungus gibi mikroorganizmalarda ve az sayıda bakteri türünde özellikle de Antarktika göllerinden izole edilen Gammaproteobakteriler de tanımlanmıştır. Ayrıca, bazı mikroorganizmalar düşük sıcaklıklarda mezofilik enzimlere göre daha aktif ve kararlı olan ve böylece düşük sıcaklıklı endüstriyel işlemler için kullanılabilecek soğuk aktif veya psikrofilik enzimler de üretebilmektedir [103] .

Bununla beraber son yıllarda antibiyotiklerin çoğuna dirençli hale gelen suşların artması nedeniyle mikrobiyal çeşitliliğin tıbbi amaçlar doğrultusunda incelenmesi de potansiyel anlamda bir öncelik haline gelmiştir. Mikrobiyal çeşitliliğin yoğun olduğu noktalarda, mikroorganizmalar bir arada yaşadıkları ve özellikle enerji kaynakları konusunda birbirleriyle rekabet edebilecek yollar geliştirdikleri için bu noktalarda tıbbi araştırmalara öncelik verilmektedir. Funguslar ve bakteriler; birçok ekstrem ortamda tanımlandıkları ve bu ortamların zorlu koşullarında hayatta kalmak ve gelişmek amacıyla özel yöntemler geliştirdiklerinden potansiyel farmasötik uygulamaları olan antibiyotikler, antitümör ilaçlar veya kolesterol düşürücü ilaçlar gibi yeni biyoaktif metabolitler için zengin bir kaynak oluşturmaktadır [88, 104,105]. Antarktika süngerleri ile ilişkili bazı fungus türlerinin de antimikrobiyal ve antitümoral aktivitelere sahip olduğu gösterilmiştir [106]. *Actinomycetes* dünya çapında en önemli antibiyotik kaynaklarından biridir ve Arktik *Actinomycetes* biyoprospektif için güçlü bir potansiyele sahiptir [107, 108]. *Streptomyces* spp. organizmasının Doğu Sibirya sedimentlerinden izole edilen *ART5* suşunun *Candida albicans*'a karşı inhibe edici aktivite gösterdiği bildirilmiştir [109].

Bunların yanısıra, biyoremidasyon için de Antarktik mikroorganizmaların kullanılabilme potansiyelleri vardır. Günümüzdeki sanayileşmiş ve sanayileşmekte olan devletlerin ekonomilerinde sentetik kimyasallara dayanan sanayilerin katkısı önemlidir. Bu sanayilerde hammaddelerin işlenmesinde kısa sürede ekonomik olarak kaliteli bir tüketici ürünü elde etmek için ucuz ve biyolojik olarak çözünmeyen birbirinden farklı kimyasal maddeler kullanılmaktadır. Bu kimyasallar zaman içinde canlılara ve doğaya zarar vermektedir. Bunlardan kurtulmak için en yeni ve güvenilir yöntemlerden biri ise biyoremidasyondur. Biyoremidasyon canlı organizmaları kullanarak kirliliğin temizlenmesi anlamındadır. Özellikle pestisitler gibi kimyasallar, tarım arazileri başta olmak üzere ormanları ve doğamızı tehdit etmektedir. Bu kirliliklerden kurtulmak için

birçok mikroorganizma tuz gölleri [110], bazik göller [111] ve tarım alanları [112] gibi birbirinden farklı lokasyonlardan izole edilerek biyoremidasyon için kullanılmıştır. Kutup çalışmaları ile birlikte kutup bölgelerinden potansiyel türler izole edilip, pestisitler gibi kirleticilere karşı biyolojik mücadele ile doğa daha temiz hale getirilebilmektedir [85].

Kutup Algleri

Antarktika'nın sınırlı karasal ekosistemlerinde, tüm fotosentetik organizmalar yaşam alanlarının ekolojisine önemli bir katkıda bulunmaktadır. Buzsuz zemin, Antarktika kıtasının sadece % 0.18'ini oluşturmaktadır. Ancak, fotosentetik yaşam bu alanla sınırlı değildir. Kıyı bölgelerde gerçekleşen alg patlamaları dolayısıyla belirli bölgelerde yeşil ve kırmızı renkler görülmektedir. Antarktika'daki kar algleri, ilk olarak 1950'lerde ve 1960'larda yapılan keşiflerle tanımlanmıştır ve o zamandan beri Antarktika'da bulunan çeşitli alg türleri incelenmektedir. Tek bir kar alg patlamasının binlerce metrekareyi kapsayabileceği düşünüldüğünde kar algleri potansiyel olarak bölgenin en önemli fotosentetik birincil üreticilerinden biridir. Bununla birlikte karasal ve deniz ekosistemlerine besin sağlanmasını da etkilemektedir. Son yıllarda küresel ısınmanın sonucu olarak, Antarktika Yarımadası'ndaki ısınma sanayi öncesi sıcaklıklara göre 1.5 °C'yi aşmıştır. Bu bakımdan özellikle kar alglerinin Antarktika'nın biyosferine nasıl uyum sağladığını ve küresel ısınmaya karşı olası tepkilerini anlamak, iklim değişikliğinin Antarktika'nın bitki örtüsü üzerindeki genel etkisini inceleme açısından çok önemlidir [113, 114].

Algal hücrelerin konsantrasyonu, binlerce hücrelik bir popülasyon mL^{-1} değerine ulaştığında, kar veya buzda renk değişikliği meydana gelir. Renk ve yoğunluğu, pigment kompozisyonuna ve popülasyon yoğunluğuna bağlıdır. Alg patlamaları ortam koşullarına göre baskın olarak içerdikleri pigment ve metabolitlerine göre kırmızı, yeşil ve sarı-kahverengi renklerde olabilir. Örneğin klorofil baskın olduğunda yeşil kar görülürken, fukoksantin gibi birincil karotenoidler baskınsa, altın-kahverengi kar ortaya çıkabilir [115].

Arktik ve Antarktik'te deniz buzu içinde en bol bulunan mikroalgal taksonlar diatomlardır (*Bacillariophyceae*). Sadece Kuzey Kutbu'nda 550'den fazla diatom türü tanımlanmıştır. Kuzey Kutbu'nda; *Fragilaria, Cylindrotheca* ve *Achnanthes* nispeten yaygın tek hücreli diatom cinsleridir, Antarktika'da ise *Amphiprora, Pinnularia,*

Pleurosigma, Synedra ve *Tropidoneis* türlerinin varlığı bildirilmiştir. Algler en çok birinci yıl buzunda, dip buzda, deniz buzunun iç kısmındaki boşluk katmanlarında ve buz boşluklarında görülmektedir. Deniz buzu içindeki mikroalgal patlama genellikle oldukça kısa ömürlüdür. Alg biyokütlesi, yetersiz ışık, düşük sıcaklık ve yüksek deniz buzu tuzluluğu nedeniyle kışın genellikle düşüktür. Alg konsantrasyonları ışık ve sıcaklık arttıkça ve tuzluluk azaldıkça ilkbaharda hızla artar [116].

III. Ulusal Antarktik bilim seferi kapsamında Antarktika'dan yeni alg suşları da izole edilmiştir. Özellikle kutuplardan alınan örneklerden literatürde ilk defa Antarktika'dan izole edilmiş *Chlorella variabilis* strain YTU.ANTARCTIC.001 (Aksesyon numarası MN372092) [117], *Auxenochlorella pyrenoidosa* strain Ozcimen.001 (Aksesyon numarası MT951391) [118], *Chlorella sorokiniana strain* Egemen.001 (Aksesyon numarası MW147167.1) [119] olarak NCBI'a yüklenerek literatüre kazandırılmıştır.

Mikroalgler, suyun bulunduğu her ortamda çoğalabilen, türlerine ve yetiştikleri ortam koşullarına göre değişik oranlarda yağ, protein ve karbonhidrat içeren bitki benzeri mikroskobik canlılardır. Bu mikroorganizmalar üstün adaptasyon mekanizmaları sayesinde Antarktika gibi ekstrem koşullara bile dayanabilmektedir. Antarktik mikroalgler, içerdikleri yüksek oranda yağ, protein ve karbonhidrat sayesinde gıda, hayvan yemi ve enerji alanlarında kullanılabilmektedir. Bu içeriklerin yanı sıra bu mikroalgler, bulundukları ortamın koşullarına adapte olabilmek için hücre içi ve hücre dışına fukoksantin, astaksantin, beta-karoten, klorofiller, çoklu doymamış yağ asitleri, steroller ve peptidler gibi birçok değerli metabolit sentezler. Bu intraselüler ve ekstraselüler metabolitler; antibiyotik, antiviral, antikanser, antifungal, antibakteriyal, antiinflamatuar ve hipokolestrolemik özellik göstermektedir. Kutup mikroalgleri bu özellikleri ile ilaç alanındaki biyoteknolojik uygulamaların yanısıra kozmetik, gıda ve gıda katkı maddesi, gübre, hayvan yemi ve enerji alanlarında da kullanılabilmektedir [120, 121, 122, 123].

Sonuç

Gezegenimizin geçmişine ait bilgiler bir saklı hazine gibi Antarktika'da bulunmaktadır. Dünyamızın özellikle kirlilik ve küresel ısınma gibi küresel sorunlarını çözmede bu kıta insanlığa yardımcı olacaktır. Dünya tatlı su kaynaklarının % 70'ini barındıran Antarktika, canlı yaşamı ve canlıların geçirdiği süreçleri öğrenme bakımından çok önemlidir. Özelllikle son yüzyılda Dünyamızın en büyük problemlerinden olan küresel

ısınma, çevre kirliliği ve çeşitli sağlık problemlerini çözmede Antarktika kıtasında araştırılan bitki, alg ve mikroorganizmalar fayda sağlayacaktır. Örnek olarak tarım arazilerinde kullanılan çeşitli sentetik kimyasal yapılı pestisitlerin topraktan temizlenmesi ve azaltılmasında Antarktika kaynaklı mikroorganizmalardan faydalanılabilmektedir. Aynı zamanda kutup algleri başta olmak üzere kıta üzerinde yaşayan canlılardan çeşitli faydalı ekstraktlar çıkartılarak bunların teröpatik etkileri sayesinde bazı hastalıkların tedavisinde kullanılabilecekleri düşünülmektedir. Asya ve Avrupa kıtasının keşisme noktasında bulunan ve sanayileşmekte olan Türkiye, kirlilik ve küresel ısınma gibi sorunlardan etkilenmektedir. Bu yüzden Antarktika temelli çalışmalardan elde edilen sonuçlar ile sorunlarımızın çözümüne yönelik projeler oluşturulmalıdır. Antarktika çalışmaları ülkemiz için stratejik ve ulusal öncelikli alanlardan olup Türk bilim insanlarının Antarktika ile ilgili araştırmalara yönlendirilmesi, bu konudaki bilimsel ve teknolojik yetkinliğin artırılmasının sağlanması ve bu konularda daha fazla bilimsel çalışmaların yapılması için desteklerin sunulması büyük önem arzetmektedir.

Teşekkür
Tüm yazarlar; Cumhurbaşkanlığı himayesinde, Sanayi ve Teknoloji Bakanlığı uhdesinde ve İTÜ Kutup Araştırmaları Uyg-Ar Merkezi koordinasyonunca gerçekleştirilen TAE-III seferinde desteklenen projelerinden dolayı teşekkürlerini sunar. Prof. Dr. Didem Özçimen, yapmış olduğu kutup çalışmalarına desteklerinden dolayı TÜBA-GEBİP Ödülü kapsamında Türkiye Bilimler Akademisi (TÜBA)'ne teşekkürlerini sunar.

Kaynaklar

1. WorldAtlas 2018. https://www.worldatlas.com/articles/what-are-the-origins-of-the-names-arctic-and-antarctica.html
2. Liddell, H. G., Scott, R., Crane, G., Stuart Jones, H. and McKenzie, R., A Greek-English Lexicon Antarctica. Oxford: Clarendon Press, 2002.
3. Encyclopædia Britannica 2019. https://www.britannica.com/place/Antarctica
4. World Geography 2019. https://www.enchantedlearning.com/geography/continents/Land.shtml
5. Scott K. N., Ice and Mineral Resources. In: Liggett D., Storey B., Cook Y., Meduna V. (eds) Exploring the Last Continent. Springer Cham., 2015.
6. Burcu Ö, Küresel Salgınlar Kutup Bölgelerinde Öğreneceklerimiz ve Geleceğimiz. Küresel Salgının Anatomisi: İnsan ve Toplumun Geleceği. 2020, Tuba Bilimler Akademisi. p:1019-1029, 978-605-2249-46-8.
7. Encyclopedia 2020. https://www.encyclopedia.com/people/history/explorers-travelers-and-conquerors-biographies/james-cook Erişim tarihi: [08.02.2021]
8. Ingrıd, H., et al., Early knowledge of Antarctica's vegetation: Expanding past and current evidence. Revista Chilena de Historia Natural, 2012. 85: p.409-418.
9. Harris, CM. and J. Meadows, Environmental management in Antarctica Instruments and institutions. Marine Pollution Bulletin, 1992. 25(9-12): p.239–249.

10. Stewart, J., Antarctica an Encyclopedia, Second edition. McFarland & Company Inc., North Carolina, U.S.A., 2011.
11. Barrett, J. E., et al., Variation in biogeochemistry and soil biodiversity across spatial scales in a polar desert ecosystem. Ecology, 2004. 85: p.3105-3118.
12. Thomas, D. N. et al., The biology of polar regions. Oxford University Press, Oxford, UK, 2008.
13. Convey, P. et al., The spatial structure of Antarctic biodiversity. Ecological monographs, 2014. 84, p. 203-244
14. Convey, P. and Stevens, M. I., Antarctic biodiversity. Science, 2007. 317 (5846): p.1877-1878.
15. Gilbert, J. A. et al., Demonstration of antifreeze protein activity in Antarctic lake bacteria. Microbiology, 2004. 150(1): p.171-180.
16. British Antarctic Survey 2020. https://www.bas.ac.uk/about/antarctica/wildlife/plants/ Erişim tarihi: [04.01.2021].
17. Amosova, A. V. et al., Molecular Cytogenetic Analysis of Deschampsia antarctica Desv. (Poaceae), Maritime Antarctic. PloS one, 2015. 10(9).
18. Ruhland, C. T. et al., The influence of ultraviolet-B radiation on growth, hydroxycinnamic acids and flavonoids of Deschampsia antarctica during Springtime ozone depletion in Antarctica. Photochemistry Photobiology, 2005. 81(5): p.1086-1093.
19. Pascual-Díaz, J.P., Serçe, S., Hradecká, I. et al. Genome size constancy in Antarctic populations of Colobanthus quitensis and Deschampsia antarctica. Polar Biol 43, 1407–1413 (2020). https://doi.org/10.1007/s00300-020-02699-y
20. Lee, J., et al., Combined analysis of the chloroplast genome and transcriptome of the Antarctic vascular plant Deschampsia antarctica Desv. PLoS One, 2014. 9(6): e101100.
21. Romero, M., et al., Leaf anatomy of Deschampsia antarctica (Poaceae) from the Maritime Antarctic and its plastic response to changes in the growth conditions. Revista Chilena de Historia Natural, 1999. 72(1):p. 411-425.
22. Barcikowski, A., et al., *Deschampsia antarctica* (Poaceae)–the only native grass from Antarctica. L. Frey (Ed.), Studies on Grasses in Poland, W. Szafer Institute of Botany, 2001. PAS, Kraków. pp. 367-377
23. Byun, M., et al., Constitutive expression of DaCBF7, an Antarctic vascular plant Deschampsia antarctica CBF homolog, resulted in improved cold tolerance in transgenic rice plants. Plant Science, 2015. 236(1)p: 61-74.
24. Amosova, A., et al., Molecular cytogenetic analysis of *Deschampsia antarctica* Desv.(Poaceae), maritime Antarctic. *PloS One, 2015.* 10(9) p: e0138878.
25. John, U., et al., Ice recrystallization inhibition proteins (IRIPs) and freeze tolerance in the cryophilic Antarctic hair grass Deschampsia antarctica E. Desv. Plant, Cell & Environment, 2009. 32(4)p: 336-348.
26. Rabert, C., et al., Expression of *Deschampsia antarctica* Desv. polypeptide with lipase activity in a *Pichia pastoris* vector. International Journal of Molecular Sciences, 2014. 15(2) p:2359-2367
27. Kucuk, C., Bitki Probiyotik Bakteriler: Bitkiler Üzerindeki Rolleri ve Uygulamalar. International Journal of Life Sciences and Biotechnology, 2019. 2(1): p.1-15.
28. Kucuk, C. and A. Almaca, Bitki gelişimini teşvik eden rizobakteriler tarafından üretilen metabolitler ve bitki gelişimine etkileri. International Journal of Life Sciences and Biotechnology, 2020. 3(1): p.81- 94.
29. Podolich, O., et al., First record of the endophytic bacteria of Deschampsia antarctica E. Desv. from two distant localities of the maritime Antarctica. bioRxiv, 2019. p: 1-15
30. Mühling, M., Woolven-Allen, J., Murrell, J.,C, Improved group-specific PCR primers for denaturing gradient gel electrophoresis analysis of the genetic diversity of complex microbial communities. The ISME Journal, 2008. 2(1):p. 379-392
31. Park, M, et al., Endophytic bacterial diversity of an Antarctic moss, Sanionia uncinata. Antarctic Science, 2013. 25(1):p 51-54.

32. Podolich, O., et al., Reviving of the endophytic bacterial community as a putative mechanism of plant resistance. Plant Soil, 2015. 388(1):p. 367-377.
33. Cuba, M. et al., Micropropagation of *Deschampsia antarctica* a frost-resistant Antarctic plant. Antarctic Science, 2005. 17(1): p.69-70.
34. Osorio, J. et al., The effects of growth regulators and a scanning electron microscope study of somatic embryogenesis in Antartic hair grass (*Deschampsia antarctica* Desv.). Polar Biology, 2014. 37: p.217-225.
35. Marely, C. D. et al., Advances of native and non-native Antarctic species to *in vitro* conservation: improvement of disinfection protocols. Scientific Reports*, 2020.* 10: p.1.
36. Cuba-Díaz, M., et al., Genome size comparison in *Colobanthus quitensis* populations show differences in species ploidy. Polar Biology, 2016., 40(7): p. 1475–1480. doi:10.1007/s00300-016-2058-z
37. Marhold, K., et al., IAPT chromosome data 30/2. Taxon, 2019. 68(5): p.1124-1130.
38. Gielwanowska, I., Bochenek, A. and Szczuka, E., Development of the pollen in the Antarctic flowering plant Colobanthus quitensis [Kunth] Bartl. Acta Agrobotanica, 2007. 60(3):p 3-8.
39. Cuba-Díaz, M., et al., Optimización de parámetros para la propagación in vitro de Colobanthus quitensis (Kunth) Bartl. Gayana Botánica, 2014. 71(1): p 58-67.
40. Zúñiga, G. E. et al., Micropropagation of Antarctic Colobanthus quitensis. Antarctic Science, 2009. 21(2): p.149-150
41. Smıth, R. I. L., Terrestrial plant biology of the sub-Antarctic and Antarctic. In: Laws, R. M., ed. Antarctic biology. Academic Press, 1984. p.61-162.
42. Moore, D. M., Studies in Colobanthus quitensis (Kunth) Bartl.and Deschampsia antarctica Desv. II. Taxonomy, distribution and relationships. British Antarctic Survey Bulletin, 1970. 23: p.63-80.
43. Convey, P., Reproduction of Antarctic flowering plants. Antarctic Science, 1996. 8: p.127-134.
44. Vera, M. L., Colonization and demographic structure of Deschampsia antarctica and Colobanthus quitensis along an altitudinal gradient on Livingston Island, South Shetland Islands, Antarctica. Polar Research, 2011. 30(1): p.7146.
45. Cannone, N. et al., S. Vascular plants changes in extreme environments: effect of multiple drivers. Climatic Change, 2016. 134(4): p.651-665.
46. Convey, P. et al., The spatial structure of Antarctic biodiversity. Ecological Monographs, 2014. 80(2): p.203-244.
47. Hughes L., Biological consequences of global warming: Is the signal already, Trends in ecology & evolution, 2000. 15(2): p.56-61.
48. Bravo, L. A. et al., Cold resistance in antarctic angiosperms. Physiologia Plantarum, 2001. 111: p.55-65.
49. Oldham , P. D. and Kindness, J., Biodiversity Research and Innovation in Antarctica and the Southern Ocean. bioRxiv, 2020.
50. Torres-Díaz M., et al., Biological Interactions and Simulated Climate Change Modulates the Ecophysiological Performance of Colobanthus quitensis in the Antarctic Ecosystem. PLoS ONE, 2016. 11(10): e0164844. https://doi.org/10.1371/journal.pone.0164844.
51. Smith, R. L., Vascular plants as bioindicators of regional warming in antarctica. Oecologia, 1994. 99: p.322-328.
52. Pérez-Torres, E. et al., The role of photochemical quenching and antioxidants in photoprotection of Deschampsia Antarctica. Functional Plant Biology, 2004. 31(7): p.731-741.
53. Rozema, J. et al. The role of uv-b radiation in aquatic and terrestrial ecosystems an experimental and functional analysis of the evolution of uv-absorbing compounds. Journal of Photochemistry and Photobiology B-biology, 2002. 66(2): p.12.

54. Salvucci, M. E. and Crafts-Brandner S. J., Relationship between the heat tolerance of photosynthesis and the thermal stability of rubisco activase in plants from contrasting thermal environments. Plant Physiology, 2004. 134: 1460-1470.
55. Gidekel, M. et al., Identification and characterization of three novel cold acclimation-responsive genes from the extremophile hair grass *Deschampsia antarctica* desv. Extremophiles, 2003. 7: p.459-469
56. German, S., Peter, J. U. And Martina, P. R., Ice recrystallisation inhibition protein or antifreeze proteins from deschampsia, lolium and festuca species of grass. WO, 2005
57. Manuel, G., et al., Plant promoter. US 7273931 B2, 2007. https://lens.org/053-289- 529-443-956
58. Bravo, L. A. and Griffith, M., Characterization of antifreeze activity in antarctic plants. Journal of Experimental Botany, 2005. 56: p.1189-1196.
59. Manuel, G., et al., Agent for cutaneous photoprotection against uva/uvb rays [Internet]. US 8357407 B2, 2013. Available: https://lens.org/004-963- 1699 859-833-206
60. Cuba-Díaz, M., et al., Advances of native and non-native Antarctic species to *in vitro* conservation: improvement of disinfection protocols. Sci Rep, 2020. **10**(3845): p1-10. https://doi.org/10.1038/s41598-020-60533-1.
61. Nemergut, D. R. et al., Structure and function of alpine and arctic soil microbial communities. Research in microbiology, 2005. 156(7): p.775-784.
62. Sabbe, K., E. et al., Benthic diatom flora of freshwater and saline lakes in the Larsemann Hills and Rauer Islands, East Antarctica. Antarctic Science, 2003. 15(2): p.227-248.
63. Convey, P. and Stevens, M. I., Antarctic biodiversity. Science, 2007. 317(5846): p.1877-1878.
64. Jakosky, B. M., et al., Subfreezing activity of microorganisms and the potential habitability of Mars'polar regions. Astrobiology, 2003, 3:2: 343-350.
65. Vincent W.F., Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. Antarctic Science, 2000. 12: p.374-385.
66. Friedmann E.I. (ed), Antarctic microbiology. John Wiley & Sons, 1993.
67. Gibson, J.A.E. et al., Biogeographic trends in Antarctic lake communities; In Trends in Antarctic Terrestrial and Limnetic Ecosystems. Bergstrom, D. M., Convey, P. and Huiskes, A.H.L. (eds.) Springer, (2006a). p.71-99.
68. Simmons, B. L., Long-term experimental warming reduces soil nematode populations in the McMurdo Dry Valleys, Antarctica. Soil Biology and Biochemistry, 2009. 41(10): 2052-2060
69. Aislabie, J. M., Jordan, S. and Barker, G. M., Relation between soil classification and bacterial diversity in soils of the Ross Sea region, Antarctica. Geoderma, 2008. 144: p.9-20.
70. Tytgat, B., et al., Bacterial community composition in relation to bedrock type and macrobiota in soils from the Sør Rondane Mountains, East Antarctica. FEMS Microbiology Ecology, 2016. 92(9).
71. Papale, M. et al., Prokaryotic assemblages within permafrost active layer at Edmonson Point (Northern Victoria Land, Antarctica). Soil Biology & Biochemistry*, 2018.* 123: p.165-179.
72. Koo, H. et al., Metagenomic Analysis of Microbial Community Compositions and Cold-Responsive Stress Genes in Selected Antarctic Lacustrine and Soil Ecosystems. Life, 2018. 8(3): 29.
73. Shivaji, S., Bacterial biodiversity, cold adaptation and biotechnological importance of bacteria occurring in Antarctica. Proceedings of the Indian National Science Academy, 2017. 83: p.327-352.
74. Laybourn-Parry, J., W. Quayle, and T., Henshaw, The biology and evolution of Antarctic saline lakes in relation to salinity and trophy. Polar Biology, 2002. 25(7): p.542-552.
75. Convey, P. et al., The spatial structure of Antarctic biodiversity. Ecological Monographs, 2014. 84: p.203-244.

76. Yergeau, E. et al., Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. FEMS Microbiology Ecology, 2007. 59: p.436-451.
77. Dennis, P. G. et al., Soil fungal community composition does not alter along latitudinal gradient through the maritime and sub- Antarctic. Fungal Ecology, 2012. 5: p.403-408.
78. Niederberger, T. D. et al., Microbial community composition in soils of Northern Victoria Land, Antarctica. Environmental Microbiology, 2008. 10: p.1713-1724.
79. Cary, S. C. et al., On the rocks: microbial ecology of Antarctic cold desert soils. Nature Reviews Microbiology, 2010. 8: p.129-138.
80. Chong, C. W. et al., Patterns in the distribution of soil bacterial 16S rRNA gene sequences from different regions of Antarctica. Geoderma, 2012. 181: p.45-55.
81. Vyverman, W. et al., Evidence for widespread endemism among Antarctic micro-organisms. Polar Science, 2010. 4: 103-113.
82. Cary, S. C. et al., On the rocks: the microbiology of Antarctic Dry Valley soils. Nature Reviews Microbiology, 2010. 8(2): 129.
83. Adams, B. J. et al., Diversity and distribution of Victoria Land biota. Soil Biology and Biochemistry, 2006. 38(10): 3003-3018.
84. Pearce, D. A. et al., Microorganisms in the atmosphere over Antarctica. FEMS Microbiology Ecology, 2009. 69(2): 143-157.
85. Kırkıncı, S. F. (2020). Antarktika kaynakli örneklerden dalapon herbisitini parçalayan bakterilerin izolasyonu, karakterizasyonu ve tanisi. Ondokuz Mayıs Universitesi Yuksek Lısans tezi, s 35
86. Ozcimen, D., et al., Isolation of Blastomonas sp. from Horseshoe Island, Skua Lake, Antarctica, YTU.POLAR.001, MN384971, NCBI GenBank, 28-August-2019
87. Ozcimen,D., et al., Isolation of Achromobacter sp. from Horseshoe Island, Skua Lake, Antarctica, YTU.KUTUP.001, MN396385, NCBI GenBank, 31-August-2019.
88. Humbert, S. et al., Molecular detection of anammox bacteria in terrestrial ecosystems: distribution and diversity. The ISME Journal, 2010. 4(3): 450.
89. Shmakova, L., N. Bondarenko, and A., Smirnov, Viable species of Flamella (Amoebozoa: Variosea) isolated from ancient arctic permafrost sediments. Protist, 2016. 167(1):p. 13–30, http://dx.doi.org/10.1016/j.protis.2015.11.001.
90. Gilichinsky, D. et al,. Bacteria in permafrost. In Psychrophiles: From Biodiversity to Biotechnology; Springer: Berlin/Heidelberg, Germany, 2008. p. 83–102, ISBN 9783540743347.
91. Rivkina, E., et al., Biogeochemistry of methane and methanogenic archaea in permafrost. FEMS Microbiol Ecol, 2007. 61 (1): p 1-15.
92. Faizutdinova, R., et al., Yeasts Isolated from Ancient Permafrost , J.D. Castello, S.O. Rogers (Eds.), Life in Ancient Ice, 2005, Princeton University Press, Princeton, NJ. P. 118-126.
93. Kochkina, G.A., et al., Survival of micromycetes and actinobacteria under conditions of long-term natural cryopreservation. Microbiology, 2001. 70(1): p. 356-364.
94. Stoupin D., Cryptic diversity within the choanoflagellate morphospecies complex Codosiga botrytis - phylogeny and morphology of ancient and modern isolates. Eur J Protistol, 2012. 48 (2): p. 263-273.
95. Ozcimen, D., et al., Isolation of Paracercomonas sp. TAE3-YTU.004 from Galindez Island, Antarctica, MW485507.1, NCBI GenBank, 22-January-2021.
96. Ozcimen, D., et al., Isolation of Flamella arnhemensis strain TAE3-YTU.007 from Galindez Island, Antarctica, MW485950.1, NCBI GenBank, 23- January-2021.
97. Ozcimen, D., et al., Isolation of Flamella balnearia strain TAE3-YTU.005 from Horseshoe Island, Skua Lake, Antarctica, MW487484.1, NCBI GenBank, 24- January-2021.
98. Ozcimen, D., et al., Isolation of Flamella arnhemensis strain TAE3-YTU.006 from Horseshoe Island, Antarctica, MW513457.1, NCBI GenBank, 30-January -2021.

99. Ozcimen, D., et al., M. Isolation of Paracercomonas sp. TAE3-YTU.008 from Horseshoe Island, Skua Lake, Antarctica, MW521096.1, NCBI GenBank, 31 -January -2021.
100. Malard, L. A. and Pearce, D. A., Microbial diversity and biogeography in Arctic soils. Environmental Microbiology Reports, 2018. 10(6): 611-625.
101. Morita, R. Y., Psychrophilic bacteria. Bacteriological reviews, 1975. 39(2): 144.
102. Whyte, L. G., Bourbonnière, L., and Greer, C. W., Biodegradation of petroleum hydrocarbons by psychrotrophic Pseudomonas strains possessing both alkane (alk) and naphthalene (nah) catabolic pathways. Applied and Environmental Microbiology, 1997. 63(9): p. 3719-3723.
103. Sepideh, P., et al., Psychrophilic enzymes: structural adaptation, pharmaceutical and industrial applications. Applied Microbiology and Biotechnology, 2021. 105(1): p.899–907. 10.1007/s00253-020-11074-0
104. Gon, O. and Heemstra, P. C., Fishes of The Southern Ocean. JLB Smith Institute of Ichthyology Grahamstown, 1990. South Africa.
105. Gostinčar, C. et al., Extremotolerance in fungi: evolution on the edge. FEMS Microbiology Ecology, 2009. 71(1): p.2-11.
106. Medema, M. H. et al., antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Research, 2011. 39(2): p.339-346.
107. Henríquez, M. et al., Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial, antitumoral and antioxidant potential. World Journal of Microbiology and Biotechnology, 2014. 30(1): p.65-76.
108. Bredholdt, H. et al., Rare actinomycete bacteria from the shallow water sediments of the Trondheim fjord, Norway: isolation, diversity and biological activity. Environmental Microbiology, 2007. 9(11): 2756-2764.
109. Liao, L. et al., Bioprospecting potential of halogenases from Arctic marine actinomycetes. BMC Microbiology, 2016. 16(1): p.34.
110. Akcay K, Kaya Y. (2020). Isolation, characterization and molecular identification of a halotolerant *Bacillus megaterium* CTBmeg1 able to grow on halogenated compounds. Biotechnol Biotechnol Equip. 2019;33(1): 945–953
111. Wahhab, B. H. A., et al., Identification and characterization of a 2, 2-dichloropropionic acid (2, 2-DCP) degrading alkalotorelant bacterium strain BHS1 isolated from Blue Lake, Turkey. Journal of Tropical Life Science, 2020. 10(3):p 245-252.
112. Edbeib, M., F., et al., Further Analysis of Burkholderia pseudomallei MF2 and Identification of Putative Dehalogenase Gene by PCR. Indonesian Journal of Chemistry, 2020. 20 (2): p 386 – 394
113. Torstensson, A., et al., Elevated temperature and decreased salinity both affect the biochemical composition of the Antarctic sea-ice diatom Nitzschia lecointei, but not increased pCO2. Polar Biol, 2019. 42(1):p 2149–2164. https://doi.org/10.1007/s00300-019-02589-y
114. Gray, A., et al., Remote sensing reveals Antarctic green snow algae as important terrestrial carbon sink. Nat Commun, 2020. 11(2527):p. 1-9. https://doi.org/10.1038/s41467-020-16018-w
115. Hoham, R., W., and D., Remias, Snow and Glacial Algae: A Review. J Phycol, 2020. 56(1):p 264–282. https://doi.org/10.1111/jpy.12952
116. Arrigo, K., R., Sea Ice Ecosystems. Ann Rev Mar Sci, 2014. 6(1):439–467. https://doi.org/10.1146/annurev-marine-010213-135103
117. Özçimen D., et al., Isolation, characterization and identification of Chlorella variabilis isolated from Horseshoe Island, Skua Lake, Antarctica, YTU.ANTARCTIC.001, MN372092, NCBI GenBank, 2019.
118. Özçimen D., et al.,Isolation, characterization and identification of Auxenochlorella pyrenoidosa strain Ozcimen.001 isolated from Horseshoe Island, Skua Lake, Antarctica, MT951391, NCBI GenBank, 2020.

119. Özçimen D., et al., Isolation, characterization and identification of Chlorella sorokiniana strain Egemen.001 isolated from Horseshoe Island, Skua Lake, Antarctica, MW147167.1, NCBI GenBank, 2020.
120. Del Campo, J., A., García-González, M., and Guerrero, M., G., Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. Applied microbiology and biotechnology, 2007. 74(6):p.1163-1174
121. Sathasivam, R., et al., Microalgae metabolites: A rich source for food and medicine. Saudi Journal of Biological Sciences, 2019. 26(4):p. 709-722.
122. Huilca, G., Licto, L., and Flores, R., Production of lipids from psychrophilic microalgae present in antarctic glaciers for the synthesis of biofuel. Revista Vínculos, 2020. 4(1):p. 35-51
123. Jha, D., et al., Microalgae-based Pharmaceuticals and Nutraceuticals: An Emerging Field with Immense Market Potential. Chem Bio Eng Reviews, 2017. 4(4):p 257-272.

Bir sonraki sayıda görüşmek ümidiyle..
Hope to see you in the next issue..

Author Guidelines

General Principles

1. The article should be submitted by the responsible author responsible as Microsoft Word (Doc, Docx).

2. The responsible author is responsible for monitoring all the processes of the article.

3. The main section headings should be bold and the first letter in capital letters, the first letter of the first word in the 2nd-degree headings should be large. If a third-degree title is required, the title should be in italics and only the first letter of the first word should be capitalized. If possible, articles with fourth-degree titles should not be used in our journal.

4. The main headings and sub-headings should not be numbered.

5. Turkish and English titles of the articles should be short, descriptive, and not more than fourteen words (except prepositions).

6. In the Pre-Control and Evaluation processes, the authors must submit the proposed corrections within 30 days at the latest. Otherwise, the article will be rejected.

7. Please click here to see the article written according to tothe rules of our journal.

Note: The general similarity rate should not exceed 20% except the bibliography part of the submitted articles. It is necessary to inform the journal editor if the rate specified in the necessary cases is exceeded. All articles submitted to the journal are screened with ithenticate plagiarism program.

8. Except for the Turkish and English titles of the work, all remaining parts should be written on the right side.

9. The text should be written on an A4-size page, in 12-font size Times New Roman, and double-spaced.

10. There should be 3 cm margin on the left, right, and top of the page.

11 Text should be written in a single column, all pages should be given a page number.
There is no page limit in publishing an article in our journal.

Names Section

A double-blind peer reviewer system is applied in our journal. Therefore, the names and addresses of authors should not be given in the main text when loading the Manuscript to the system. Author names and contact information should be written on a separate cover page. The cover page is available in the article submission section of our journal.

Abstract

The article can be written in Turkish and English. English and Turkish abstracts should be written at the beginning of the Turkish manuscripts.
The abstract should contain brief and clear information about the purpose, method , and results of the article. 10 font size, single line spacing and maximum 300 words should be written. No reference should be made to the "Abstract".
Keywords: Keywords should be 10 font sizes, minimum 3, and maximum of 5 words. Keywords must be separated with a comma (,) sign and should be in lowercase.

Introduction

The sections of the main part of the study should be written in 14 font sizes; Bold and the first letter should be capitalized; Article should have Introduction, Methods, Results, Discussion, and Conclusion sections. The second level titles should be written in the left, in 12 font sizes, the first letter of the first word should be capitalized, bold, and numbered. A line in the previous paragraph must be separated by a space.

Figures and Tables

In the Turkish article for Figures and Tables;" Şekil", "Tablo"; whereas in The English article "Fig", "Table" should be used (Table 1, Fig 1).
Figure and Table words should be written in bold, and at the end of the description of figures and tables should not have a dot (.)
Figures, graphics, photographs and the like should be written under Figures and written with 11 fonts. Figure and Table should be given in the main document in the relevant places, should not be uploaded as separate files or should not be added to the end of the text.

Examples;

Table 1 Possible effects of genetically modified organisms
Table 2 Methods of tissue culture in tomato plants
Fig. 1 Amount of GMO products in the world
Fig. 2 Countries with the highest number of GM cultivation in the world

Citation Inside the Text

In the text, the author should be numbered. Name and year should not be specified.
Example: Potatoes produced on a global scale are used in many basic areas [1, 2, 3]. Fifty percent of primarily produced potatoes are used for fresh consumption, such as baking, frying, boiling [4, 5, 6, 7].

References

The references section should be written in 10 font sizes and without hanging indentations. In the references section, italics should not be written except in italic words such as "in vivo, in vitro, ex-situ" and species names. References should be written according to the "Chicago style". Besides, there is an endnote style preparing for "International Journal of Life Sciences and Biotechnology". You can prepare your references using that style. For Endnote style, you can reach it by clicking on the endnote at the bottom right part of the main page of the journal.
Examples

Example of an article with 1 author;

Marakli, S., A Brief Review of Molecular Markers to Analyze Medicinally Important Plants. International Journal of Life Sciences and Biotechnology, 2018. 1 (1): p. 29-36.

Example of an article with 2 authors;

Kocacaliskan, I. and I. Tailor, Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. The Journal of Horticultural Science and Biotechnology, 2001. 76 (4): p. 436-440.

Example of an article with 3 authors;

Segura-Aguilar, J., I. Hakman, and J. Rydström, The effect of 5OH-1,4-naphthoquinone on Norway spruce seeds during germination. Plant Physiology, 1992. 100 (4): p. 1955-1961.

Example of articles with 4 or more authors

Arasoglu, T., et al., Synthesis, characterization, and antibacterial activity of juglone encapsulated PLGA nanoparticles. Journal of applied microbiology, 2017. 123 (6): p. 1407-1419.

Example for the book;

Kocalishkan, I., Allelopathy. 2006, Ankara, Turkey: Our Office Press-In Turkish.

Example for Book Chapter;

Kaya, Y., F.Z. Huyop, and M.F. Edbeib, Genetic Diversity in Plants, in Advances in Biosciences, F.Z. Huyop and S. Mohammed, Editors. 2019, Penerbit UTM Press. Malaysia: Malaysia. p. 04-24.

Ethical Principles and Publication Policy

Ethical standards for publication exist to ensure high-quality scientific publications, public trust in scientific findings, and that people receive credit for their ideas. In addition, the authors are encouraged to follow the ethic guidelines of the Committee on Publication Ethics (COPE) which can be viewed on the COPE website.

International Journal of Life Sciences and Biotechnology (Int J. Life Sci. Biotechnol.) is an electronic peer-reviewed international journal trying to have the highest standards of publication ethics. For that, we affirm the following principles of the Publication Ethics and Malpractice Statement.

If malpractice is discovered at any time even after the publication, the articles not in accordance with these standards will be removed from the publication. Int J. Life Sci. Biotechnol. is checking all papers in a double-blind peer-review process. We also check for plagiarisms, research fabrication, falsification, and improper use of any organisms in research. We will also report any cases of suspected plagiarism or duplicate publishing. Int J. Life Sci. Biotechnol. reserves the right to use plagiarism detecting software to screen submitted papers at all times.

Author's responsibilities: The author or authors must guarantee that they have written a unique study. Moreover, they must make sure that the article has not been submitted and evaluated elsewhere at the same time. Literature of the other researchers, all contributors and sources (including online sites) should be appropriately credited and referenced. All submitted manuscripts should be edited for language. All references should be cited without been copied or plagiarized. If needed, any financial sources or another conflict of interest should be disclosed. In order to correct the paper, any significant error or inaccuracy in the published works should be notified by the related authors. An author agrees to the license agreement before submitting the article. All articles must be submitted using the online submission procedure. Submitting a paper simultaneously to more than one publication at a time is a violation of publications' ethics.

Editorial responsibilities: Editors along with Editor-in-Chief and Editorial Board have publication decisions. Editors must guarantee a fair double-blind peer-review of the submitted articles for publication. They have to prevent any potential conflict of interests between the author and editors and reviewers. They have to guarantee the confidentiality of submitted articles before publishing. Editor-in-Chief will coordinate the work of the editors.

Peer review/responsibility for the reviewers: They have to review the manuscripts based on content without regard to ethnic origin, gender, sexual orientation, citizenship, religious belief, or political philosophy of the authors. They have to guarantee the confidentiality of submitted articles before publishing. They have to report any plagiarisms, research fabrication, falsification, and improper use of any organisms in research to the editors and/or the Editor-in-Chief. They have to review the papers objectively and state their opinions clearly in the forms and on the paper. A reviewer having inadequate time or feeling unqualified should notify the editors as soon as possible and excuse her/himself from the review process.

Plagiarism

All journals published by IJLSB are committed to publishing only original material, i.e., material that has neither been published elsewhere nor is under review elsewhere. Manuscripts that are found to have been plagiarized from a manuscript by other authors, whether published or unpublished, will incur plagiarism sanctions.

Manuscripts are checked by Ithenticate Plagiarism System.

Duplicate Submission

Manuscripts that are found to have been published elsewhere, or to be under review elsewhere, will incur duplicate submission/publication sanctions. If authors have used their own previously published work or work that is currently under review, as the basis for a submitted manuscript, they are required to cite the previous work and indicate how their submitted manuscript offers novel contributions beyond those of the previous work.

Citation Manipulation

Submitted manuscripts that are found to include citations whose primary purpose is to increase the number of citations to a given author's work or articles published in a particular journal, will incur citation manipulation sanctions.

Data Fabrication and Falsification

Submitted manuscripts that are found to have either fabricated or falsified experimental results, including the manipulation of images, will incur data fabrication and falsification sanctions.

Improper Author Contribution or Attribution

All listed authors must have made a significant scientific contribution to the research in the manuscript and approved all its claims. It is important to list everyone who made a significant scientific contribution, including students and laboratory technicians.

Redundant Publications

Redundant publications involve the inappropriate division of study outcomes into several articles.

Sanctions

In the event that there are documented violations of any of the above-mentioned policies in any journal, regardless of whether or not the violations occurred in a journal published by Int J. Life Sci. Biotechnol., the following sanctions will be applied:

Immediate rejection of the infringing manuscript.

Immediate rejection of every other manuscript submitted to any journal published by Int J. Life Sci. Biotechnol. by any of the authors of the infringing manuscript.

The prohibition against all of the authors for any new submissions to any journal published by IJLSB, either individually or in combination with other authors of the infringing manuscript, as well as in combination with any other authors. This prohibition will be imposed for a minimum of 36 months. Prohibition against all of the authors from serving on the Editorial Board of any journal published by Int J. Life Sci. Biotechnol.

In cases where the violations of the above policies are found to be particularly egregious, the publisher reserves the right to impose additional sanctions beyond those described above.

Publication Charge

"International Journal of Life Sciences and Biotechnology" is an Open Access Journal and does not charge any printing charges from authors, during article delivery, assessment and printing stages.

Aim and Scope

International Journal of Life Sciences and Biotechnology (Int J. Life Sci. Biotechnol.) is an international peer-reviewed journal that publishes original articles from all biology and molecular biology studies, particularly in the fields of life sciences and biotechnology. The language of publication is Turkish and English. Also the main objective of Int J. Life Sci. Biotechnol. is to provide quality publications to scientists, researchers, and engineers from both academia and industry who want to communicate the latest developments and practices in their field.

Int J. Life Sci. Biotechnol. publishes original papers in various fields of Life Sciences and Biotechnology that covers, but is not limited to, the following areas:

- Agricultural Biotechnology

- Animal (livestock and fish production, physiology, breeding and genetics, biotechnology, etc),

- Animal Biotechnology

- Biochemical Genetics,

- Biochemistry

- Biodiversity and biodiscovery

- Bioinformatics and system biology

- Biology and Molecular Biology,

- Bioremediation and biodegradation

- Biotechnology

-Bioethics (Life Sciences and Biotechnology)

- Botany,

- Evolution and Population Genetics,

- Food Biotechnology

- Genetic engineering and cloning

- Lichens

- Genetics,

- Biotechnological product and Halal Food

- Industrial Biotechnology

- Medical Biotechnology

- Molecular Genetics

- Plant (Plant production, physiology, breeding and genetics, biotechnology, agronomy, horticulture, plant protection, etc.),

- Plant Biotechnology

- Soil (soil ecology, physics, and chemistry, etc)

-Polar Science (Life Sciences and Biotechnology)

DECLARATION: This work is part of Gülfidan KUYUMCU's MA thesis. Articles on 27-29 September 2017 held in Bayburt in Turkey was presented as a Oral Presentation I. International Organic Agriculture and Biodiversity Symposium

Genetic Analysis Related To Organized Genetic Changes in Potato And Processed Potatoes

Gulfidan Kuyumcu[1*], Muhammed Majed Abed[2]

Author Addresses: [1] Samsun Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, Samsun / Turkey
[2] Samsun Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, Samsun / Turkey

*Corresponding Autor: Gulfidan Kuyumcu, e-mail: ijlsb@intsa.org

Please write the e-mail addresses and Orcid ID numbers of all authors **(required fields)**

Author 1 mail and Orcid ID:

Author 2 mail and Orcid ID:

Author 3 mail and Orcid ID:

Copyright Transfer Agreement

INTERNATIONAL JOURNAL OF LIFE SCIENCES SCIENCE AND BIOTECHNOLOGY (IJLSB)

Title:

Authors:

This form must be signed by all the authors and sent by you to publish your article at "International Journal of Life Sciences Scıence And Biotechnology (IJLSB)".

By signing this document, the following conditions are considered acceptable:

1. The fact that all publishing rights belong "International Journal of Life Sciences Scıence And Biotechnology,
2. Prepared according to the Writing Rules and Publishing Policies of "International Journal of Life Sciences Scıence And Biotechnology",
3. Article original and not previously published at home or abroad or is not under consideration for publication,
4. It belongs to us by the scientific and ethical responsibility is not reached other authors; All the authors a-re aware of the work and responsibilities of other authors, first author of the article that I have to accept me as I declare;

Authors Name Surname	Date	Signature

Ethics Committee Report

Article If an Ethics Committee Report is required to conduct a research on animals and humans and to conduct this research, it is mandatory that the **Ethics Committee Report be scanned and uploaded as a PDF file**. Otherwise, the article is returned to the author at the preliminary examination and other stages.

1.REVIEWER SUGGESTION

DEGREE:

FIRST NAME:

LAST NAME:

MAIL ADDRESS:

INSTITUTION:

DEPARTMENT:

SUBJECTS:

2.REVIEWER SUGGESTION

DEGREE:

FIRST NAME:

LAST NAME:

MAIL ADDRESS:

INSTITUTION:

DEPARTMENT:

SUBJECTS:

3.REVIEWER SUGGESTION

DEGREE:

FIRST NAME:

LAST NAME:

MAİL ADDRESS:

INSTITUTION:

DEPARTMENT:

SUBJECTS: