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Analysis of relations with members of Antalya province fisheries cooperatives

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Abstract

Producer organizations, which are structures that are also accepted as the development criteria of countries nowadays, are established to provide social and economic needs of their members. Because the organizations are multi-purpose, they are preferred by producers as they undertake many tasks from regulating fisheries market to protecting rights of producers and from providing training services related to production and sales to meeting the needs. For this reason, in this research, face-to-face surveys were conducted with 75 members of 14 active fisheries cooperatives in Antalya. The survey application period, which is research's data collection phase, was planned as fishing season and occur between November 2017 and March 2018. In the study, sample size of fisheries cooperative members was determined by simple random sampling and factor analysis method, one of the multivariate statistical analyzes, was used to determine the factors forming relationship between cooperatives and members. According to research results; although the main reason why to participate in cooperatives is aim of obtaining economic and technical support, activities of cooperatives are considered insufficient by members. Conversely, though sufficient support is not given, conditions are limited, and success is not achieved as much as expected, efforts to stand and increasing memberships of cooperatives is considered as a positive result in terms of fisherman organisation in Antalya.

Keywords: Fisheries cooperatives, Organisations, Fisheries, Antalya

Introduction

The producer organization has important functions in fisheries production, storage, transportation, domestic and foreign markets in eliminating unfair competition between small and large enterprises. It is only possible with an effective organization, members can obtain production inputs at affordable prices, to increase their income, and to market their products in good conditions (İnan, 2008). For this reason, organization, which is accepted as one of the indicators of the level of development of countries, is of great importance for the fisheries sector of our country, as it is in different sectors. However, besides that, one of the most important problems of the fisheries sector is producer organizations.

Fisheries cooperatives established by Law No. 1163 undertake many tasks such as regulating the fisheries market, protecting the rights of producers and meeting their needs.

Member composition of the organization formed by the fisheries sector in Turkey is mostly small-scale fishermen (Ünal et al., 2009, Yılmaz 2009, Olguner et al., 2015). However, the fishermen's organization in Turkey still has not reached the desired level (Ünal and Yercan 2006). For this reason, it is emphasized in many studies that organizations, most of which are created by small-scale fishermen, are ineffective organizations (Zengin and Güngör 2017) and should be supported because they cannot provide full performance (Kurtoğlu 2006, Akyol and Ceyhan 2010, Dartay and Canpolat 2017, Yılmaz and Şen 2018).

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Today, there are 572 fisheries cooperatives and 31241 members in these cooperatives in our country. Although the fisheries cooperatives in Antalya province constitute 24,5% of the total fisheries cooperatives, their number is increasing day by day (Anonim, 2021). In Antalya province, which has significant contributions to the fisheries sector, there are 14 fisheries cooperatives with 694 members and 1 Antalya Regional Fisheries Cooperative Union, which was formed by the merger of 10 cooperatives. Although the province of Antalya, chosen as the study area, has a good fisheries history, the lack of organizational culture, the insufficient effectiveness of cooperatives in fisheries activities and the problems experienced in organization and member relations are of great importance.

In this study, it is aimed to reveal the factors affecting the organization of fishermen in Antalya through fisheries cooperatives. In this context, the effect of the variables that lead fishermen who are members in fisheries cooperatives in Antalya to organize has been examined. As a result of the research, it has been tried to offer solutions in order to eliminate the existing and potential problems in the organization of fishermen.

Material and Method

Since the main population of the study consists of fishermen who are members of fisheries cooperatives in Antalya Province, face-to-face surveys were conducted one of the research methods for primary data. The survey application period, which is research's data collection phase, was planned as fishing season and occur between November 2017 and March 2018.

For this purpose, pilot survey studies were conducted by using the records obtained from the Ministry of Agriculture and Forestry Antalya Directorate of Provincial, Antalya Fisheries Cooperatives and public institutions.

After evaluating the pilot survey data, necessary arrangements were made in accordance with the purpose of the study and the Neyman method was used for the statistical analysis of the survey data obtained in determining the sample volume of the cooperative members who own the fishing boats.

The simple random sampling formula used with this method (Yamane, 1967).

$$n = \frac{(N\ z^2\ p\ q)}{(N\ d^2)\ +\ (z^2\ p\ q)}$$

Where;

n: Number of samples,

N: The total number of units of the sampling frame,

z: The standard normal value found depending on the chosen confidence level (the study was studied with 99% confidence)

p: The probability of the event of interest (as the study basically tried to measure the organizational tendencies of the members and the organizational perception depending on it, it was defined as the probability that any selected fisherman would find the producer's organization successful)

q: Probability that the event of interest will not occur (defined as the probability that any selected fisherman will find the producer's organization a member to fail)

d: Shows the accepted sensitivity in the sampling (worked with 4% deviation in the study)

In the research, it is planned to obtain data with the problems of the members about fishing, support requests, reasons for being a member, the level of awareness, their perceptions, thoughts, evaluations and suggestions about the cooperative. A five-point Likert scale was used for questions about these situations.

During the analysis of the data, the frequency and tables for discrete variables were prepared using the SPSS 22 program and presented as a summary. In addition, factor analysis was also used, which aims to obtain a small number of identifiable significant variables from a large number of variables measuring the same structure among the variables (Kalaycı, 2005; Kleinbaum et al., 1997).

In factor analysis, a correlation matrix is created for all variables in the first stage, and in the second stage, factors are rotated to maximize the relationship between factors and variables by extracting the variables from the correlation matrix based on the correlation coefficients (Özdamar, 2010).

$$X1\text{-}M1 = L11F1+L12F2+.....L1kFk+ \varepsilon 1$$
$$X2\text{-}M2 = L21F1+L22F2+.....L2kFk+ \varepsilon 2$$
$$XP\text{-}MP = LP1F1+LP1F2+.....LPkFk+ \varepsilon P$$

In the equation, Lij = coefficient of factors (factor load), i:variable, j:specifies factor load (weight). The new variables derived in the analysis are expressed as "Factors". It is aimed to reveal the random factors that reflect the classification, which are not observed from the variable in the data matrix (P), which are observed with the analysis and are correlated (X), but are revealed by the combination of the variables.

For this reason, in the research, the reasons for the organization of the members of the fisheries cooperatives, which have many variables, and their thoughts about the organization, were tried to be determined by obtaining significant and few variables by factor analysis method. In factor analysis, measurement is made with the Kaiser Meyer-Oklin (KMO) test to determine the degree of suitability of the analysis of explanatory variables. The KMO sampling adequate criterion is an index used to compare the magnitude of the observed correlation coefficients with the size of the partial correlation coefficient. Considering that the applicability of the factor analysis technique decreases as the KMO value decreases, a KMO value of 0.90 is excellent, good at 0.80, moderate at 0.70, low at 0.60, and unacceptable if it is below 0.50. is evaluated as. The main criteria taken into account when deciding on the number of factors are the eigenvalue and variance criteria. Generally, factors with an eigenvalue above 1 are chosen in practice (Joseph vd. 2009).

In this research, the reasons for the members of the fisheries cooperatives to become members of the cooperative were collected under 26 headings and factor analysis was applied to the data obtained using the likert scale.

Results and Discussion

In Antalya Province, there are 14 fisheries cooperatives on the 640 km long coastline from Kaş district to Gazipaşa district (Table 1.).

According to the records obtained from the Antalya Provincial Directorate of Agriculture and Forestry, the newest established fisheries cooperatives in the province according to the year of establishment are Kemer Fisheries Cooperative and Serik Fisheries Cooperative, the oldest established Yeşil Antalya Fisheries Cooperative, Alanya Fishery Cooperative, Kaş Fisheries Cooperative. Cooperative, Denizyaka Fisheries Cooperative and Manavgat Fisheries Cooperative (Table 1.).

Table 1. Fisheries cooperatives interviewed and their establishment years

District Name	Cooperative Name	Foundation Year
Muratpaşa	Yeşil Antalya Fisheries Cooperative	1990
Konyaaltı	Yeni Liman Fisheries Cooperative	1995
Konyaaltı	Kemer Fisheries Cooperative	2016
Aksu	Aksu Fisheries Cooperative	2010
Alanya	Alanya Fisheries Cooperative	1990
Finike	Finike Fisheries Cooperative	1995
Gazipaşa	Gazipaşa Fisheries Cooperative	2004
Kaş	Kaş Fisheries Cooperative	1990
Kaş	Kalkan Fisheries Cooperative	2004
Manavgat	Denizyaka Fisheries Cooperative	1990
Manavgat	Manavgat Fisheries Cooperative	1990
Manavgat	Side Fisheries Cooperative	2015
Serik	Belek Fisheries Cooperative	2004
Serik	Serik Fisheries Cooperative	2016

It is possible to come across many studies on the socio-demographic characteristics of the members of fisheries cooperatives. Some of these are Doğan and Gönülal (2011), Karademir and Emin Arat (2014), Dartay and Canpolat (2017), Çımat and Duran (2018).

In this study, which was conducted in Antalya, the ages of the members of the aquaculture cooperatives ranged from 26 to 70, and the average age was 50, when the ages of the members were examined from socio-demographic characteristics. According to the results in Table 2, the majority of the population of Fisheries Cooperative members is young/middle-aged. As a matter of fact, in the master's thesis study conducted by Göncüoğlu (2008), it was emphasized that 22.9% of the young age common group and 40.6% of the middle age group. It was concluded that most of the members of Antalya Province Fisheries Cooperatives are in the young/middle-aged segment, as in this study.

It was determined that 93.3% of the Fisheries Cooperative members participating in the survey were married (Table 2.). According to this situation; It can be said that most of the fisheries cooperative members are married.

When the educational status of the cooperative members surveyed in the research is examined, it is seen that the formal education period of the members is low. Members have received formal education mainly at primary level (Table 2). However, it was determined that some of them continued to secondary school and high school in various ways. As a result of this analysis, as in the results obtained in a study conducted in 2014, the education level of the cooperative members is similarly low (Karademir and Arat, 2014).

Cooperative members are grouped as families of 2-3, families of 4, and families of 5-8 people, according to the family population size. According to the results of the analysis made in this context, it has been determined that the members mostly consist of families of 4 with a ratio of 45% (Table 2.).

When the housing status of the fisheries cooperative members interviewed was examined, it was determined that 73.3% of them were homeowners according to the results of the analysis (Table 2.). Rental prices of Fisheries Cooperative members, who are renters, vary between 250 TL and 1000 TL.

Most of the fisheries cooperative members have social security. As a matter of fact, Table 2 shows that 76% of the members have social security. The majority of those who do not have social security work in the tourism sector, which was active during the fishing ban period. The members have social security during the tourism season and their social security ends again when the season ends.

Table 2. Socio-demographic characteristics of the fisheries cooperative members interviewed

Range of Age	**Number**	**%**
26-45	24	32,0
46-55	28	37,3
56+	23	30,7
Total	**75**	**100,0**
Marital status		
Married	70	93,3
Single	5	6,7
Total	**75**	**100,0**
Education		
Illiterate	1	1,3
Primary School	31	41,4
Middle School	21	28,0
High School	16	21,3
University	6	8,0
Total	**75**	**100,0**
Family Size		
2-3	19	25,3
4	34	45,4
4+	22	29,3
Total	**75**	**100,0**
Housing Status		
Host	55	73,3
Renter	20	26,7
Total	**75**	**100,0**
Social Security Status		
Available	57	76,0
Absent	18	24,0
Total	**75**	**100,0**

As seen in Table 3, the memberships of the members of the fishery cooperatives to the cooperative vary between 1 year and 28 years, and the average membership period is 11 years. In another study conducted in Istanbul, it was reported that the majority of cooperative members have a membership period of 1-10 years (Karademir and Emin Arat 2014). It has been determined that most of the membership periods of fisheries cooperatives in Antalya are between 1-10 years. The fact that the establishment of the cooperative was newly established during the said period also has an effect.

Table 3. Cooperative membership durations of the fisheries cooperative members interviewed

Cooperative membership durations (Year)	**Number**	**%**
1-10	39	52,0
11-20	28	37,3
21-28	8	10,7
Toplam	**75**	**100,0**

In the research, it was determined that the majority of the members of fisheries cooperatives, with a rate of 68%, were pleased with being a member (Table 4).

Table 4. The pleased of being a member of the fisheries cooperative members interviewed

Satisfaction with being a member	**Number**	**%**
Very pleased	7	9,3
Pleased	51	68,0
Partially pleased	14	18,7
Not pleased	2	2,7
Not pleased at all	1	1,3
Total	**75**	**100,0**

In the study, the signs of success of the cooperative according to the members of the fishery cooperatives were tried to be explained with 5-point Likert scale questions. Among the signs of success of the members' cooperatives, they answered that there is unity and solidarity between the members and the managers of the cooperative, planned and conscious work, good marketing, and product processing. In addition, answers were received from the members that the management team is strong and good, the cooperative has no debt, the companies and traders cannot defraud the producers, the organization is good and they work honestly (Table 5). In this context, it is revealed that among the signs of success of the members' cooperatives, "the unity and solidarity between the members and the cooperative managers, the strong and good management team, honest and planned and conscious work" are more important.

Table 5. The reasons for the success of the cooperative according to the interviewed fisheries cooperative members

The reasons fort he success	**Ave rage**	**Std. deviation**	**Never Agree**		**Don't agree**		**Undeci ded**		**Agree**		**Totally agree**		**Total**	
			N	%	n	%	n	%	n	%	n	%		%
There is unity and solidarity between the members and cooperative managers	4,11	0,649	1	1,3	2	2,7	-	-	57	76,0	15	20,0	75	100,0
Planned and conscious work	3,99	0,762	3	4,0	1	1,3	1	1,3	59	78,7	11	14,7	75	100,0
Good marketing	3,92	0,834	3	4,0	2	2,7	5	6,7	53	70,7	12	16,0	75	100,0
The product is being processed	3,71	0,941	5	6,7	3	4,0	8	10,7	52	69,3	7	9,3	75	100,0
Strong and good management team	4,08	0,653	1	1,3	1	1,3	4	5,3	54	72,0	15	20,0	75	100,0
The cooperative has no debt	3,84	0,823	2	2,7	5	6,7	5	6,7	54	72,0	9	12,0	75	100,0
Inability of companies and traders to defraud manufacturers	3,76	0,803	3	4,0	3	4,0	8	10,7	56	74,7	5	6,7	75	100,0
Good organization	3,96	0,829	3	4,0	2	2,7	3	4,0	54	72,0	13	17,3	75	100,0
Working honestly	4,07	0,684	1	1,3	2	2,7	3	4,0	54	72,0	15	20,0	75	100,0
Return of activities	3,87	0,827	3	4,0	3	4,0	4	5,3	56	74,7	9	12,0	75	100,0
Regular payments of members	3,80	1,273	5	6,7	6	8,0	6	8,0	50	66,7	8	10,7	75	100,0

In the study, factor analysis, which is a multivariate statistical analysis, was applied in order to obtain a small number of identifiable and significant variables from a large number of variables that measure the same structure among the variables of the survey results of the cooperative members, and the thoughts of the members about the organization they belong to and the reasons for being a member were examined.

In the research, the reasons for the members of the fisheries cooperatives interviewed to be a member of the cooperative was examined with 26 questions using a 5-point Likert scale. Factors related to these causes are given in Table 6.

As a result of the analysis, the Cronbach alpha value was found to be 0.87. It is important for the reliability of the test that the value found is close to 1. The factors related to the reasons for becoming a member in the cooperative are given in Table 6.

In the factor analysis, the eigenvalue was taken as a criterion and 4 factors with values greater than 0.40 were determined. While these 4 factors explained 74% of the variance, these factors were summarized as cooperative activities, economic support of the cooperative, technical support of the cooperative, and contributing to the unity and solidarity of the cooperative.

According to this result, the members of the cooperative preferred the first factor, primarily, because it is a fair administration, a democratic (equal voice) administration, it benefits me, it has a very good control and control system, it provides development, it enables us to act jointly (producers, etc.). Because, according to the results of the reason for being a member was grouped as "cooperative activities".

In factor 2, the cooperative members primarily preferred the options, because the sale of the product is guaranteed (easily

marketable, use of marketing services), i sell the product at a good price (high profit), i obtain inputs at low prices, to reduce risk and use available resources (sales and storage space, workforce, they gave the opportunity to use the inputs in the best way. According to the results of the analysis, the reason for being a member was grouped as “economic support of the cooperative”.

In Factor 3, the reason for becoming a member is grouped as "technical support of the cooperative", according to the analysis result, since the cooperative members prefer their options primarily because it is easy to find credit, provides technical support for production, can supply consumer goods cheaply and supports educational activities.

In the 4th factor, the cooperative members preferred their options because we increase our economic power by acting together and the idea that unity is stronger. According to the results of the analysis, the reason for being a member is grouped as "contributing to the unity and solidarity of the cooperative" (Table 6.).

Table 6. Factor weights obtained as a result of factor analysis of the reasons for the members of the fisheries cooperative interviewed to be a member of the cooperative.

Reasons to become members	**Factor Weights**			
	1	**2**	**3**	**4**
Because I sell the product at a good price (high profit)		0,856		
As the marketing activity		0,887		
Because the sale of the product is guaranteed (because it can be easily marketed, benefit from marketing services)		0,881		
Since I procure the inputs at a low price		0,770		
To take advantage of the support provided	0,512	0,479		
Because the risk is low (to reduce the risk)		0,668		
Because it's easy to find a loan			0,826	
Since I can obtain consumables cheaply			0,757	
Since it provides technical support for production		0,416	0,771	
As we increase our economic power by acting together				0,775
Since it allows the best use of available resources (Sales and storage space, workforce, inputs)		0,617	0,402	
Since there is an open and transparent management in the cooperative	0,799			
Since it supports educational activities	0,476		0,570	
Since it is sensitive to social responsibilities (Creating public opinion)	0,783			
Since it is a democratic (equal voice) form of government	0,843			
Since it is an independent structure	0,860			
Since it enables us to act jointly (Solidarity between producers)	0,701			
Because it provides development	0,732			
To ensure the continuity of fisheries	0,700			
Because of its influence in the region	0,709			
Because it gives reputation around me	0,491		0,568	
Managers are honest and have moral values	0,800			
From the idea that unity is strength	0,682			0,544
Because it is a fair administration	0,868			
Because it benefits me	0,809			
Because it has a very good control and control system	0,814			
Eigenvalue	**12,159**	**4,256**	**1,551**	**1,268**
Variance	**34,823**	**18,591**	**14,082**	**6,479**
Cumulative Variance	**34,823**	**53,414**	**67,495**	**73,975**
Cronbach α		**0,870**		

Again, the opinions of the members of the fisheries cooperatives interviewed about the cooperative, in which a 5-point Likert scale was used, were examined with 45 questions and factor analysis was applied with the information obtained. The Cronbach alpha value was found to be 0.79. The factors related to the reasons for becoming a member in the cooperative are given in Table 7.

In the factor analysis, the eigenvalue was taken as a criterion and 5 factors with a value greater than 0.40 were determined. These 5 factors explained 68.9% of the variance. These factors are summarized as the activities of the cooperative management, the success of the head of the cooperative and his assistants, the interaction of the cooperative, the importance that the member gives to the cooperative and the sustainability of the membership.

According to this result, the cooperative members preferred options in the first factor, primarily, the general assembly meeting time and day are announced, every issue is discussed in detail at the general assembly, participation in the general assembly is high, decisions are taken democratically in the general assembly, the cooperative activity report is clearly and detailed. According to the results of the analysis, their thoughts about the cooperative they are a member in were grouped as "activities of the cooperative management".

In the second factor, the cooperative members preferred the options: the head of the cooperative is successful, the head of the cooperative is educated and knowledgeable, the cooperative employees do their job well, the head of the cooperative is good at speaking, the head of the cooperative is experienced. According to the results of the analysis, their thoughts about the cooperative they are a member in were grouped as "the success of the cooperative president and his assistants".

In the 3rd factor, the cooperative members preferred the options that the cooperative develops fisheries in the region, the cooperative contributes to the development of the region, the cooperative supports the infrastructure works of the region, the cooperative has cooperation and cooperation with other similar cooperatives. According to the results of the analysis, their thoughts about the cooperative they are a member in were grouped as "cooperative interaction".

Cooperative members are happy to be a member of the cooperative in factor 4, the members trust the cooperative, I was impressed by the family members and fishermen when they decided to become a member, cooperative membership is important in our family and I recommend them to my environment. According to the results of the analysis, their thoughts about the cooperative they are a member of were grouped as "the importance that the member gives to cooperatives".

In factor 5, while the cooperative members prefer the options, i will continue to be a member in the future, the cooperative general assembly is held regularly and on time, according to the analysis result, the option of being a member of the cooperative is determined to be inversely related and their thoughts about the cooperative they are a member in are “sustainability of the membership” grouped as.

The opinions of the fisheries cooperative members interviewed about the cooperative were compiled from various sources and examined with 45 questions. The 5 factors obtained as a result of the factor analysis were tried to be summarized as the activities of the cooperative management, the success of the head of the cooperative and his assistants, the interaction of the cooperative, the importance given by the member to the cooperative and the sustainability of the membership (Table 7.).

Table 7. Factor weights obtained as a result of factor analysis of the opinions of the fisheries cooperative members interviewed about the cooperative

Thoughts	Factor Weights				
	1	**2**	**3**	**4**	**5**
I will continue to be a member in the futures	0,495				0,526
I recommend it to my environment				0,635	
I will be in administration in the future.		0,450			
Cooperative president is experienced		0,712			
Cooperative president is educated, knowledgeable	0,403	0,803			
Cooperative president's speech is good	0,506	0,719			
Cooperative management consists of honest, reliable people	0,644	0,552			
I find the cooperative successful		0,640	0,466		
Cooperative can actually be even more successful					0,677
Cooperative does not distinguish between members	0,759				
Thanks to the cooperative, my income level increased			0,648		
The cooperative gave me self-confidence					
Cooperative president is successful		0,830			
The board is working successfully	0,413	0,641	0,437		
Cooperative workers do their job well	0,416	0,727			
Cooperative manager and his assistants are successful	0,477	0,683			
The cooperative management has good communication with its members	0,671	0,493			
The buildings of the cooperative are sufficient			0,628		
I can easily meet with cooperative managers whenever i want		0,667			
I trust the cooperative	0,682				
Cooperative membership is important in our family				0,627	
There is no unfair advantage and corruption in the cooperative	0,601				
Cooperative general assembly is held regularly and on time	0,533			0,586	0,478
There is no fight in the general assembly work	0,528			0,631	
General assembly meeting time and day are announced	0,850				
High attendance at the general assembly	0,787				
The cooperative activity report is presented in an understandable and detailed manner	0,757		0,421		
Every issue is discussed in detail at the general assembly	0,810				
Decisions in the general assembly are taken democratically	0,768				
Necessary records (books) are kept regularly in the cooperative	0,714				
I am happy to be a member of the cooperative				0,662	
I have experience in cooperative			0,615	0,494	
Influenced by family members when deciding to become member			0,405	0,607	
I was impressed with other fishermen when deciding to become a member				0,608	
I will be a member of other agricultural organizations (Chamber of Agriculture, TKK etc.)				0,463	
Members trust the cooperative	0,449			0,671	
I like to collaborate		0,607			
It is easy to become a member in the cooperative					-0,508
The cooperative supports the infrastructure works of the region			0,559		
The cooperative contributes to the development of the region			0,731		
Cooperative is independent				0,681	
The state supports the cooperative			0,682		
The cooperative develops fisheries in the region			0,779		
The cooperative has cooperation and cooperation with other similar cooperatives.			0,527		
Increases the income of cooperative members			0,587	0,529	
Eigenvalue	**19,758**	**3,968**	**3,153**	**2,203**	**1,919**
Variance	**20,872**	**17,018**	**13,649**	**12,162**	**5,189**
Cumulative Variance	**20,872**	**37,890**	**51,539**	**63,702**	**68,891**
Cronbach α		**0,791**			

Conclution and Recommendation

According to the research results; it is observed that the fishermen engaged in hunting activities in Antalya are small-scale enterprises. For this reason, the members stated that they needed state support.

In the study, the evaluation of the success criteria of the producer organization that the members are involved in was examined. In this context, when evaluated by scoring the success criteria of the cooperative, which includes Fisheries Cooperatives Members; it has been revealed that more importance is given to the unity and solidarity between the members and the cooperative managers with a maximum of 4.11 out of 5, then the honest, strong and good management staff, and the planned and conscious work.

Although it is determined in the research that 68% of the members are satisfied with being a member of the cooperative they are affiliated with, the members evaluate the activities of the cooperatives as insufficient. The members have a general view that the organizations cannot fully realize the expected success due to the limited organizational conditions. According to the aforementioned members, in order for Fisheries Cooperatives to be successful, their problems must be solved and their needs met. Among the suggestions that come to the fore are the state support and the unity and solidarity of the members and their awareness.

The factor analysis method was also used in the study. The reasons for members to become members of producer organizations were examined by the research method. According to the results of the analysis, the factors affecting the reasons for the members of fisheries cooperatives to become a member of the cooperative; the technical and economic support of the cooperative has been determined as being included in the unity and solidarity of the cooperative and benefiting from cooperative activities. As a matter of fact, it has been revealed that the producers in the research region can more easily solve many procedures that need to be fulfilled by becoming members with producer organizations.

In the research, the factors affecting the thoughts and evaluations of the members about the organization they are involved in were also examined with factor analysis. According to the results of the analysis, the factors affecting the thoughts and evaluations of the members of the fisheries cooperative about the cooperative; the success of the head of the cooperative and his assistants, the interaction of the cooperative with the members, the importance that the member gives to the cooperative, the activities carried out by the cooperative management.

In the study, although EU-type organizations are taken as an example for the producer organizations in our country, it has been revealed that many of these factors cannot be met by the organizations and the desired success cannot be achieved due to the structural and functional differences with the producer organizations in the EU. Fisheries organizations in the European Union; it protects the producer and directs the fisheries market. In addition, the elements that make up the organization in the producer organization in the EU are complementary to each other with their duties and responsibilities. Producer organizations, which are structurally different from our country, also carry out lobbying activities in order to give direction to agricultural policy. Agricultural Cooperatives in the EU can even come together to establish new producer organizations to benefit from the EU funds used to regulate the markets. In our country, as in EU countries, it would be beneficial to make legal arrangements that will bring producers together and facilitate organization.

Solving the problems that make the activities of producer organizations inadequate in the long term can be achieved by the joint work of producer organizations, ministries and universities with the aim of rational and sustainable fishing. In addition, there is a need for more studies that will produce solutions to the problems in the field of organization. Insufficient studies on the organization of fishermen make it important to support these studies.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Ploidy estimation in pepper and eggplant via stomata characteristics

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Abstract

The most reliable methods to confirm the ploidy level in plants are to count the chromosomes or to measure the DNA in the cells by flow cytometry. However, these methods are laborious, time consuming, and require special equipment. In this study, the reliability of stomatal characteristics in confirming the ploidy level was investigated in haploid and spontaneous double haploid (SDH) pepper and eggplant plants. Stomatal characteristics were measured using a digital camera and related software from light microscope images in 100 samples at each ploidy level. Stomatal density, guard cell width and length were measured in random fields of view, and chloroplasts were counted. Mean stomatal lengths were determined as 28.34 µm and 40.39 µm, and mean stomatal widths were determined as 22.52 µm and 29.50 µm respectively for haploid and SDH plants in pepper. The stomatal density was 10.71 in haploid and 27.07 in SDH. Average stomatal lengths were determined as 22.32 µm and 32.00 µm, and mean stomatal widths were determined as 17.36 µm and 22.32 µm, respectively, in haploid and SDH eggplant. The stomatal density of eggplants were found to be 29.20 in haploid plants and 12.61 in SDHs. Chloroplast numbers in guard cells of SDH plants were determined to be 2 fold more than haploids. In haploid and SDH peppers 9.93 and 18.66 chloroplasts were counted, respectively, and 6.39 and 11.19 chloroplasts were counted in eggplants, respectively. There were positive relationships between stomatal size and chloroplast number and ploidy level, and negative relationships between stomatal density and ploidy level, which can be presented as an early marker to determination ploidy levels in both species.

Keywords: chloroplast, ploidy level, stomata, anther culture

Introduction

Haploid plants have very important place in plant breeding because they contain only one series of alleles at each locus allows to reveal recessive mutations and to obtain 100% homozygous pure lines in one generation by doubling the chromosome numbers. Since haploid plants cannot form gametes, they are unfertile and do not produce seeds. In order to be used in breeding programs, they must be transformed into productive double haploid (DH). Plants obtained by doubled haploid techniques can be haploid or spontaneous double haploid (SDH) (Gyulai *et al*., 2000, Alremi *et al.*, 2014, Keleş et al., 2015, Ari *et al*., 2016, Çömlekçioğlu & Ellialtıoğlu, 2018). Spontaneous genome doubling is largely dependent on plant species, and it has been reported that spontaneous genome doubling is greater than 90% in some species, while some species are reported to be resistant to spontaneous genome doubling (Mir *et al*., 2021). Grozeva *et al*. (2021), obtained 100% haploid in some genotypes and 100% SDH in some genotypes by anther culture. As the average of 17 genotypes, 59.9% of the plants were haploid and 40.1% were diploid. In previous studies on eggplant, SDH rate reported as 15.4% by Dumas de Vaulx & Chambonnet (1982), 25.6% by Rotino (1996) and 46.4% by Salas et al (2011).

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In eggplant anther culture stimulated microspores develop into callus. The most of the callus tissues were found to be mixoploid with different ploidy types ranging from haploid to tetraploid. However, 60%-70% of the regenerated plants (Corral-Martinez & Segui-Simarro, 2012; Rivas-Sendra *et al*., 2015) were confirmed to be SDH by flow cytometry. For this reason, ploidy levels of plants must be verified quickly before chromosome doubling is performed. The most reliable method is to count the chromosomes or to measure the DNA in the cells by flow cytometry. However, these methods are difficult, time consuming and require special equipment. Therefore methods to determine ploidy in a large number of plants in a short time gain importance.

It has been shown by many researchers that haploid plants have smaller stomata and lower stomatal density than diploids (Omidbaigi *et al*., 2010; Głowacka *et al*., 2010; Hannweg et al., 2013; Xie, 2015; Widoretno, 2016; Comlekcioğlu & Ozden, 2019). Chloroplast count, DNA content, stomatal size and morphological observation were compared for haploid and diploid pepper (Abak *et al*., 1998), haploid and diploid watermelon (Sarı *et al* 1999), diploid and tetraploid watermelon (Şimşek *et al*., 2013) plants. It has been reported that the ploidy level in plants can be selected quite practically as a result of using the obtained morphological and cytological data together. The chloroplast numbers in stomatal guard cells show significant differences according to species (Lawson, 2009). While most researchers reported that different ploidies have similar chloroplast numbers, Jacobs & Yoder (1989) reported no similarity between genetically similar diploid and tetraploid tomato chloroplast numbers. On the other hand, stomata size is not controlled only by genome size. Stomata size shows significant variation according to leaves, plants and ecological factors (McGoey *et al*., 2014, Tekin & Yılmaz, 2018). It has been reported that neither stomatal density nor stomatal size can be used to determine the ploidy level of diploid and tetraploid watermelon (Jaskani *et al*., 2005), and mixoploid and tetraploid ginger (Soonthornkalump et al., 2017).

The rapid increasing of the use of doubled haploid methods in plant breeding has also increased the need for rapid ploidy screening of the obtained plants. In this study, the relationship between stomatal densities, stomatal size, number of chloroplasts in guard cells and ploidy level in plants obtained by anther culture method in pepper and eggplant were re-examined.

Material and Method

This study was carried out in the greenhouse and tissue culture laboratories of United Genetics Vegetable Seeds Company (Mustafakemalpaşa, Bursa, Turkey). The pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena*) genotypes tested in the study are the breeding lines of the company.

Morphological traits such as leaves size, plant height, vigor, internodes height, and fertility, presence of pollen and seeded fruit of regenerated androgenic plants were compared with the donor plants. Regenerates were grouped as haploid or SDH based on morphological observation. In both species, 100 plants were studied in each ploidy group. Stomata were visualized using a Leica DMLB light microscope. Images were captured and measured using a digital camera and related software.

Stomata Count

Young leaves that have completed their development at the shoot tip (usually 4-5 leaves from top to bottom) were used. The leaf membranes taken from the lower epidermis (middle part of the leaf) of the leaves were placed on the slide and a drop of $AgNO_3$ (silver nitrate) solution was dropped on it (AgNO3 makes stomata and chloroplasts appear brighter). Stomata in 1 random microscopic field of view were counted at 10X40 magnification.

Stoma sizes

The width and length of 100 stomata, from each ploidy level, were measured as µm.

Number of chloroplasts

Chloroplasts in guard cells of 100 stomata counted at each ploidy level were counted.

The experiment was carried out in a randomized block design with 100 replications (plants). The data were subjected to analysis of variance (ANOVA) using Tarist (Açıkgöz *et al.*, 2004) and biplot (principal component method) by Minitab 17 Statistical Software (Anderson, 1998).. Mean separation was performed by Fisher's Least Significance Difference (LSD) ($p < 0.01$).

Results and Discussions

It was determined that there were significant differences according to ploidy levels in terms of all traits examined in both plant species ($P < 0.001$). The summary of analysis of variance (ANOVA) of pepper and eggplant for stomatal characteristics were presented in Table 1 and Table 2, respectively.

The highest F value was calculated in chloroplast number for pepper (Table 1). It is understood that there is a very high difference in the number of chloroplasts according to ploidy levels. Likewise, it was determined that there was a more significant difference between the haploid and SDH plants in terms of both the number of stomata and the stomatal length compared to the stomatal width, and they were more reliable traits to confirm of ploidy level than the stomatal width.

In eggplant, the highest F value was determined in the number of stomata according to ploidy levels. This is followed by stomatal length and chloroplast number. In eggplant, the lowest F value was calculated in stomatal width as in pepper.

Higher stomata density was found in haploid plants compared to diploids. Haploid plants had 2.5 times more stomata in pepper than diploid ones and 2.3 times more in eggplant. Similar stomatal numbers were determined in pepper and eggplant at the same ploidy levels. The average number of stomata per unit area was 27.07 in haploid pepper, 29.20 in eggplant, 10.71 in diploid pepper and 12.61 in eggplant.

Haploid and SDH plants also showed significant differences in stomatal sizes. It was determined that the mean stomatal width was 22.52 µm in haploid pepper, 17.36 µm in eggplant, and 29.50 µm and 22.32 µm in diploid pepper and eggplant, respectively, and the difference between ploidy levels was significant. The width of stomata was 1.3 times wider than haploids in DH plants in both species.

Table 1. Summary of ANOVA for pepper stomatal characteristics

Source of Variation	Degrees of Freedom	Number of Stomata		Stomata Width		Stomata Length		Number of Chloroplasts	
		Mean Square	*F* –Value	Mean Square	*F* –Value	Mean Square	*F* –Value	Mean Square	*F* –Value
Replication	99	23.16	0.85 ns	7.51	0.85 ns	15.07	0.84 ns	6.35	1.29 ns
Ploidy Level	1	13382.48	488.43**	2436.30	275.66**	7258.80	404.45**	3810.65	773.29 **
Error	99	27.40		8.84		17.95		4.93	
Total	199	92.40		20.37		52.90		24.76	

** Significant at alfa level 1% ns; non-significant

Tablo 2. Summary of ANOVA for pepper stomatal characteristics

Source of Variation	Degrees of Freedom	Number of Stomata Stoma Sayısı		Stomata Width Stoma Eni		Stomata Length Stoma Uzunluğu		Number of Chloroplasts	
		Mean Square	*F* –Value	Mean Square	*F* –Value	Mean Square	*F* –Value	Mean Square	*F* –Value
Replication	99	17.88	0.91 ns	6.98	1.30 ns	12.58	1.13 ns	3.08	1.10 ns
Ploidy Level	1	13761.41	702.22**	1234.30	229.73**	4681.06	419.10**	1152.00	410.25**
Error	99	19.60		5.37		11.17		2.81	
Total	199	87.80		12.36		35.34		8.72	

** Significant at alfa level 1% ns; non-significant

The mean stomatal length was 28.34 µm in haploid pepper plants and 40.39 µm in diploids, and 22.32 µm and 32.00 µm in eggplant haploid and diploid plants, respectively. DH plants were found to be 1.4 times longer than haploids in both pepper and eggplant in stomatal length as well as in stomatal width. However, 252% more stomata were found in pepper and 231% more in eggplant compared to DH plants in haploid plants (Table 3).

The stomatal dimensions and chloroplast images of haploid and SDH plants of pepper and eggplant are presented in Figure 1 and Figure 2.

Tablo 3. Certain stomatal characteristics of haploid and SDH plants of pepper and eggplant

Plant Species	Ploidy Level	Number of Stomata	Stomata Width µm	Stomata Length µm	Number of Chloroplasts
Capsicum annuum	Haploid	27.07 a	22.52 b	28.34 b	9.93 b
	Double Haploid	10.71 b	29.50 a	40.39 a	18.66 a
	LSD %1	1.94	1.10	1.57	0.83
Solanum melongena	Haploid	29.20 a	17.36 b	22.32 b	6.39 b
	Double Haploid	12.61 b	22.32 a	32.00 a	11.19 a
	LSD %1	1.64	0.86	1.24	0.62

Means followed by the same letter in column do not differ according to least significance difference (LSD) test (P≤ 0.01). Similarly, Abak *et al* (1998) reported that stomatal density and especially the number of chloroplasts in guard cells, in androgenic pepper plants determined to be haploid and SDH by root tip chromosome count, are reliable traits for the estimation of ploidy level from a particular genotype. Significant differences have been detected in stomatal cell density, size and number of chloroplasts of diploid and tetraploid plants of *Solanum aethiopicum* (PI 636107), known as Ethiopian eggplant. In plants whose ploidy level was confirmed by flow cytometry, stomatal length and diameter were positively correlated with ploidy level, and the number of stomata negatively correlated (Sakhanokho & Islam-Faridi 2014). Soonthornkalump *et al*. (2017) reported that the stomatal length and the number of chloroplasts per stomata were higher in tetraploid ginger plants than diploid plants, whereas the highest stomatal density was found in diploid plants. It has been reported that the stomatal diameter of diploid plants is significantly lower than that of tetraploid plants. The stomatal length increased and stomatal density decreased with the increase of ploidy level in also *Arabidopsis thaliana* plants (Robinson *et al*. 2018). Comlekcioglu & Ozden (2019) determined that the stomatal density of gooseberry plants, which were determined to be diploid and tetraploid by flow cytometry, showed significant differences according to the ploidy level, and that diploid plants had more than twice stomata density (175.53 and 77.73, respectively) compared to tetraploids. Diploid and tetraploid plants showed clear differences in stomatal sizes.

It should be kept in mind that the stomatal width may differ significantly depending on whether the stoma is open or closed

during the taking of the leaf epidermis layer for measurement. While measuring, care should be taken to measure in closed stomata or to take samples from leaves incubated for a certain period of time in a controlled environment for CO_2, humidity, and light (Monda *et al*., 2011). If the stoma is open or closed, the stoma length remains the same, making it a reliable (stable) traits (Beaulieu *et al*., 2008).

While the average number of chloroplasts in guard cells was very similar between the same ploidy plants in both species, increase of the number of chloroplasts in SDH plants was significant. It was determined that the chloroplast numbers in the haploid and DH plant guard cells were significantly different according to the ploidy levels in both pepper and eggplant. On average, 9.93 chloroplasts were counted in guard cells in haploid peppers, while 18.66 chloroplasts were counted with an increase of 87.91% in diploid plants. The number of chloroplasts in eggplant was determined as 6.39 in haploid plants, and 11.19 (75.11% more) chloroplasts were counted in DH plants (Table 3). This shows that chloroplast number is the most reliable marker among the investigated traits in determining the ploidy level.

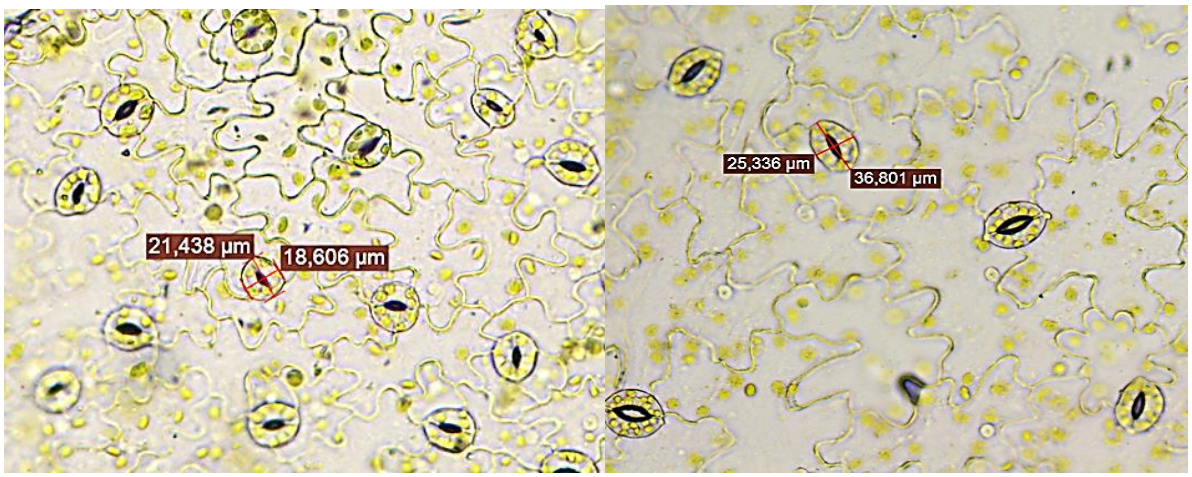

Figure 1. Stomata sizes and chloroplasts in haploid (left) and SDH (right) peppers.

Figure 2. Stomata sizes and chloroplasts in haploid (left) and SDH (right) eggplant.

Our results were fully consistent with the results of Qin & Rotino (1995) for pepper with an average of 9.93 chloroplasts per stoma in haploid and with an average of 18.66 chloroplasts in diploid. The researchers measured the number of chloroplasts per stoma and the length of the stomata in 17 androgenic plants (whose ploidy level have been confirmed by chromosome count) obtained by anther culture from three pepper genotypes. It was found that the plants grouped according to the chloroplast number were the same as the ploidy level defined by the root tip chromosome count. The number of chloroplasts in haploid plants varied between 8.3-11.0 and the average was 9.3 chloroplasts. Garcia-Arias *et al*. (2018) reported that there is a significant correlation between chromosome number and chloroplast number, which can be an indirect and effective indicator of ploidy in gooseberry plants. While there were 5.2 chloroplasts on average in haploid (2x=24) plants, it ranged from 7 to 10 (mean 9.3) in tetraploid (4x=48) donor plants and 4-32 in mixoploid plants. Mixoploid plants with 48 chromosomes presented more than 7 chloroplasts as did tetraploid plants. It has been reported that if a flow cytometer is not available, stomatal size and density will allow a quick evaluation, especially when working with large numbers of plants. Matteij et al., (1992) reported that genetically similar 2x, 4x, 6x and 8x potato plants were able to distinguish accurately by their chloroplast numbers in guard cells. Tepe et al., (2002) reported that there is a high correlation between ploidy level and the number of stomata in the leaf unit area in mint plants. When stomata counts and chromosome counts were evaluated together, it was concluded that there was a high correlation between these two traits. They found that as the number of chromosomes increased, the cell size increased and the number of stomatal cells per leaf unit area decreased.

In Brassica species, the number of chloroplasts in guard cells have been reported to be 4.2-7.8 and 7.9-13.6 for *B. rapa* haploids and diploids, respectively, 7.5-12.4 and 14.1-20.3 for *B. napus* amphihaploids and amphidiploids, respectively and 7.7-9.9, 11.7- 7.9 and 18.0-26.5 for *B. oleracea* haploids, diploids and tetraploids, respectively. No significant effect of plant vegetative or generative developmental stage or growth temperature on chloroplast number was determined (Monakhos *et al*., 2014). The size of the stomata and the number of chloroplasts were significantly increased in the polyploid plants when compared to the diploids of ginger for which ploidy confirm by flow cytometry. With the help of stomata size and chloroplast numbers, tetraploid and diploid plants were determined, but tetraploid and mixoploid plants could not be confirmed (Soonthornkalump *et al*., 2017).

Alsahlany *et al*. (2019) compared the chloroplast count, genome-wide Single Nucleotide Polymorphism genotyping and flow cytometry methods to determine ploidy in diploid and tetraploid potato genotypes. It has been reported that three ploidy determination methods give the same results for all evaluated plants and that chloroplast count can be used as a reliable and inexpensive method for determining the ploidy level.

While Kramer & Bamberg (2019) suggested chloroplast counting using iodine-based staining for potato, they found that different traits could be fast and reliable for estimating ploidy with different methods and stomatal length was as accurate as chloroplast counts, and scoring faster. They observed that the number of stomata per unit leaf area indicates the ploidy level, with tetraploids having more than diploids, but suggested that the length of guard cells is easier to measure.

When the correlation of ploidy level and stomatal characteristics was evaluated in both species, a significant positive correlation was determined between stomatal size and chloroplast number, and a significant negative correlation was determined between ploidy level and stomatal density (Table 4 and Table 5).

Table 4. Correlation between ploidy level and stomatal characteristics in pepper

	Ploidy level	**Stomata number**	**Stomata width**	**Stomata length**
Ploidi level				
Stomata number	-0.853**			
Stomata width	0.775**	-0.667**		
Stomata length	0.830**	-0.728**	0.792**	
Choloroplast number	0.879**	-0.741**	0.788**	0.817**

** Significant at alfa level 1%

Table 5. Correlation between ploidy level and stomatal characteristics in eggplant

	Ploidy level	**Stomata number**	**Stomata width**	**Stomata length**
Ploidi level				
Stomata number	-0.888**			
Stomata width	0.708**	-0.670**		
Stomata length	0.816**	-0.787**	0.790**	
Choloroplast number	0.815**	-0.742**	0.710**	0.807**

** Significant at alfa level 1%

In both species, a negative relationship was found between the number of stomata and the size of stomata and the number of chloroplasts. It was determined that there was a positive relationship between stomatal sizes and the number of chloroplasts in guard cells, and these properties were stable and reliable parameters. It has been confirmed once again that ploidy is an important factor in stoma characteristics. PC1 (principal component) 82.9%, and PC2 8.5% constituted 91.4% of the total variation between stomatal characteristics and ploidy level in pepper and PC1 81.3%, and PC2 8.3% constituted 89.6% of the total variation in eggplant (Figure 3 and Figure 4).

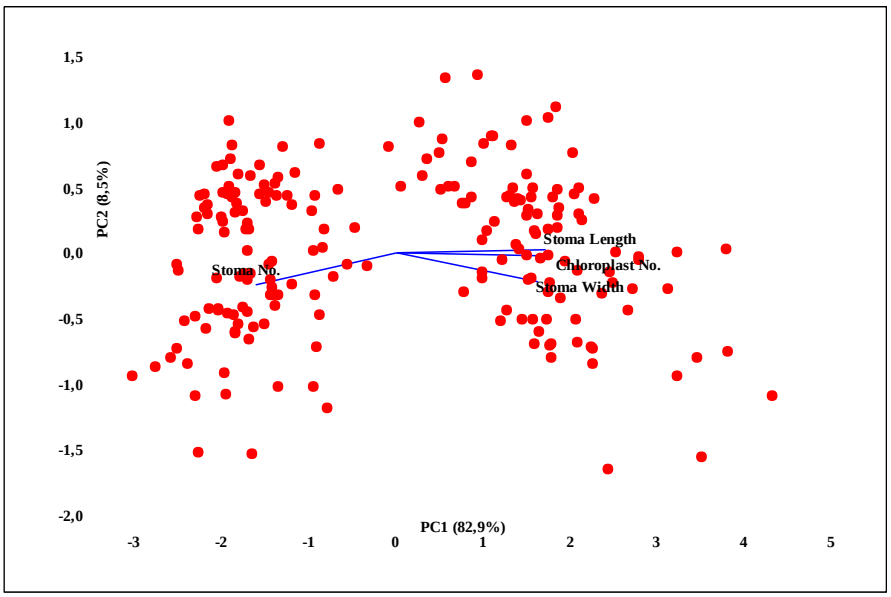

Figure 3. The relationships between ploidy level and stomatal characteristics according to biplot analysis of principal components in pepper

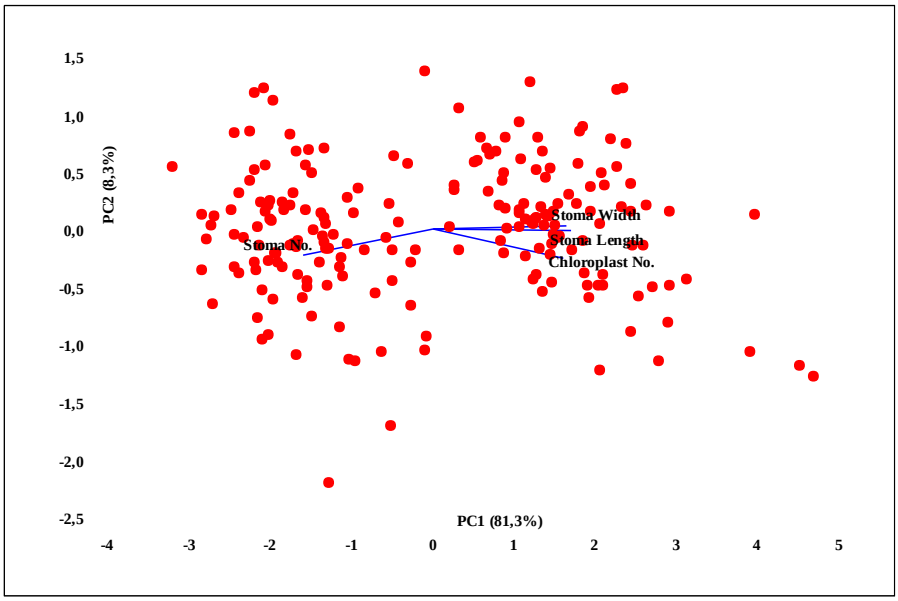

Figure 4. The relationships between ploidy level and stomatal characteristics according to biplot analysis of principal components in eggplant

Conclusions

Haploid plant needs to undergo chromosome duplication to obtain a fertile plant. For successful use of DH plants in plant breeding, it is important to confirm the status of the ploidy level. The chromosome doubling can be spontaneous or induced. There are advantages and disadvantages to using any method to determine ploidy. Chromosome counting is undoubtedly the most reliable method for determining the ploidy level in plants.

However, it was concluded that stomatal characteristics, especially chloroplast numbers, can be used safely in making the first groupings in case of a large number of plants. Pre-ploidy screening based on stomatal characteristics of androgenic plants is useful for reducing population size and can accelerate breeding programs. In the absence of a flow cytometer, stomatal features other than mixoploids can be used to successfully confirm haploid and DH plants. Allows a quick and reliable evaluation when working with a large number of plants. Identifying haploid and diploid plants with epidermal stoma characteristics is faster and cheaper than chromosome counting or flow cytometry, and is an important alternative that does not require expensive equipment.

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Author Contributions: HB; planned of overall research; NC, SSE: statistical analyses, writing-review and editing: FNA, MAY; collected and prepared of samples and laboratory analysis.

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Identification, genetic diversity and biological control of dollar spot disease caused by *Sclerotinia homoeocarpa* on golf courses in Turkey

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Abstract

Dollar spot caused by *Sclerotinia homoeocarpa*, can create damage to warm and cool-season turfgrasses, is a significant disease. In the study, 13 golf courses in 5 provinces of Turkey were surveyed for dollar spot disease caused by *Sclerotinia homoeocarpa*. During the survey, samples were taken from leaves with cream spots, framed by a reddish-brown edge and from areas of light yellow small patches. Identifications of *Sclerotinia* isolates were performed by DNA sequencing analysis. Five bacterial strains were examined to detect their antifungal influences against the dollar spot by using the seed coating method in greenhouse conditions. As a result of isolations from infected plants from 13 golf courses, 7 *Sclerotinia homoeocarpa* (Sh) isolates were obtained. Pathogenicity tests performed in the greenhouse ranged from 81.03% to 90.75%. The consequence of biologic control studies, *Pseudonomas putida* 88cfp, *Pseudomonas putida* 166fp, and *Bacillus cereus* 44bac were found efficient the ratio of 92.99%, 88.71%, and 87.50% respectively. In further studies, effective bacterial strains (88cfp, 166fp, and 44bac) should be carried out in large golf courses.

Keywords: *Sclerotinia homoeocarpa*, turfgrass, virulence, biological control

Introduction

In recent years, Turkey has become a golf center by bringing together golf lovers around the world with international golf facilities and tournaments. There are golf courses in the provinces of Muğla, Istanbul, Ankara, Aydın, and Samsun, especially in Antalya. Particularly Belek Town of Antalya creates a unique golf tourism potential with its cultural, historical, and natural structure as well as qualified golf courses and facilities and hosts international tournaments. The establishment and protection of such areas around the world have become a million-dollar industry. Maintaining and ensuring the continuity of such turf areas requires great effort, cost, and expertise. Millions of dollars are spent annually in the world and in Turkey in recent years only for the maintenance of golf courses and the control against diseases. In addition, additional expenses are made to repair and renew heavily damaged grasses.

These efforts and expenses can only be paid for if the plants are healthy and the grass fields are long-lasting. For all these reasons, there is the excessive and indiscriminate use of fungicide in such areas, especially in Turkey. It is thought that these practices will increase problems such as soil and groundwater pollution.

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One of the diseases that has been a problem for some years in the golf courses of the south and west coasts of Turkey and requires struggle is the 'dollar spot' disease. However, a comprehensive survey of the disease in golf courses in Turkey was carried out for the first time with this study.

Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) is a major disease that affects grass areas. The disease causes crucial damages, especially to highly preserved golf courses (Goodman and Burpee 1991, Viji et al. 2004; Liberti et al. 2012). The first symptoms of the disease are yellow-green blotches on the leaves. Later, it turns into cream-colored spots with a reddish-brown border and these spots get larger over time. Dollar spot symptoms vary with the types of grass and the nature of the turf. In short mown areas such as golf courses, the disease is manifested as coin-sized, round, cream-light yellow patches with a diameter of 2 to 8 cm. For this reason, the disease has been called the "Dollar Point" disease (Smiley 1992). As a result of recent technological advances, researchers have observed that there are subtle morphological and genetic differences between species and within the genus of *Sclerotinia* and there are subtle differences in reproductive structures and they agree that the identification and taxonomy of this species should be reconsidered (Jackson 1973; Kohn and Grenville 1989; Novak and Kohn 1991; Carbone and Kohn 1993; Holst-Jensen et al. 1997; Vargas and Powell 1997). In a study by Liberti et al. (2012) they separated *S. homoeocarpa* isolates into F-type and C-type based on the morphological characteristics of their fungus and they supported it molecularly Salgado-Salazar et al. (2018) reported that four different species that give rise to "dollar spot" disease in grass areas. These species that are in the genus *Clarireedia* were named *C. jacksonii, C. monteithiana, C. homoeocarpa,* and *C. bennettii*. The use of fungicides has increased in turfgrass areas, especially in golf courses in the world. This situation led to pollution of the environment, groundwater, and seas, deterioration of human health, and formation of pathogen endurance (Balcı and Gedikli 2012). For these reasons, alternative methods are needed which are less harmful to the environment. The use of beneficial microorganisms can be alternative or complementary practices to the existing harmful practices used in the control of the disease. The most prevalent beneficial microorganisms used in the control of plant pathogens are *Bacillus* and *Pseudomonas* species. This study, it was aimed to identify *S. homoeocarpa* isolates isolated from golf courses in Turkey, to determine their genetic differences and virulence, and to determine the effects of some local bacterial isolates against the disease under greenhouse conditions.

Materials and Methods

Survey and isolation of the pathogen

Sixty-two diseased turfgrass plants were picked up from the 13 golf courses in Antalya (9), İstanbul (1), Ankara (1), Aydın (1), and Muğla (1) Provinces in 2015. Infected plant samples were surface disinfested for 40-50 s in 1 % sodium hypochlorite (NaOCl), rinsed for 40s in sterile water, and put PDA (Potato Dextrose Agar) (Difco, USA) added 50 mg gentamicin per ml. They were then kept at 25°C for 4-5 days under fluorescent light conditions for 12 hours day and 12 hours night.

Pathogenicity Tests

The tests were carried out in a greenhouse with all isolates. Twenty creeping bentgrass (*Agrostis stolonifera* L.) seeds were sowed in the pots (10 cm in diameter) included the sterilized (two consecutive days at 121 ºC for 45 minutes) soil, sand, and burnt fertilizer mix (2:1:1). After emergence, the grass was cut to a height of 1,5-2 cm.. Isolates were incubated on PDA (Difco, USA) at 25°C for 1 week. Inoculums were prepared consisted of mixing 500 ml of rye grains with 100 ml of deionized water in heat-resisting bottles. Then, these bottles with rye grains were autoclaved twice at 90ºC for 90 min on each of 2 consecutive days. Each bottle was inoculated with 15 discs (5mm diameter), containing the fungi, cultured for 8-10 days at 25 ºC under fluorescent light at 12-h day and night cycles. After incubation, five infested rye grains have put on the surface of the tall fescues. Pots were covered with polyethylene bags after inoculation. The pots were placed under continuous at 27±1 °C and relative humidity of 92% to 95%. There were three replicate pots for treatment. The control consisted of pots without inoculum. One week after inoculation, the bags were removed, and severity of disease was evaluated on a scale of 0 to 5: 0= 100% healthy plants, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= 51-80% and 5= 100% diseased plants (Mocioni et al. 2011). Disease severity values were calculated using these scales and the following formula.

Disease Severity= Σ [(the number of samples in the scale with different disease grades x scale value) / highest scale value x total number of samples observed]×100 (Townsend and Heuberger 1943)

Bacterial isolates

The bacteria (44bac, 88bfp, 88cfp, 215b and166fp) used in this study were obtained from tomato and cucumber rhizosphere in a previous study (Aşkın 2008), and 44bac and 88bfp were found effective on *Sclerotium rolfsii*. Diagnosis of the five antagonistic bacteria used in the study was done by the molecular method in our previous study (Ünal et. al. 2019).

Molecular identifications of fungal isolates

Fungal DNA isolation was performed by using QIAGEN Blood and Tissue Kit, according to the manufacturer's procedure. The polymerase chain reaction was done using primers ITS-1 and ITS-4 (White et al. 1990). PCR (The polymerase chain reaction) was performed in a 50 µl reaction mixture containing 25 µl GoTaq® Hot Start Green Master mix (2×) (Promega, USA), 13 µl sterile double-distilled water, 4 µl BSA, 2 µl forward primer (10 mM), 2 µl reverse primer (10 mM), 4 µl template DNA. The PCR cycling protocol consisted of initial denaturation at 94 ºC for 4 min, followed by 30 cycles of 94 ºC for 45 s, 55 ºC for 45 s, and 72 ºC for 2 min, and a final elongation step of 72 ºC for 10 min.

Molecular identifications of bacteria (166fp, 215b, 44bac, 88bpf, 88cpf) were made in another study (Ünal et al. 2019).

Genetic diversity

In this study, the sequenced isolates' ITS regions composed of ITS1, ITS2, and 5.8S were aligned by using ClustalX, and also phylogenetic tree was constituted by using the Maximum Likelihood method and Jukes-Cantor model (Jukes and Cantor 1969) in MEGA 7 (Kumar et al. 2016). The tree was constituted to scale, which was based on branch lengths measured in the number of substitutions per site. This analysis involved 8 nucleotide sequences. *Sclerotinia sclerotium* isolate was used as an external isolate. Complete deletion option and bootstrap test with 1000 replications were used in this program (ie. all positions containing gaps and missing data were eliminated).

Bacterial inoculum

Suspensions of 1×10^8 cfu/mL concentration were prepared from each bacterial strain developed in PDW (Potato dextrose water) and their measurements were made with a spectrophotometer. After sterilizing the seed surfaces, they were immersed in bacterial solutions and kept for 12 hours (Aşkın 2008).

Biocontrol assays

Biocontrol assays were performed using turfgrass seeds mixture containing cv. *Lolium perenne, Festuca arundinacea, Cynodon dactylon* and the virulent *S. homoeocarpa* species (Sh 4). Sterilized garden soil: burnt manure: river sand (2: 1: 1) mixture was used in the experiments. The fungal inoculum was prepared by developing in rye grains as in pathogenicity study. Bacteria were applied by coating to the seeds. Studies were conducted on both sterilized and non-sterilized soils in three applications: (1): Planting uncoated grass seeds in inoculated soils (positive control) (2): Planting uncoated grass seeds in non-inoculated soils (negative control), (3): Planting coated grass seeds in inoculated soils.

Coated with the antagonist bacteria and uncoated grass seeds were sowed at a depth of 2 cm as 30 seeds per 10 cm in diameter pots. The pots were put in greenhouse conditions containing 12 hours of light, 12 hours of darkness, and 24 ± 1 ºC temperature. When the plants germinated and reached 1 cm, five infested rye grains were placed on the surface of the plants. Pots were covered with a polyethylene bag after inoculation. The pots were placed under continuous at 25°C and relative humidity of about 95%. After the inoculation, 0-5 scale was used after 25 days according to disease development status (Mocioni et al. 2011). These scale values were converted to disease severity values using the diseases severity formula.

Statistical analysis

Variance analyzes were carried out using SPSS GLM statistical program to determine the differences in both virulence level of isolates and disease rates in biocontrol assay. Disease ratios obtained according to scales were applied to the Towsend-Heuberger formula to calculate disease severity, and the activity of bacterial isolates with the Abbott formula was determined from disease severity values. Disease severity was compared by Tukey multiple comparison test on these ratios.

Results

During the survey phase of the study, a total of 62 samples were collected from 13 golf courses in 5 provinces in Turkey. As a result of isolations from leaves that have cream color blotches surrounded by dark red color (Fig. 1a) and from plants on small yellowed circular patches (Fig. 1b), seven *S. homoeocarpa* isolates were obtained that was based on both colony morphologies and rDNA internal transcribed spacer (ITS) region sequences (Table 1). The colony color of all isolates on PDA was initially white but after 7-10 days became black-white color (Fig. 1 c). 'Y-shaped branching ' in fungus hyphae was observed (Fig. 1d) on a light microscope.

Figure 1. *Sclerotinia homoeocarpa*: symptoms on leaves on 'Rough' area (a) 'dollar spot' symptoms on 'Green' area (b) in a golf course, colony appearance on PDA (c), 'Y-shaped branching' on hypha (d)

As a result of the study, amplicons displayed by gel transilluminator were found to be approximately 550 bp which is specific to *S. homoeocarpa.* The sequences of all isolates were 99-100% similar to those of *S. homoeocarpa* deposited in the NCBI database. Average disease severity values of *S. homoeocarpa* isolates in pathogenicity tests carried out ranged from 81.03 to 90.75%. The most virulent isolate was Sh 4 isolated from Antalya province with 90.75% disease severity (Table 1).

Table 1. Origin, turf composition, and disease severity values of *Sclerotinia homoeocarpa* (Sh) isolates isolated from golf courses

Isolate numbers	Origin	Turfgrass Composition	*Disease severity (%)
Sh1	Antalya	*Cynodon dactylon* *Lolium perenne* *Poa trivialis*	90,66±2,54a
Sh2	İstanbul	*Festuca arundinacea* *Lolium perenne* *Poa pratensis* *Agrostis stolonifera*	86,18±2,34ab
Sh3	Antalya	*Agrostis stolonifera* *Cynodon dactylon*	81,03±1,84b
Sh4	Antalya	*Agrostis stolonifera* *Cynodon dactylon*	90,75±2,02a
Sh5	Muğla	*Cynodon dactylon* *Lolium perenne* *Poa trivialis*	81,10±3,03b
Sh6	Antalya	*Cynodon dactylon* *Lolium perenne* *Poa trivialis*	86,70±1,41ab
Sh7	Antalya	*Cynodon dactylon* *Lolium perenne* *Agrostis stolonifera*	90,70±0,87a

*There is no difference between the values expressed in the same letter, P<0.0001

Data obtained with ITS 1 and ITS4 primers on the 7 isolates of *Sclerotinia homoeocarpa* were used to produce the dendrogram shown in Fig. 2. *S. homoeocarpa* species took part in the same cluster on the tree.

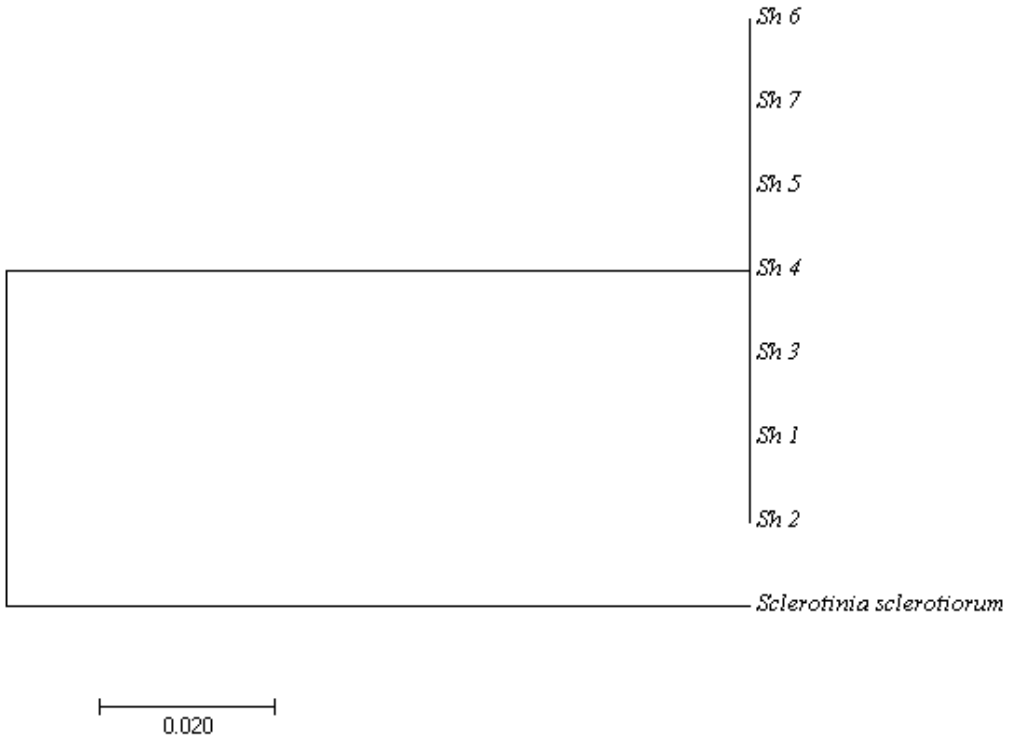

Figure 2 Maximum likelihood tree showing the relationship among *Scleratinia homoeocarpa* (*Sh*) isolates obtained from turfgrasses in golf courses

In greenhouse experiments, all bacterial isolates were found to be effective when compared to the severity of the disease in control. The lowest disease severities were measured (P<0.0001) in the treatment of *Pseudomonas putida* 88cpf, *Pseudomonas putida* 166fp, and *Bacillus cereus* 44bac as 6.13%, 9.87%, and 10.93% respectively. The highest disease severity was measured in the treatment of *Paenibacillus* sp. 215b as 37.33% when compared to the disease severity value in control. The highest protection effect was observed on isolate *Pseudomonas putida* 88cpf (92.99%). The isolates *Pseudomonas putida* 166fp (88.71) and *Bacillus cereus* 44bac (87.50) followed it (Fig. 3, Table 2). During the evaluations, it has been observed that antagonist bacteria have a positive effect on plant growth and quality.

Table 2. Effects of bacterial strains against the disease of *Sclerotinia homoeocarpa* on turfgrass

Treatments	**Disease severity *(%)**	**Efficacy (%)**
Pseudomonas putida 88cpf	6.13 ± 1.578^{e}	92.99
Pseudomonas putida 166fp	$9.87 \pm 1.578^{c\,d}$	88.71
Bacillus cereus 44bac	10.93 ± 1.578^{cd}	87.50
Stenotrophomonas rhizophila 88bpf	17.07 ± 1.578^{c}	80.48
Paenibacillus sp.215b	37.33 ± 1.578^{b}	57,32
(+) Control	87.47 ± 1.578^{a}	-
(-) Control	0.00	

*There is no difference between the values expressed in the same letter, P<0.0001

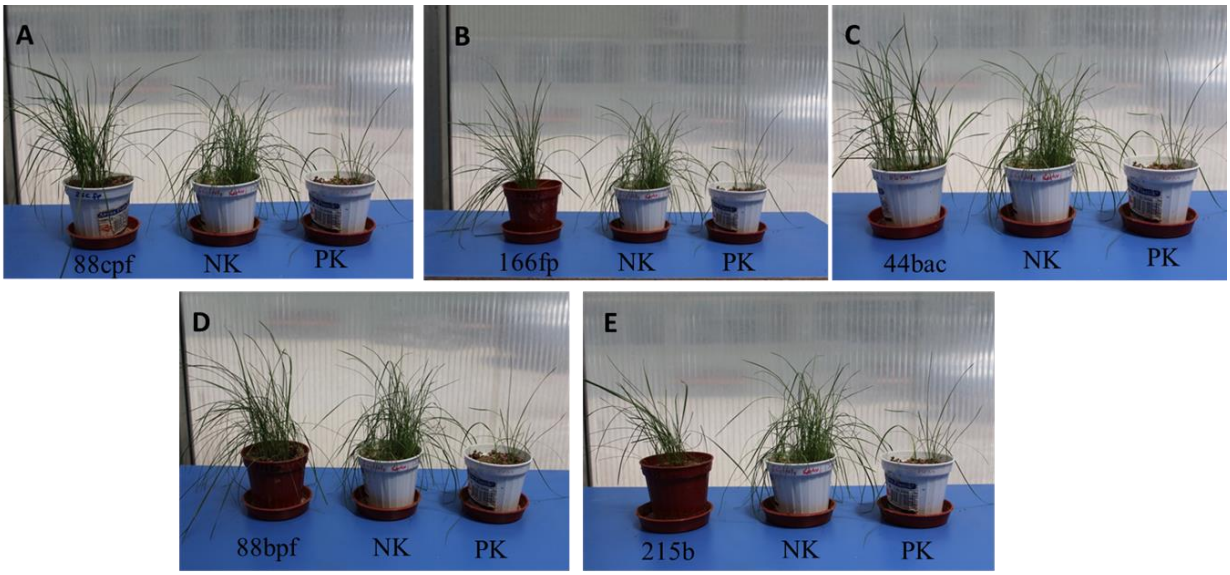

Figure 3. Effects of bacterial strains against *Sclerotinia homoeocarpa* in greenhouse experiments: **A**. *P. putida* 88cpf, **B.** *P. putida* 166fp; **C.** *B. cereus* 44bac, **D.** *Stenotrophomonas rhizophila* 88bpf, **E.** *Paenibacillus* sp. 215b

Discussion

In the studies on the control of the 'Dollar spot' disease in the world, various biological control strategies have been investigated besides chemical control. These studies are mostly in the form of practice of organic materials and nutrients to alert as living naturally microorganisms in the phyllosphere or direct application of bacteria and fungi that are known to suppress the disease. Although most biological control strategies evaluated to date are less efficient than fungicides, some of them deserve further research. In some of these studies, some commercial organic materials such as organic manure, compost, and mud have been investigated for the suppression of dollar spot diseases, and some compost, organic fertilizers, and mud have been observed to decrease the severity of dollar spot disease, but it has been reported by the researchers that further investigation is needed to clarify the effects of these materials in reducing the *S. homoeocarpa* and to determine the mechanisms of action. (Landschoot and McNitt 1997; Liu et al. 1995; Nelson and Craft 1991a; Landschoot and McNitt 1997). The effects of many fungal and

bacterial microorganisms have also been investigated for the control of the disease and different results have been obtained. *Trichoderma* species were mostly used in biological control studies of dollar spot disease as fungal microorganisms. *Trichoderma harzianum* and *Fusarium heterosporium* were detected as the most effective fungi. In a study conducted in Turkey, on the biological control of the disease, *Trichoderma harzianum* (TRIC8) isolate was found to be 65.60% effective against dollar spot disease in field conditions (Aşkın et al. 2019). In studies with antagonist bacteria, the highest effect was detected in *Pseudomonas* species. *Pseudomonas fluorescens* Migula and *P. lindbergii* ATCC 31099 strains were found effective to dollar spot disease on Kentucky bluegrass (*Poa pratensis* L.) under controlled conditions (Hodges et al. 1994; Rodriguez and Pfender 1997). In this study, it was concluded that pyrrolnitrin, an antibiotic produced by *P. fluorescens*, inhibited *S. homoeocarpa*. (Rodriguez and Pfender 1997). Similarly, in this study, *Pseudonas putida* (88cfp) and *Pseudomonas putida* (166fp) were among the most effective bacterial species. In this study, apart from *Pseudomonas* spp., a *Bacillus cereus* isolate (44 Bac) also showed high efficacy against the disease. In some studies on the biological control of the disease, *Enterobacter cloacae* (EcCT-501) was effective only in young grass areas (Nelson and Craft 1991b), while *Streptomyces* sp (Schumann and Reuter 1993). The use of "transferable hypovirulence" to suppress the disease has been reported (Zhou and Boland 1997, 1998).

Conclusions

With this study, *S. homoecarpa* fungi causing "Dollar spot" disease was isolated and identified in large golf areas of Turkey for the first time. In addition to these, virulences of these isolates, their phylogenetic analyses, and biologic control opportunities with some domestic bacterial isolates were investigated. In further studies, biological control studies of the disease using these bacterial strains should be carried out in large golf courses. Formulation studies of effective isolates in the field should also be carried out.

Authors' contributions

FU survey, isolation, identification, pathogenicity, phylogenetic analysis, biological control studies of *S. homoeocarpa* isolates. Designed the study and wrote the manuscript with input from all authors, AA and EK provision of bacterial isolates and biological control studies, MY analyzed the data (Statistic analyses), read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests

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An investigation on the effect of nano-ZnO application on cadmium phytoextraction by safflower

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Abstract

Nowadays, soils contaminated with heavy metals are one of the biggest environmental pollution problems in the world. Phytoextraction method is the most effective and well-known plant remediation method that can be used to clean-up agricultural soils contaminated with heavy metals. Nanoparticle (NP) applications have recently been introduced to remove pollutants, promote plant growth and improve pollutant phyto-availability to improve the effectiveness of this method. In this study, it is aimed to use phytoextraction method and nanomaterial together for the cleaning of cadmium (Cd) contaminated growth media and to investigate the effects of nanomaterial on plant properties. For this purpose, a hydroponic culture was planned and zinc oxide nanoparticles (ZnO-NP), a nanomaterial which was determined by OECD as a priority, was used for the experiment. As safflower (*Carthamus tinctorius*) can be grown in different ecological conditions, it was selected. Safflower seeds were germinated in a mixture of peat-perlite (1:1), and after 2-3 leaves, they were transferred to the Hoagland nutrient solution. In order to see the effects of Cd × ZnO-NP applications, morphological observations of the plants were made and chlorophyll contents were measured before the harvest by applying ZnO-NP and (0-3-6 mg / L) Cd in increasing doses (0, 5, 10 mg / L) to the nutrient solution. Plants were harvested 20 days after the transplanting. Shoot and root dry weights of plants, Zn, and Cd concentrations were determined. The results showed that Cd accumulation of the plant increased due to increasing doses of ZnO-NP. In the shoot of the safflower plant, Cd has accumulated 5.2 to 8.7 times more Cd than the hyperaccumulation critical threshold value (100 µg / g). The research showed that the safflower can have a promising Cd phytoremediation potential in the environment.

Key words: Phytoextraction, cadmium, ZnO nanoparticle, safflower, hydroponic culture.

Introduction

Soil is the primary source of agricultural production. Unfortunately, soils are polluted especially because of human activities (misuse of soils, industrial activities, excessive use of chemical fertilizers and pesticides, erosion, polluted waters, nuclear accidents, urban wastes, mining, etc.). Because of this, agricultural production areas are decreasing, ecological balance is disrupted and pollutants are included in the food chain and threatening living organisms or causing their extinction (Esetlili and Anaç, 2015). The most common pollutants in agricultural soils are heavy metals, which are among the inorganic pollutants (Çağlarırmak et al., 2010; Dağhan and Öztürk, 2015; Köleli et al., 2018). Unlike organic pollutants, heavy metals remain intact even if they change form in their environment (Köleli et al., 2018). Since these pollutants persistent in the soil, they limit the growth of microorganisms and plants, inhibit the development of plant roots and leaves, and can destroy the soil ecosystem health. Heavy metals enter into the food chain through plants from contaminated soil and water; also threaten animals and human beings' life (Zhu et al., 2019).

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Heavy metals such as lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni), mercury (Hg), which the living and non-living environment are exposed in nature are defined as metals with a density higher than 5 g/cm^3 (Seven et al., 2018). Cadmium is one of the most dangerous heavy metals and it is a non-essential element for plants. This heavy metal is presented seventh of substance in the Substance Priority List (included 275 substances), which is prepared by the Agency for Toxic Substances and Disease Registry of United State (US-ATSDR) (ATSDR, 2021). In general, as little as 0.05 to 0.2 mg/kg dry weight Cd concentrations cause toxic effect in plant tissues (Kabata-Pendias, 2000). Over that, doses of leaf chlorophyll pigments damage, chlorosis and necrosis appears in plant leaves, and biomass production of shoot and roots decreases, plant length reduces in plants (Rizwan et al., 2019a). Therefore, especially the problem of Cd contaminated soil should be addressed urgently.

Phytoextraction method is used for cleaning soils contaminated with heavy metals. This method is more economical than many methods involving physical, chemical, thermal and biological processes, is environmentally friendly, does not require special equipment, and allows the reuse of the reclamation area. The plant which is used in phytoextraction should grow fast, have deep rooting and high shoot biomass production, accumulate high metal in its harvestable parts, tolerate heavy metal accumulated and be easily harvested. This method is very effective for low pollutant concentrations (Daghan et al., 2012; Köleli et al., 2018; Dağhan, 2019).

Phytoextraction is known as an environmentally friendly cleaning method. However, it takes a long time to clean-up the heavy metal contaminated areas. Therefore, the combined use of plants and nanomaterials in environmental management has been popular approach, since some nanomaterials can support plant seed germination and plant growth (Song et al., 2019). Nanomaterials could play a role in phytoremediation technology by removing pollutants, promoting plant growth, and increasing pollutant's phyto availability (Song et al., 2019).

Zinc oxide nanoparticles are the most well-known and widely used nanoparticle in all metallic nanoparticles. Due to their high metal adsorption capacity, ZnO-NPs are used to rehabilitate metal-contaminated water and soil (Hua et al., 2012; Tang et al., 2014; Hussain et al., 2021). The appropriate concentration of ZnO-NPs can significantly increase the phyto-accumulation potential of heavy metals in metal-contaminated water sources. It has been reported that ZnO-NPs can be used as potential supports for phytoremediation in heavy metal-contaminated areas (Hussain et al., 2021).

The hypothesis of this study was using ZnO-NP to stimulate plant growth while alleviating Cd toxicity on plant and on the other side enhancement Cd uptake by plants. For this aim phytoextraction method and ZnO-NPs were used together to clean-up Cd from contaminated growth media to investigate the effects of ZnO-NPs treatments on Cd phytoextraction by safflower plants.

Materials and Methods

Material

In this study, the ZnO-NPs were synthesized with the sol-gel method (Gokhale et al., 2009), which was modified by Dr. Birol Karakaya. Zinc oxide nanoparticle was chosen as a nanoparticle material in this research because it has been determined by the Organization for Economic Cooperation and Development (OECD) as a priority nanomaterial (OECD, 2013).

Dinçer variety of safflower (*Carthamus tinctorius*) was used as plant material in the experiment. Plant seeds were purchased from Eskişehir Transition Zone Agricultural Research Institute.

Method

Characterization of ZnO-NPs

Structural properties, shapes, particle sizes and elemental compositions of ZnO-NPs, which were synthesized according to the sol-gel method, were determined by using scanning electron microscopy with energy-dispersive X-Ray Spectroscopy (SEM-EDX) (Carl Zeiss, Supra 55).

In addition, the crystal structures of ZnO-NPs were determined by using X-ray diffraction analysis (XRD) RadB-DMAX II Computer Controlled X-ray Diffractometer device.

Hydroponic experiment

Safflower seeds were germinated in the peat and perlite mixture (1:1, w/w) medium. When the seedlings were had 2-3 leaves and rooted slightly, they were transferred into the Hoagland nutrient solution (Hoagland and Arnon, 1950) medium (pH 5.2). The macro and micro nutrients and their concentrations in the Hoagland nutrient solution were as follows: 1 mM KH_2PO_4, 3 mM KNO_3, 0.25 mM $MgSO_4.7H_2O$, 2 mM $Ca(NO_3)_2.4H_2O$, $2.5x10^{-2}$ mM KCl, 1 µM $MnSO_4.H_2O$, 0.25 µM $CuSO_4.H_2O$, 0.25 µM $(NH_4)_6Mo_7O_{24}$, 0.125 µM H_3BO_3, 0.1 mM Fe-EDTA.

The seedlings were transferred to the 4.5 L polyethylene pots full with Hoagland solution. Safflower seedlings were placed separately as 2 plants in each pot. The experiment was set-up in completely randomized design with three replications in the factorial arrangement (3x3). The nanomaterial was applied to Hoagland nutrient solution in the form of ZnO and at doses of 0, 5, 10 mg/L. Cadmium was applied to the solution in the form of $CdSO_4.8H_2O$ at doses of 0-3-6 mg/L. Cadmium doses were determined considering the toxic limit value (3 mg/L) of Cd in the plant. The nutrient solution was changed every 2-3 days. Plants were grown in nutrient solution medium under controlled conditions (16/8 hours light/dark, 25/20 ^oC temperature and 60% humidity, light intensity 10 Klux) for 20 days. Before harvest, the chlorophyll content of leaf was measured with Konica-Minolta SPAD-502 chlorophyll meter as soil plant analysis development value (SPAD value) (Dağhan, 2018).

The plant samples were harvested as the shoot and root, washed with distilled water, and then dried at 65 °C until they reach a constant weight. Then the samples were ground and homogenized by means of agate mill (Retsch MM-301 Mixer Mill, Retsch, Nordrhein-Westfalen, Germany) for analysis.

Plant analysis

The ground plant materials were digested with acids (HNO_3 and H_2O_2) in the microwave oven (MarsXpress 6 CEM, Matthews, USA) (Müftüoğlu et al., 2012). In this method, 0.2 g plant sample were digested for elemental analysis with 2 mL deionized water, 2 mL 35% H_2O_2 and 5 mL 65% HNO_3 for 45 minutes in the microwave oven.

The total Cd and Zn concentrations of the digest were measured by using Atomic Absorption Spectrophotometer (AAS, NovAA 350, Analytic Jena).

Statistical analysis

The data set were subjected to ANOVA by using the SPSS-20 statistical analysis package program. The mean separation were performed by the Duncan' multiple range test at $p<0.05$ probability level.

Results and Discussions

Characterization of ZnO-NPs

Scanning electron microscope (SEM) image of the ZnO-NPs material is given in Figure 1. According to the image, ZnO-NPs have spherical shape and the size of ZnO-NPs was below 100 nm and the smallest size was 44.69 nm. In the EDX analysis results, mostly Zn and ZnO peaks were observed and most of them were in ZnO form (Figure 2).

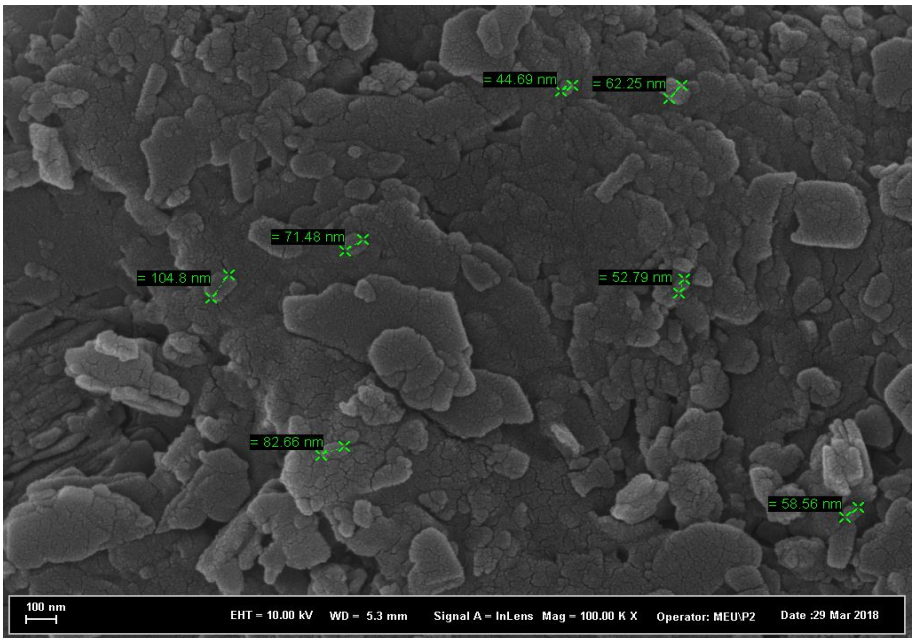

Figure 1. Scanning Electron Microscope (SEM) image of ZnO-NPs (×100K)

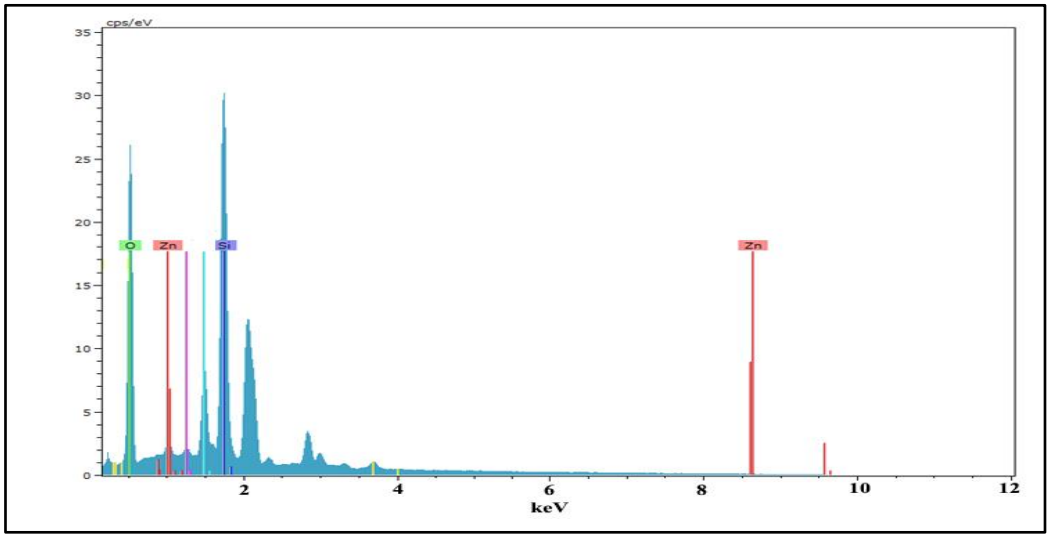

Figure 2. SEM-EDX spectrum of ZnO-NPs

The crystal structures of ZnO-NPs were analyzed by XRD (Figure 3). The results show that the corresponding sharp peaks at 100°, 002 101°, 102° 200°, 110° and 112° at 2θ are the peaks showing the crystal structure of ZnO-NPs (Figure 3). Debye-Scherrer's equation (Eren ve Baran, 2019):

$$D = K\lambda / (\beta \cos\theta) \quad \text{(Eq:1)}$$

From this equation, the size of the nanoparticle crystals was calculated as 24.89 nm, which is consistent with the zeta potential results. As expressed in inequality, D= Size of the particle (nm), K= Constant (0.90), λ = Wavelength of X-ray (1.5406 °A), β = Half the value of the highest peak as radian unit (FWHM), θ = Refraction angle.

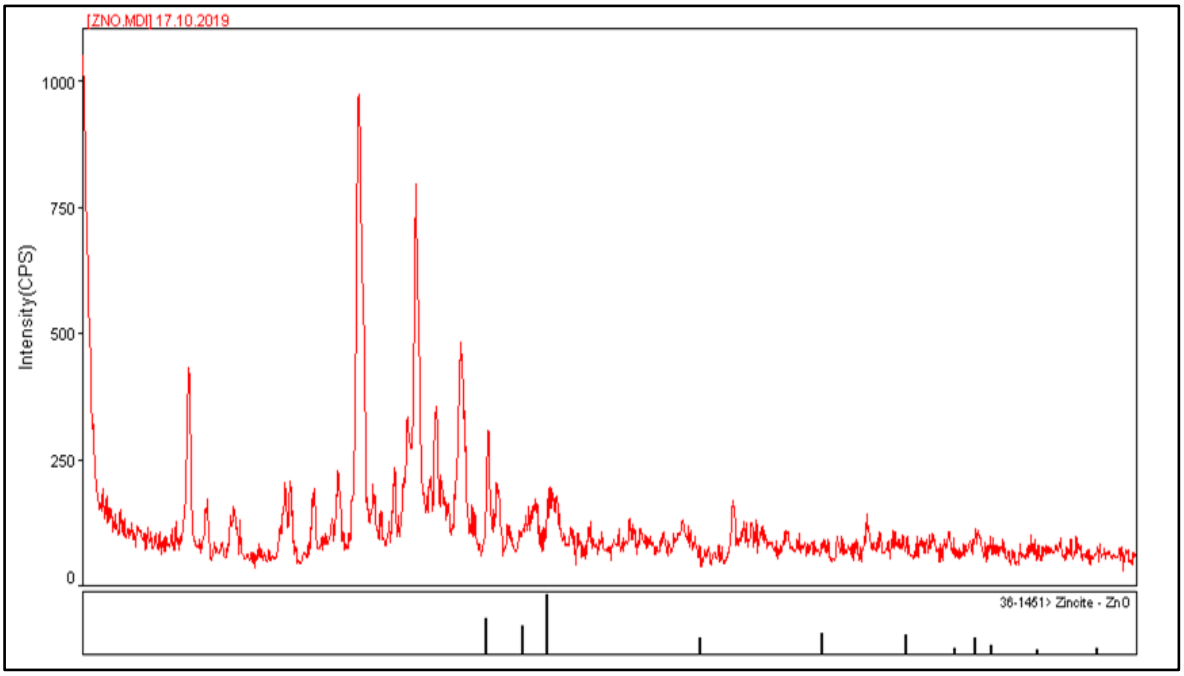

Figure 3. ZnO-NP synthesized by XRD green light spectra method

Morphological Observation

The plants treated with increasing doses of Cd without ZnO-NP were compared to the control; the severe toxic effect of Cd was observed (Fig. 4. A). While the control plant had high shoot biomass with healthy leaves, it was observed that chlorosis and necrosis appeared in Cd-treated plants (Fig. 4. A). In plants treated with 5 and 10 mg ZnO-NP/L, growth reduction was observed in plants compared to control, severe chlorosis and stunting growth at 3 mg Cd/kg doses, and severe necrosis and stunting at 6 mg Cd/kg doses were apparent (Fig. 4. B and C).

On the other hand, it was observed that the plants treated with increasing doses of ZnO-NP and without Cd had grown well and their leaves were dark green (Fig. 4. D). However, it has been determined that the plants become stunted, the leaves become smaller, and very severe chlorosis appears in the old leaves with Cd applications (Fig. 4. E and F). The applications of ZnO-NP had positively affected the plant growth while Cd had negatively affected the plants growth.

Chlorophyll Content

The effects of ZnO-NP (0, 5 and 10 mg/L) and Cd (0, 3 and 6 mg/L) application at increasing doses on the chlorophyll content, root and shoot dry weights of the safflower plant were investigated in hydroponic culture experiment conducted under the control environment conditions.

Table 1 shows that ZnO-NP and Cd applications were significant ($p<0.01$) according to the variance analysis results of leaf chlorophyll (SPAD Unit) contents of safflower plant. The highest chlorophyll content (42.9 SPAD units) was obtained from plants treated with 10 mg/L ZnO-NP without Cd treatment. The lowest chlorophyll value (6.23 SPAD units) was obtained from 6 mg/L Cd application without ZnO-NP. Exposure of ZnO-NPs has positively affected leaf chlorophyll content and morphological observations supported this result.

Dry Weights

Higher biomass production of shoot and root under metal stress is the one of the main criteria for determination of phytoremediation potential of a plant. The effect of ZnO-NP and Cd applications on the shoot dry weights of the safflower plant was found to be statistically significant (Table 1). The highest dry weight of the shoot was obtained at 5 and 10 mg/L ZnO-NP (0.505 g and 0.498 g, respectively) doses without Cd treatment. The effects of ZnO-NP and Cd applications on the dry weight of shoots were found to be statistically significant at $p<0.01$. The highest root dry weight (0.118 g/pot) was determined in control plant without Cd treatment. In addition, it was determined that low-dose ZnO-NP applications increased the shoot biomass ($ZnO\text{-}NP_0$: 0.422, $ZnO\text{-}NP_5$: 0.505 and $ZnO\text{-}NP_{10}$: 0.498) compared to control plants (Table 1).

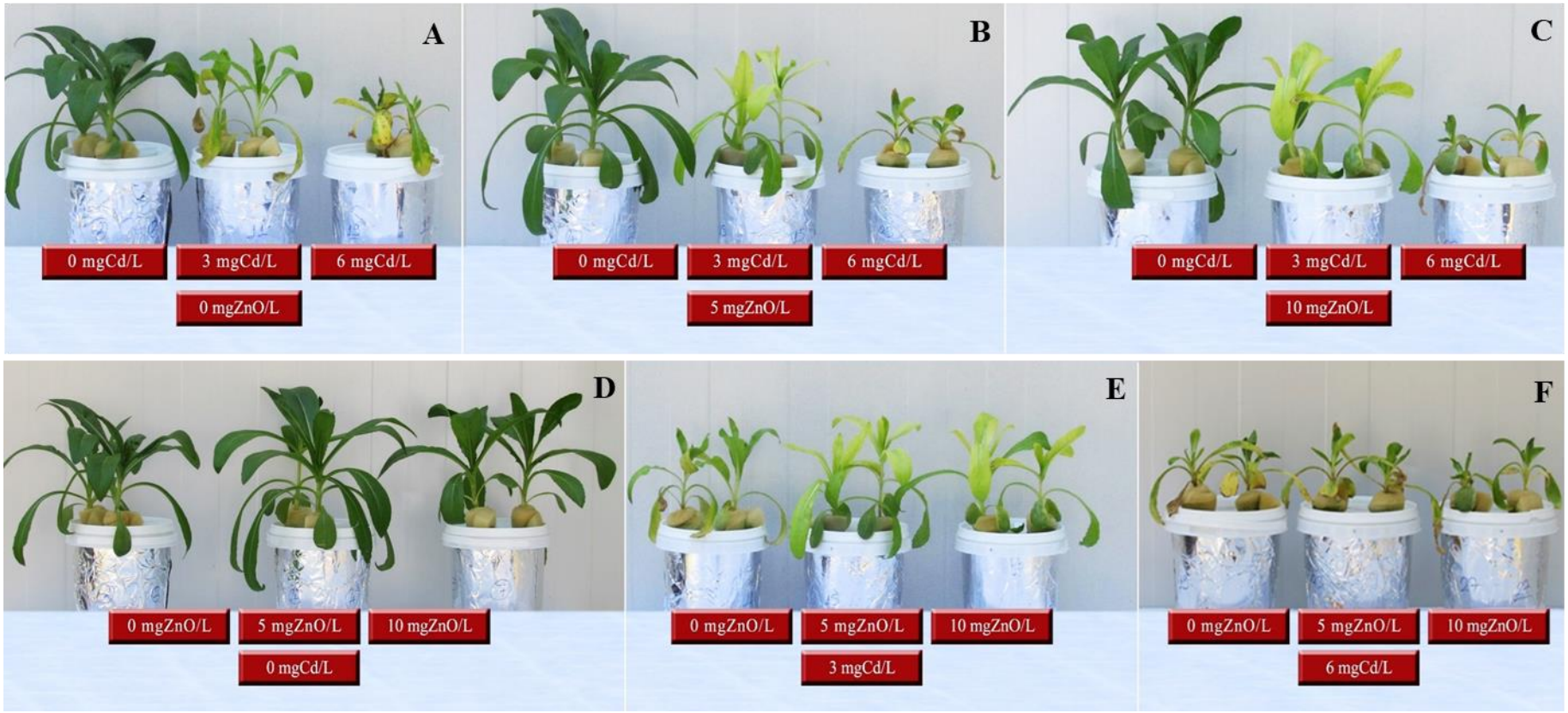

Figure 4. Morphological changes of safflower plant under ZnO-NP and Cd treatments

The dry weights of root was decreased with increasing dose of Cd. The effects of Cd applications on root dry weight of safflower were found to be statistically significant ($p<0.01$). However, ZnO-NP applications were not significantly influenced the root biomass. The enhancement of dry weight in safflower with ZnO-NPs treatment could be related to improving growth with increasing chlorophyll content. On the other hand, Cd application severely decreased shoot and root dry weights. However, plants' growth and chlorophyll content depend on the application dose of ZnO-NPs. Because overdose of ZnO-NP application may cause toxicity on plants.

Various researchers (Zhang et al., 2019; Khan et al., 2019; Venkatachalam et al., 2017) have reported the positive effect of ZnO-NP and toxic effects of Cd on plants. Zhang et al. (2019) reported that, comparing to the control, rice biomass increased by 13-22% and 25-43% at 2.5 Cd mg/kg and 5.0 mg Cd/kg treatments, respectively, with ZnO-NP applications (0, 25, 50 and 100 mg/kg). In addition, increasing rates of ZnO-NP applications increased the dry weight of the wheat plant grown in Cd contaminated soil and decreased the oxidative stress in the plant (Khan et al., 2019). Venkatachalam et al. (2017) compared the individual effects of Cd and Pb applications with and without ZnO-NPs applications and reported an increased growth tolerance index.

Table 1. The effect of increasing doses of ZnO-NP and Cd applications on the chlorophyll content, shoot and root dry weights of the safflower (n=3)

Doses (mg/L)		Chlorophyll Concentration	Shoot	Root
		(SPAD Unit)	Dry Weight (g)	
ZnO-NP$_0$	**Cd$_0$**	42.3ab	0.422b	0.118a
	Cd$_3$	21.4c	0.181c	0.050c
	Cd$_6$	6.23e	0.125d	0.018d
ZnO-NP$_5$	**Cd$_0$**	41.5b	0.505a	0.114a
	Cd$_3$	8.50d	0.213c	0.071b
	Cd$_6$	8.80d	0.115d	0.027d
ZnO-NP$_{10}$	**Cd$_0$**	42.9a	0.498a	0.106a
	Cd$_3$	8.20d	0.202c	0.066b
	Cd$_6$	8.90d	0.116d	0.029d
ZnO-NP		67.7**	5.62**	2.49
Cd		5.6**	563**	264.8**

**: $p<0.01$ *: $p<0.05$, n.s: not significant

Zinc and Cadmium concentrations of plant

Cadmium toxicity in plants may be alleviated, to some extent, by Zn treatments (Rizwan et al., 2019b). Because Zn is an essential plant nutrient and it has many important function in the metabolic processes of plants (Marschner, 1995). Cadmium is a divalent ion (Cd^{+2}) and behave very like Zn^{2+} and therefore depending on their concentrations in soil solution Cd may compete with Zn uptake by plant roots. Köleli et al. (2004) reported that Cd toxicity could be more hazardous under Zn deficiency.

The Zn and Cd concentrations of shoots and roots of the safflower plant were shown in Table 2. The effects of both treatments (ZnO-NP and Cd) on Zn and Cd concentrations of plant shoot were found to be statistically significant ($p<0.01$). In shoots, the lowest Zn concentration (6.20 mg/kg) was determined in 0 mg Cd/L and 0 mg ZnO-NP/L applications, while the highest Zn concentration (142.5 mg/kg) was determined in 10 mg ZnO-NP/L + 3 mg Cd/L applications. It was determined that the root Zn concentration of the safflower plant ranged from 19.6 mg/kg at 0 mg ZnO-NP + 3 mg Cd/L to 162.3 mg/kg at 10 mg ZnO-NP + 6 mg Cd/L (Table 2). With the increase in ZnO-NP and Cd doses, the Zn concentration of the roots also increased.

Table 2. The effect of increasing doses of ZnO-NP and Cd applications on the Zn and Cd concentrations of shoot and root (n=3)

Doses (mg/L)		Shoot		Root	
		Zn	Cd	Zn	Cd
		(mg/kg)			
ZnO-NP$_0$	Cd$_0$	6.20h	0.00e	22.1f	0.00e
	Cd$_3$	23.5g	534d	19.6f	1417c
	Cd$_6$	37.4f	654c	28.9f	2441a
ZnO-NP$_5$	Cd$_0$	39.1f	0.00e	64.6d	0.00e
	Cd$_3$	121.6b	524d	41.8e	1188d
	Cd$_6$	79.7d	878a	83.0c	2420a
ZnO-NP$_{10}$	Cd$_0$	51.4e	0.00e	99.0b	0.00e
	Cd$_3$	142.5a	520d	76.3c	1144d
	Cd$_6$	100.0c	743b	162.3a	1994b
ZnO-NP		276**	13.24**	441**	20.7**
Cd		183**	1526**	118**	1822**

**: $p<0.01$ *: $p<0.05$, n.s: not significant

The effects of ZnO-NP and Cd treatments on the shoot and root Cd concentrations of the safflower plant were significant ($p<0.01$). The highest Cd concentration (878 mg/kg) of shoot was obtained from the 5 mg ZnO-NP/L + 6 mg Cd/L doses. The root Cd concentrations were increased with Cd applications (Table 2). The highest Cd concentration in the plant root (2441 mg/kg) was obtained from 6 mg Cd/L without ZnO-NP application.

Hyperaccumulator plants can accumulate more than 100 mg/kg (0.01%) Cd in their shoots, which are also defined as Cd hyperaccumulator plants (Van der Ent et al., 2013). While normal plants can accumulate very low amounts of Cd (0.03-5.0 mg Cd/kg in many plant species) in their aboveground parts.

However, despite such a toxic amount, the safflower plant accumulated 5.2 to 8.7 times higher Cd than the hyperaccumulation threshold value in its harvestable parts (Table 2). This result shows that the Cd phytoremediation potential of the safflower plant is high. Gowayed (2017) reported that the Zn or Cd concentrations of shoots were less than the root concentration with both (ZnO-NP and Cd) applications, and this could be due to the chemical and physical similarities that cause interaction between Zn and Cd. Khan et al. (2019) found an enhancement in root translocation factor with the increasing dose of ZnO-NP (0 - 25 - 50 and 100 mg/kg) applications to the Cd contaminated soil. Keller et al. (2015) reported that the translocation of metals from roots to the aboveground part of the plant restricted when the higher accumulation of metal in roots. On the other hand, at low metal concentrations, the plant defense system may not be active and higher amounts of metals may be transferred to the aboveground part of the plant (Khan et al., 2019). On contrary to our findings, Angelova et al. (2016) found that the safflower plant, which was subjected to varieties of heavy metals (Pb, Zn and Cd), was accumulated less heavy metals in the roots than the aboveground parts. The differences in results may be because Zn and Cd may dissimilarly interact with each other under different conditions, and the Zn application may increase or equilibrate the phytotoxicity of Cd (Khurana et al., 2012).

Conclusion

In this study, the use of the phytoextraction method and ZnO-NPs together to improve Cd uptake by safflower plants were investigated. The results showed that Cd accumulation of the plant increased due to increasing doses of ZnO-NP. In the shoots of the plant Cd has accumulated 5.2 to 8.7 times more than the hyperaccumulation critical threshold value (100 µg/g). This investigation showed that the safflower plant has high phytoremediation potential to clean-up Cd contaminated soil.

The use of metal-based nanoparticles such as ZnO-NP to increase the efficiency of the phytoextraction method is an idea that has emerged with the development of nanotechnology and phytoremediation technology. However, the use of these two technologies together may bring some additional challenges in field practices. Not knowing the possible environmental risk that metal-based nanoparticles to be used may create in the soil ecosystem is worrisome. It is also important to investigate the toxic effects of the nanoparticles to be applied for animals, plants and microbial communities in the soil. Thus, more research is needed regarding environmental risks of nanoparticles.

Author Contributions

Dağhan H. devised and supervised, the main conceptual ideas, proof outline of the project and wrote the current manuscript. Yentür F. A. carried out the hydroponic experiment, analyzed plant materials.

Conflict and Interest

Authors declare no conflict and interest.

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The role of foliar applications of boron and gibberallic acid (GA_3) on yield and quality in different strawberry types

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Abstract

This study was carried out to investigate the effects of boric acid and giberallic acid (GA_3) applications on yield and quality in strawberry cultivars. In the experiment, 5 different doses of boric acid (Control, 100, 200, 300, 400 and 500 ppm) and GA_3 (Control, 20, 40, 60, 80 and 100 ppm) were applied. As a result of the applications, the average number of flowers was determined between 29.9-50.7. Fruit weights in the experiment varied between 17.1-22.3 g. Brix value varied between 6.3-7.9%. The pH values of the fruits were found between 2.5-2.9. Titratable acidity value was measured between 0.5-1.1 percent. It was determined that the applications made a difference in terms of yield and quality in strawberry cultivars.

Keywords: strawberry, application, boric acid, GA_3, quality

Introduction

Strawberry (*Fragaria spp*.) belonging to *Rosaceae* family is of the most popular fruits due to attractive appearance, aroma and flavor (Kepenek et al., 2002), exhibiting a wide array of variation within the species regarding different planting times, table and industrial production in different ecologies of the world. Corresponding to the obtaining the desired characteristics of the relevant plant species, breeding programs have been employed in many regions of the world in this regard. Of the targets to be desired, the relevant programs aim at increasing the yield and quality of the fruits. Herewith the programs, the recent researches have addressed on the concept of fruit quality and extended to the regulation of nutritional value but it is worthy to note that the phenomenon "quality characteristics" of fruits is a complex term and not easy to define objectively. The former reports have clearly revealed that the quality associated traits are genetically or environmentally dependent, as the clearly reported (Perkins-Veazie, 1995; Prior et al., 1998; O'Connor et al., 2002).

Today, the most important factor in the importance of strawberry cultivation has been the economic growth of strawberries in different climatic and soil conditions. In addition, the income obtained from the unit area in strawberry cultivation is quite high in relative to other products. Strawberries have a good market advantage as they ripen in periods when fresh fruit is scarce (Kıyga, 2009). The ability to grow strawberries in almost every region of our country allows the strawberry fruit to be available in the market for a longer period of time. The fact that strawberry is available for sale, especially when other fruits are not available in the market, provides a good source of income to the producers, while at the same time it is a pleasant species that appeals to the palate and meets the fruit needs of the consumers (Eti, 2006).

Important quality characteristics of strawberries are fruit size, flesh firmness, fruit shape, amount of water soluble solids (SSC), water-soluble dry matter/acid ratio, total sugars (glucose, fructose, sucrose) and acidity (Azodanlou et al., 2003).

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Of the growth regulating substances in plants; gibberellins have been associated with the vegetative growth, fruit size, seed and bud dormancy break. It has been reported that the number of flowers in strawberry cultivars is an important parameter, albeit indirectly, in the estimation of yield (Strik and Practor, 1988). Gibberellins, on the other hand, stimulate pollen germination and pollen tube growth, and promote flowering and fruit set as a result of the external effect of gibberellins on the flower. Gibberellins affect many physiological and morphological activities (Kacar et al., 2006; Baktır, 2010; Kumar et al., 2013; Gündoğdu et al., 2019).

Boron element is prominent in strawberry nutrition as of the microelements required for plant growth and productivity. Carbohydrate, boron, which has an important role in the synthesis of phenolic compounds and nucleic acid, is one of the essential elements for plant growth. As a matter of fact, it is necessary for the healthy development of fruit set in strawberries. The plant's request should be taken into account in boron fertilization. Distorted fruit formations in strawberries are associated with many factors. It is known by researchers that malformed fruit formation is caused by insufficient pollination and fertilization and this situation is related to boron nutrition (Çakıcı and Arslan, 2012; Özkaya et al., 2017; Özkutlu et al., 2017).

Corresponding to the manifested impacts of boron and gibberallic acid; we hypothesized that the exogenous treatments of boron and gibberelic acid would increase and improve the quality traits of the strawberry fruits, in relative to non-treated plants. We also hypothesized that both cultivars of strawberry would differ in their responses against the relevant treatments, as the clearly reported that some traits of the plants are dependent on genetic structure. The aim of this study is to determine how different doses of boric acid and GA_3 applications affect yield and quality in Albion and Sabrina strawberry cultivars. For this purpose, 5 different doses of boric acid (Control, 100, 200, 300, 400 and 500 ppm) and GA_3(Control, 20, 40, 60, 80 and 100 ppm) were applied.

Material and Methods

Material

The research was carried out in a low tunnel strawberry greenhouse. Two cultivars were preferred as strawberry cultivars, Albion from neutral cultivars and Sabrina from short-day cultivars. Frigo seedlings were planted on the bobbins in a triangle shape at 30x30 cm intervals. In the strawberry garden, the first flowers and stolons of the strawberry varieties grown in order to ensure strong root development in the first year were plucked.

Method

Fruit samples were taken during the harvest period (between March and May) from this strawberry greenhouse, which was in full yield in 2020. The fruits were harvested when they were fully red in color. The samples taken were brought to the laboratory of the Vocational School located in the Sivaslı district of Uşak province, and the pomological analyzes of the fruit samples were made immediately and stored at -20 ^oC for the remaining biochemical analyzes.

Different doses of boric acid and GA_3 were used in the study. Applications were prepared in the laboratory of the Vocational School located in Sivaslı district of Uşak province. The research consists of a total of 10 repeated fertilizer applications and control groups. It consists of 5 replications of boric acid andGA_3 and control groups. Preparation of boric acid and GA_3; first, 50 ml of pure alcohol was put into small beakers. Then, Boric acid and GA_3 were weighed on a precision balance. After weighing, it was mixed in 50 ml of pure alcohol for each replication and mixed until it dissolved. Finally, for all replications, the samples were poured into bottles containing 950 ml of pure and shaken until thoroughly mixed. Boric acid and GA_3 amounts in Table 1. has also been given. Strawberry varieties selected for the experiment were determined as five replications and control groups.Twenty plants were used for each replication.

Table 1. Aplication mounts of Boric acid and GA_3

Boric acid application amounts	**GA_3application amounts**
Control	Control
100ppm= 0.1 g per 1 liter of pure water	20ppm= 0.02 g per 1 liter of pure water
200ppm= 0.2 g per 1 liter of pure water	40ppm= 0.04 g per 1 liter of pure water
300ppm= 0.3 g per 1 liter of pure water	60ppm= 0.06 g per 1 liter of pure water
400ppm= 0.4 g per 1 liter of pure water	80ppm= 0.08 g per 1 liter of pure water
500ppm= 0.5 g per 1 liter of pure water	100ppm= 0.1 g per 1 liter of pure water

First Flowering and Full Flowering

It was recorded as the first flowering date when 5% of the plants in the plot bloom and the full bloom date when 75% of them bloom (Özkaplan, 2010).

Harvest time

The first date on which the fruits in the plot were harvested was determined as the beginning of the harvest and the last harvest as the end of the harvest. The fruits were harvested when they got their full color.

Yield Per Plant

From the beginning to the end of the harvest, the fruits obtained from all plants were measured with a digital scale sensitive to 0.01 grams.

Fruit Width and Length

The average width and length of 10 randomly picked fruits were determined by measuring with a digital caliper with 0.01 mm precision.

Fruit Weight

During the harvest periods, 10 randomly picked fruits for each replication were weighed on a digital scale with an accuracy of 0.01 grams, and the average fruit weight was calculated according to the results obtained.

Total soluble solids content (TSS)

For each replication, the juices of 10 randomly taken fruits were determined by hand refractometer.

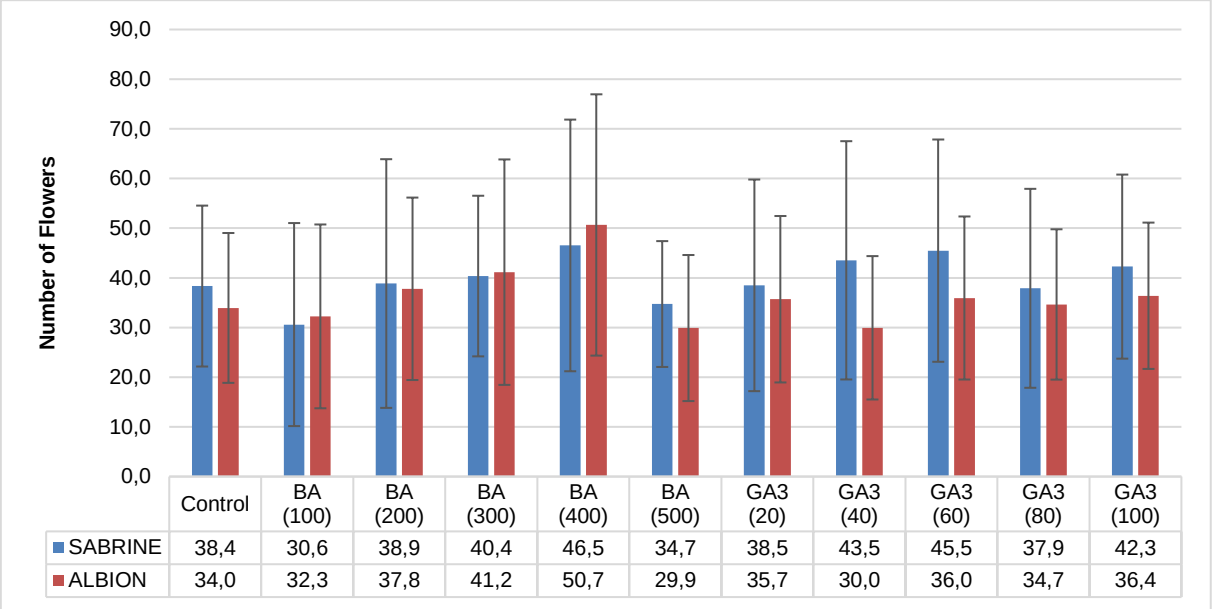
pH %
The juice of 10 randomly taken fruits was squeezed to obtain 10 ml of juice and was measured with a pH meter.

Titratable acidity values (%)
It was calculated in terms of citric acid by titration technique in 10 ml fruit juice obtained from random fruits.

Statistical analysis
Raw data of the experiments was summarized in Microsoft Excel and figures were prepared to better present the results. Then, the data was subjected to the analysis of variance and the mean separation was performed with Tukey's HSD test at $p < 0.05$. Furthermore, the "corrplot" package of R was used to perform correlation analysis.

Results and Discussion
Plant Growing
The development of strawberry plants was monitored twice a week during the growing period in the field where the research was conducted. The first trial started on 25.03.2020 and was carried out at 10-day intervals. In the meantime, the first flowering started with the first application and observations were made about it. After the last Boric and GA_3 application, full bloom was reached and flower count was done.

Number of Flowers
Various observations were made during the flowering period. The number of flowers was observed according to the amount of doses applied in the experiment. Flower counting was done at the last blooming. It was observed that the highest flower amount of Sabrina variety was in boric acid (400 ppm) application with an average of 46.5, and the lowest flower amount was observed in boric acid (100 ppm) application with an average of 30.6. It was observed that the highest average flower amount of Albion variety was in boric acid (400 ppm) application with 50.7, and the lowest average flower amount was observed in boric acid (500 ppm) application with 29.9 (Figure 1).

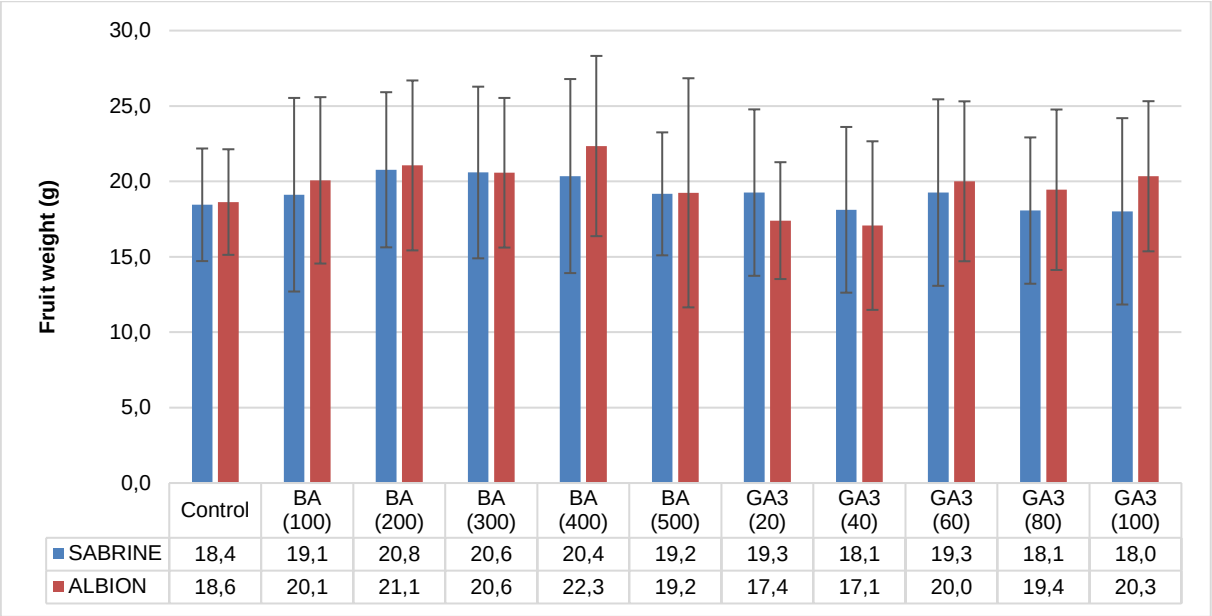


	Control	BA (100)	BA (200)	BA (300)	BA (400)	BA (500)	GA3 (20)	GA3 (40)	GA3 (60)	GA3 (80)	GA3 (100)
SABRINE	38,4	30,6	38,9	40,4	46,5	34,7	38,5	43,5	45,5	37,9	42,3
ALBION	34,0	32,3	37,8	41,2	50,7	29,9	35,7	30,0	36,0	34,7	36,4

Figure1. Number of flowers

It has been reported that the number of flowers in strawberry cultivars is an important parameter in the estimation of yield, albeit indirectly (Strik and Practor, 1988). In a study, the effects of different organic applications on yield and some quality parameters of Camarosa and Fern strawberry cultivars were investigated. In this study, the number of flowers in the applications was found to be statistically significant and nitrogen fertilizer application and green manure + farm manure + humic acid + foliar fertilizer applications formed the highest values. In a two-year study, it was determined that the number of flowers varied between varieties (34.46- 44.06) (Polat and Çelik, 2008).

Fruit Weights
It was observed that the highest average weight amount in Sabrina was in boric acid (200 ppm) application with 20.8 g, and the lowest average weight amount was GA_3(100 ppm) with 18.0 g. In the weight measurements made in Albion variety, it was seen that the highest average weight was in boric acid (400 ppm) application with 22.3 g, and the lowest average weight amount was GA_3 (40ppm) with 17.1 g (Figure 2).



	Control	BA (100)	BA (200)	BA (300)	BA (400)	BA (500)	GA3 (20)	GA3 (40)	GA3 (60)	GA3 (80)	GA3 (100)
SABRINE	18,4	19,1	20,8	20,6	20,4	19,2	19,3	18,1	19,3	18,1	18,0
ALBION	18,6	20,1	21,1	20,6	22,3	19,2	17,4	17,1	20,0	19,4	20,3

Figure2. Fruit weight of strawberry fruits

Polat and Çelik (2008) investigated the effects of different organic applications on yield and some quality parameters of Camarosa and Fern strawberry cultivars. In this study, the highest yield value was obtained from the application of green manure + farm manure + humic acid + foliar manure (Fern: 177.07 g/plant, Camarosa: 133.9 g/plant).The effects of different GA3 applications on the fruit quality of Seascape strawberry cultivar grown in Bolu ecological conditions were investigated. The effect of GA3 application prepared in two different doses (50 ppm and 100 ppm) on fruit quality parameters was determined. In the results of working; It was determined that the fruits applied 50 ppm GA3 had the lowest pH value. However, the highest fruit weight (46.01 g) was determined from the same application. It is also reported that GA3 application does not cause a significant change in aroma, taste and juice values. (Gundogdu et al., 2017).It was aimed to determine the yield and some fruit quality characteristics of two different strawberry cultivars (Festival and Camarosa) grown in the ecological conditions of Aydın/Sultanhisar district in a two-year study. In the results of working; In the second year, yield values of both cultivars decreased, while average fruit weight, fruit width and fruit length increased (Bayram, 2020).In the study conducted by Gündüz and Özdemir (2012); The effects of different two-year growing environments on the yield and fruit quality characteristics of some strawberry genotypes were investigated. In the results of working; The fruits with the highest weight are among the fruits of the Camarosa variety grown in the open, respectively, according to the years; (11.9 g and 11.3 g) reported that they were obtained.

Fruit Width and Length (mm)

In the fruit width measurements of Sabrina cultivar, it was measured that the highest value was in boric acid (200ppm) application with 34.9mm, and the lowest fruit width value was observed in GA_3 (100ppm) application with 32.4mm.It was observed that the highest value was 35.0mm in boric acid (400 ppm) fruit width measurements made in Albion variety, and the lowest value was 33.1mm in GA_3(20 ppm) and GA_3(40 ppm) applications in Albion variety (Figure 3). In the height measurements of Sabrine cultivar, it was determined that the highest value was in the application of boric acid (200 ppm) with 46.2 mm, and the lowest value of fruit length was in the control group, GA_3 (20 ppm) and GA_3 (100 ppm) applications with 43.1 mm. In the fruit length measurements made in the Albion cultivar, it was found that the highest value was in the application of GA_3 (60ppm) with 47.0mm, and the lowest fruit length value was GA_3 (40ppm) with 41.6mm (Figure 4).

As a result of the study aiming to determine the physicochemical properties of three strawberry cultivars (Rubygem, Camarosa, Amiga) grown in a commercially produced orchard in Köprübaşı district of Manisa; Fruit weight of Rubygem (28.35 g) and Amiga (28.11 g) strawberry cultivars was found to be higher than Camarosa cultivar (23.45 g). Fruit length of Amiga strawberry variety was the longest with 49.91 mm, and Camarosa variety was the shortest with 42.02 mm. Titratable acidity and pH values of strawberry fruits did not show significant differences according to the cultivars, they ranged between (0.56-0.62 g 100 mL-1), (4.15-4.32), respectively. It has been reported that the strawberry fruits grown in this region are large, the inner fullness is Rubygem and Amiga, the hardness is Amiga, the total amount of phenolic substances is the highest in Rubygem, while no significant differences are observed in terms of the chemical composition of the fruit (Türk and Şen, 2020).

The effects of seedling type and growing medium on fruit quality were investigated in soilless strawberry (Fragariaxananassa Duch.) cultivation in a glass greenhouse under the ecological conditions of the Mediterranean Region. In the experiment, as the seedling type, tube seedling and frigo seedling; Peat (T), Perlite (P), Coconut Peat (H), Volcanic Tuff (V) and their mixtures were tested as growing medium. As a result of this study; It varies according to trial years and growing environments. In terms of growing environment, average fruit length and fruit width values were determined in the highest (H+V) environment (Adak and Pekmezci, 2012).

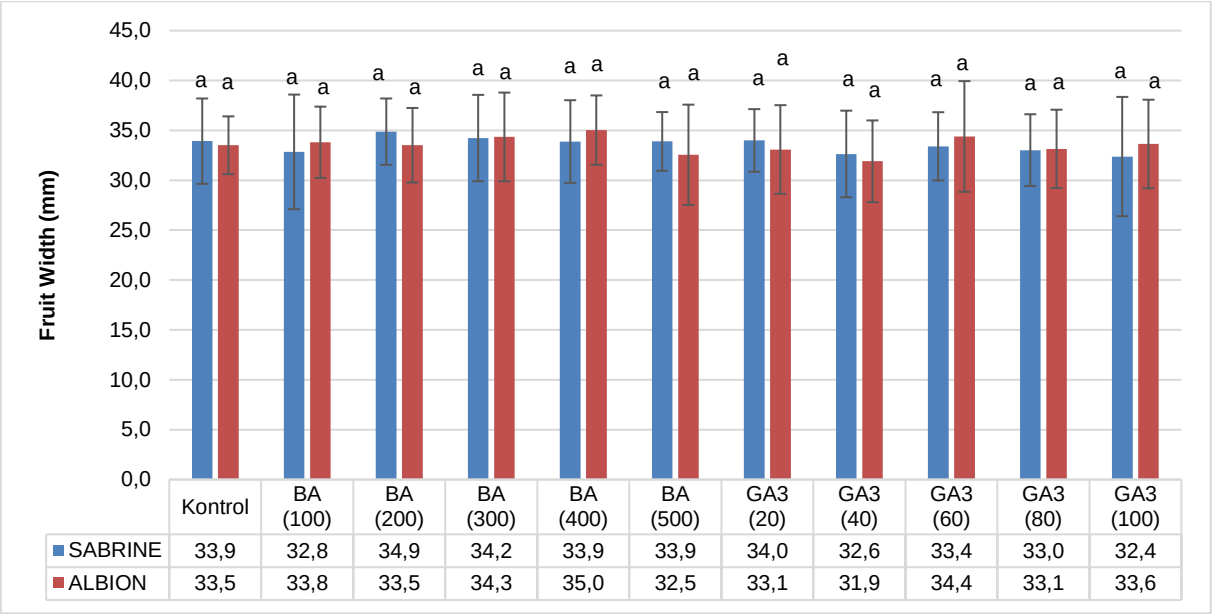


Figure 3. Fruit width of strawberry fruits (mm)

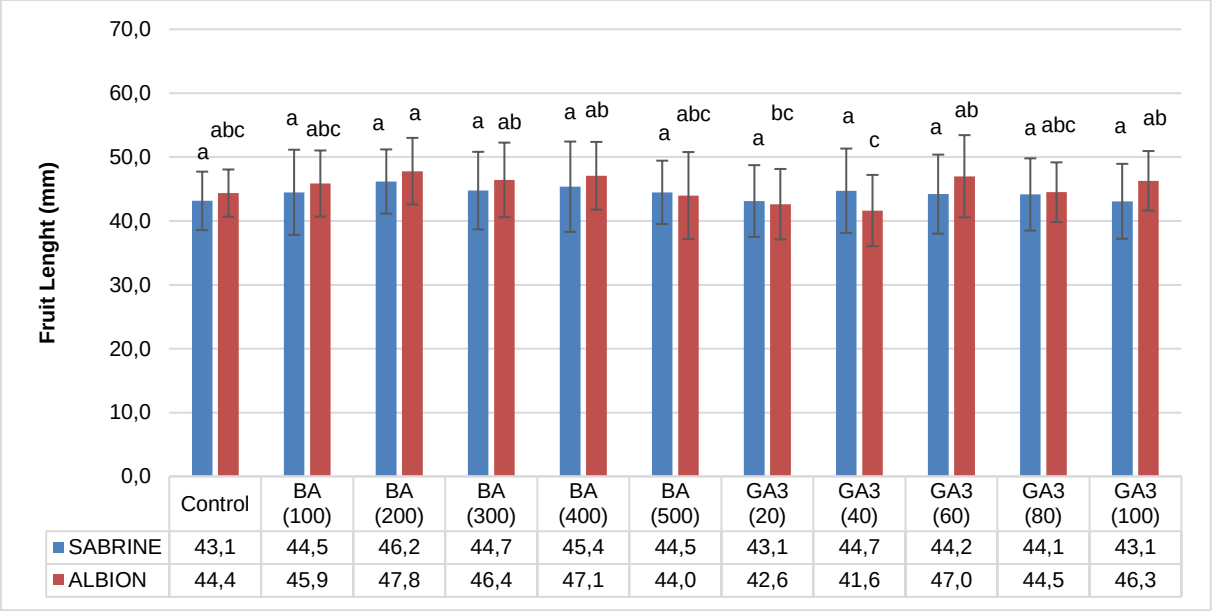
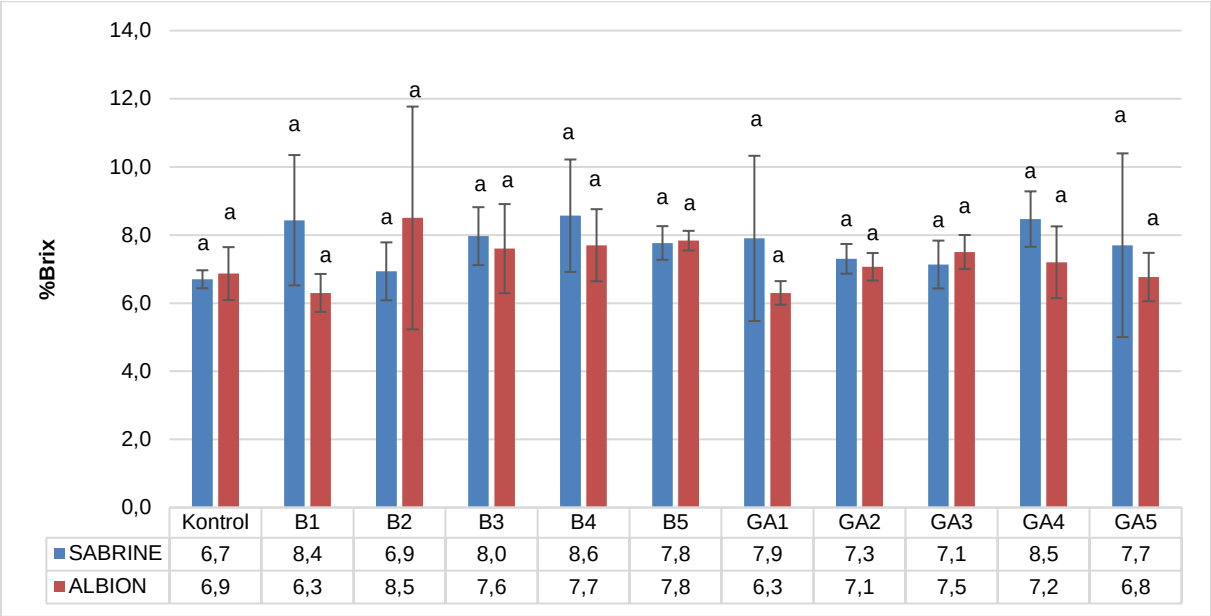


Figure 4. Fruit length of strawberry fruits (mm)

Total Soluble Solids Content (TSS) -Brix values (%)

It was determined that the highest brix amount measurements made in Sabrine cultivar were in GA_3 (20ppm) application with 7.9%, and the lowest brix amount was in the control group with 6.7%. In the measurements made in Albion variety, the highest value of brix amount was measured to be boric acid (500ppm) with 7.8%, and the lowest amount of brix was measured to be boric acid (100ppm) with 6.3% (Figure 5).



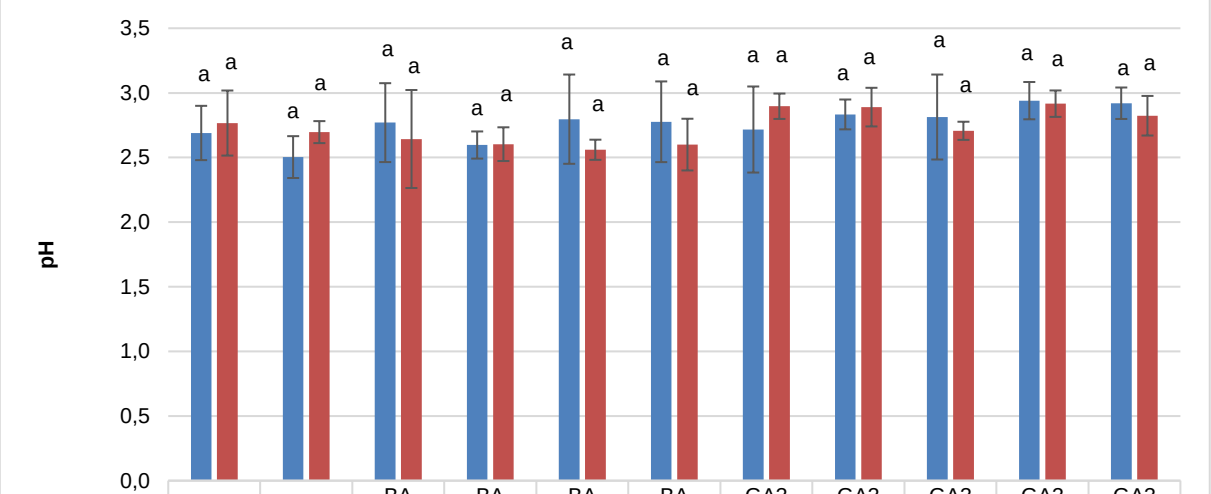
	Kontrol	B1	B2	B3	B4	B5	GA1	GA2	GA3	GA4	GA5
SABRINE	6,7	8,4	6,9	8,0	8,6	7,8	7,9	7,3	7,1	8,5	7,7
ALBION	6,9	6,3	8,5	7,6	7,7	7,8	6,3	7,1	7,5	7,2	6,8

Figure 5. Brix value of strawberry fruits (%)

It was aimed to determine the effects of growing 3 strawberry varieties (Sweet Charlie, Camarosa and Kabarla) on yield and quality, including 6 different mulch applications (black, transparent, yellow, straw, sawdust and hazelnut husk) in Persembe (Ordu) ecology. As a result of the research; The highest fruit weight was obtained from the straw mulch with the Kabarla variety (24.83 g) and with the Camarosa variety (23.51 g). Width (TSS) amount was obtained with straw mulch (6.61% brix) in Camarosa variety. The highest value in terms of titratable acidity was obtained in transparent mulch (0.50%) in Sweet Charlie variety. The highest pH value was obtained from Camarosa variety in yellow mulch (5.88). Camarosa and Sweet Charlie had better performance than Kabarla variety, and the best results in terms of yield were obtained from Camarosa variety and transparent, husk and straw mulches (Özkaplan, 2010).

pH%

It was found that the highest pH value of Sabrine was 2.9 in GA_3 (80 ppm and 100 ppm) applications, and the lowest pH value was 2.5 in boric acid (100 ppm) applications. In the pH measurements made in the Albion variety, it was found that the highest value was in GA_3 (20ppm, 40ppm and 80 ppm) applications with 2.9, and the lowest value was in boric acid (200ppm, 300ppm, 400ppm and 500ppm) applications with 2.6 (Figure 6).



	Control	BA (100	BA (200)	BA (300)	BA (400)	BA (500)	GA3 (20)	GA3 (40)	GA3 (60)	GA3 (80)	GA3 (100)
SABRINE	2,7	2,5	2,8	2,6	2,8	2,8	2,7	2,8	2,8	2,9	2,9
ALBION	2,8	2,7	2,6	2,6	2,6	2,6	2,9	2,9	2,7	2,9	2,8

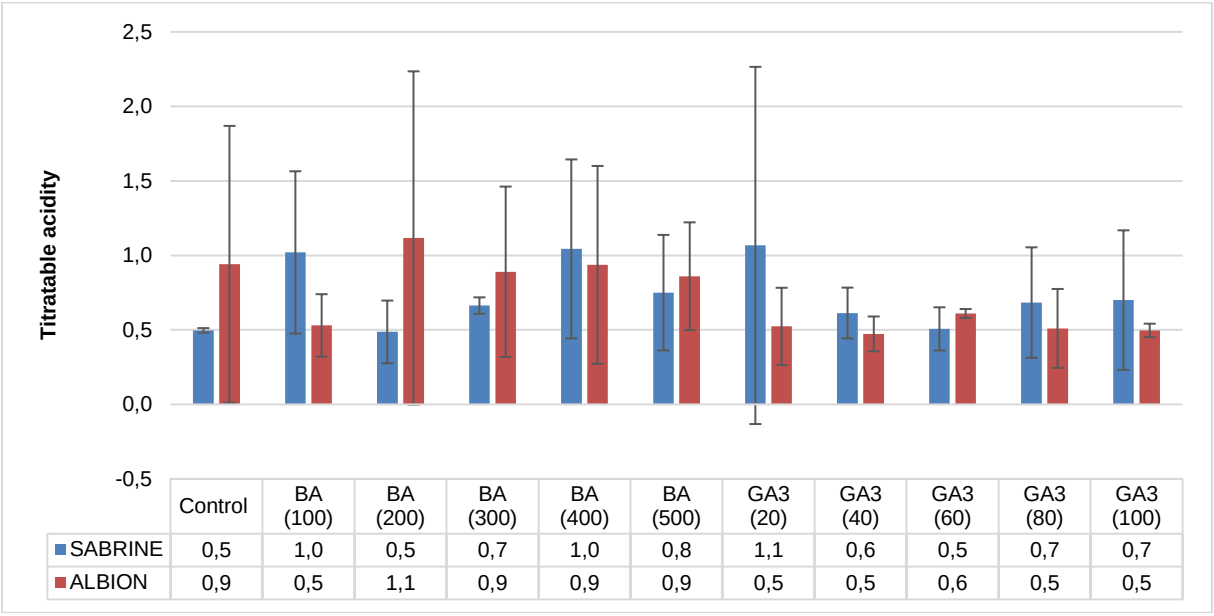
Figure 6. pH of strawberry fruits (%)

The effects of seedling type and growing medium on fruit quality were investigated in the cultivation of soilless strawberry (*Fragaria x ananassa Duch*.) in a glass greenhouse in the ecological conditions of the Mediterranean Region. In the experiment, as the seedling type, tube seedling and frigo seedling; As an alternative, mixtures of peat (T), perlite (P), coconut peat (H), volcanic tuff (V) were tested. As a result of this study; It varies according to trial years and growing

environments. There was no significant difference between seedling types in terms of titratable acidity and pH values; It has been reported that the acidity value varies between 1.12% and 1.14% and the pH values vary between 3.21 and 3.33. In terms of growing environment, average fruit length and fruit width values were determined in the highest (H+V) environment. It has been reported that frigo seedlings are advantageous in terms of seedling type, and (H) and (H+V) environments are advantageous in growing media (Adak and Pekmezci, 2012).

Titratable Acidity Values (%)

In the measurements made in Sabrine variety, the highest acidity value was obtained with 1.1% in GA_3 (20ppm) application, while the lowest acidity value was obtained from the control group and GA_3 (60ppm) applications. In the measurements made in Albion variety, it was determined that the highest acidity value was in boric acid (40ppm) application with 1.1%, and the lowest acidity value was 0.5 in boric acid (100ppm) and GA_3 (20ppm, 40ppm, 80ppm, 100 ppm) applications (Figure 7).



	Control	BA (100)	BA (200)	BA (300)	BA (400)	BA (500)	GA3 (20)	GA3 (40)	GA3 (60)	GA3 (80)	GA3 (100)
SABRINE	0,5	1,0	0,5	0,7	1,0	0,8	1,1	0,6	0,5	0,7	0,7
ALBION	0,9	0,5	1,1	0,9	0,9	0,9	0,5	0,5	0,6	0,5	0,5

Figure 7. Titratable acidity values of strawberry fruits

Çakıcı and Arslan (2012) investigated the effects of foliar potassium, boron and zinc applications on yield and quality of Camarosa strawberry cultivar. As a result of the study; It was determined that potassium, zinc and boron applied foliar in Camarosa strawberry cultivar had a positive effect on yield and quality characteristics. However, it was reported that the titratable acid content of foliar applications varied between 1.06% and 1.19% and it did not have a statistically significant effect.

The effects of different GA3 applications on the fruit quality of Seascape strawberry cultivar grown in Bolu ecological conditions were investigated. The effect of GA3 application prepared in two different doses (50 ppm and 100 ppm) on fruit quality parameters was determined. In the results of working; The highest fruit acidity (1.11%) was determined in the fruits applied 50 ppm GA3 (Gundogdu et al., 2017).

Conclusion

Different results were observed in Albion and Sabrina cultivars with different doses of GA_3 and Boric acid depending on the amount of doses applied in the experiment.In this study; the highest flower number of Sabrina and Albion cultivars was obtained from boric acid (400 ppm) application. Based on the yield parameter, the highest fruit weight in Sabrina cultivar was determined in boric acid (200 ppm) application. In the Albion cultivar, the highest fruit weight was determined in boric acid (400 ppm) application.It was determined that the highest values were obtained from boric acid (200ppm) application in the fruit width and length measurements made in Sabrina cultivar. However, in the fruit width measurements made in Albion variety, the highest value was obtained from boric acid (400 ppm) application, while the highest fruit length value was obtained from GA_3 (60 pmm) application.As a result; For Sabrina and Albion cultivars, doses of 200-400 ppm are recommended, which positively affects the yield in boric acid application, but when GA_3 applications are considered, it shows a wide variation between cultivars and applications.

Conflict of Interest

The authors are declared that they have no conflict for this research article.

Author Contribution

A.O: Field work, laboratory work, article writing; A.M.Ç.: laboratory work, article writing; V.O.;Field work and article writing

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Determination of precipitation-quality relationship by different statistical methods in bread wheat (*Triticum aestivum* L.)

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Abstract

In this study, monthly precipitations between 2007-2018 in Eskişehir, Konya, Afyonkarahisar, Uşak and Kütahya provinces were examined and their effects on protein content, zeleny sedimentation (MSDS), thousand seed weight and test weight, constituting of the quality components in wheat, were revealed. Monthly precipitations affecting these quality components were determined by using correlation analysis, principle component analysis (PCA), stepwise regression analysis, path analysis and decision tree analysis. In the research; the effects of precipitations falling in September, October, November, March, April, May and June and total precipitation on the quality components (protein content, zeleny sedimentation (MSDS), thousand seed weight and test weight) in Eskişehir, Konya, Afyonkarahisar, Uşak and Kütahya were determined. It is also aimed to determine effective monthly precipitations on the quality components by determining different analysis programs. As a result; March precipitation, April precipitation, June precipitation, October precipitation and total precipitation were determined as significant precipitations affecting the quality components (protein content, macro sedimentation (MSDS), thousand seed weight and test weight) in bread wheat. Increasing June precipitation, October precipitation and total precipitation affect the quality of bread wheat increase seed weight and test weight, while it causes a relative decrease in protein content and MSDS. Uşak and Kütahya provinces were found to be superior regions for thousand seed weight and test weight, whereas, Eskişehir and Konya provinces were determined as better provinces for zeleny sedimentation (MSDS) and protein content.

Keywords: Bread wheat, quality, rainfall, monthly rainfall, protein content, zeleny sedimentation (MSDS), thousand seed weight and test weight, statistical methods

Introduction

Wheat is known as one of the most cultivated crop with the largest acreage and production in the world. The highest production is done in Asia, Europe and America, and wheat cultivation is mostly done in the northern hemisphere (Feldman, 2001; Shewry; 2009). On the other hand, due to its wide adaptability, wheat could be grown anywhere in the world without any problems except the poles. Moreover, wheat, as an important crop for nutrition industry and trade of countries in the world, has been occupying an important place in the nutrition of the future (Slafer et. al, 1996; Shewry; 2009). Since there is no possibility to add any hectare to the existing cultivation areas in the world, to meet the need of food the increasing population in the future; only one possibility is to increase the yield obtained from the unit area. This is only possible with the application of optimum cultivation techniques and the use of high yielding and quality genotypes.

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Registered genotype refers to a genotype that is high yielding and quality, resistant to biotic and abiotic stresses and stable in terms of these characteristics (Calderini et. a. 1997; Tiwari, and Lal, 2014; Fasahat et al., 2015; Jaruchai et al., 2018). Wheat meets a very important part of the daily calorie required in human nutrition and constitutes the main food of the country as many carbohydrates and proteins in the world. High adaptability, easy storability, long-term preservation of quality, easy cultivation and high yield and quality are the indicators of why wheat is used more in the world (Peterson et al., 1992; Curic et al. 2001). Yield and quality in wheat are under the threat of biotic and abiotic stresses as well as genetic potential. Therefore, yield and quality are shaped under the influence of genotype x environment interaction. The external biotic and abiotic stresses, that wheat is exposed to during the growing period, affect the quality as well as the yield. It has been revealed by many researchers that the quality of wheat is affected by at least 50 percent of external factors such as biotic and abiotic stresses (Wang et al., 2004; Wang et. al. 2014; Baraki et al., 2014; Spaldon et al., 2017). In wheat breeding, the quality concept consists of protein content, zeleny sedimentation (MSDS), thousand seed weight and test weight, and genotypes with high values for these components are accepted as quality varieties (Tipples et. al. 1981; Leibinger and Reiners 2001). Since the concept of quality is affected by environmental conditions as well as genotypic effect, it is greatly affected by the prevailing precipitation. (Burnett and Clarke 2002), excess/insufficient rainfall during the growing period significantly determines the amount of protein content, zeleny sedimentation (MSDS), thousand seed weight and test weight in wheat. In the researches, it was determined that with increasing rainfall, test weight and thousand seed weight increase while relative decrease occur in protein content and MSDS (Zwingelberg, 1961; Panozzo and Eagles 2000). This shows that the amount of precipitation significantly affects wheat quality. In addition to this, monthly precipitations in certain months are effective during the season in wheat, they determine the quality level to be obtained for protein content, zeleny sedimentation (MSDS), thousand seed weight and test weight. In this study, monthly precipitations between 2007-2018 in Eskişehir, Konya, Afyonkarahisar, Uşak and Kütahya provinces were examined and their effects on protein content, zeleny sedimentation (MSDS), thousand seed weight and test weight were revealed by using correlation analysis, principle component analysis (PCA), stepwise regression analysis, path analysis and decision tree analysis.

Materials and Methods

In this study, the effects of precipitations in September, October, November, March, April, May and June, (these months are known as plant growing period) and total precipitations between 2007-2018 in Eskişehir, Konya, Afyonkarahisar, Uşak and Kütahya provinces on quality components (protein content, zeleny sedimentation (MSDS), thousand seed weight and test weight) were determined, and the effective monthly precipitations were revealed by different analysis programs. Monthly and total precipitations of provinces were taken from Eskişehir 3rd Regional Directorate of Meteorology (2007-2018). Data for quality components (protein content, zeleny sedimentation (MSDS), thousand seed weight and test weight) that were the average values, obtained from the Regional Yield Trials, carried out in five provinces between 2007-2018. The monthly and annual precipitation values of the examined locations and the maximum, minimum and average values of the quality components are given in Table 1.

Table 1. Monthly and annual precipitation values of the examined locations and maximum, minimum and average values of quality components.

Variable	Mean	Minimum	Maximum	Variable	Mean	Minimum	Maximum
March	41,37±18,97	9,41	91,26	**November**	37,04±21,25	0,10	125,00
April	42,33±23,25	6,13	104,98	**Total Ra.**	471,42±95,43	225,9	885,3
May	52,32±27,39	5,76	124,21	**Test We.**	77,04±1,86	70,64	82,11
June	45,83±38,45	1,94	170,56	**Zel.Sed. (MSDS)**	39,11±8,11	18,34	51,75
September	25,79±32,38	0.21	134,57	**Thou. Seed We.**	34,71±2,62	21,42	41,57
October	46,23±27,79	0,45	105,93	**Protein**	12,44±0,97	7,93	17,01

Total annual precipitation average in the region was determined as 471,42±95,43 mm. The precipitations of March, April, May, June, September, October and November are respectively; 41,37±18,97, 42,33±23,25, 52,32±27,39, 45,83±38,45, 25,79±32,38, 46,23±27,79 and 37,04±21,25, respectively. Again, average test weight, zeleny sedimentation (MSDS), thousand seed weight and protein content values was determined as; 77,04±1,86, 39,11±8,11, 34,71±2,62 and 12,44±0,97, respectively.

Results and Discussion

Quality is a phenomenon that has been increasing its importance in wheat production in recent years; the need for high quality genotypes and their use is increasing. So, it is no longer important how much wheat is produced, but also how much high quality wheat seeds are produced. By addressing the issue in this way, the society's need for quality products is also answered. Therefore, a product rich in protein is obtained and presented to the society. Since, the quality criterion is highly influenced by the environment, genotypes having different quality levels could be obtained from year to year, from region to region, so quality has to be considered as a variable concept. While saying this, it is impossible to say that the quality is affected by the environment and the quality characteristics of the varieties should not be taken into account. Although it is affected by the environment and the quality varies between certain values, the use of high quality varieties is important in terms of increasing the quality (Guarda et al., 2004); therefore, quality has to be considered as a variable concept. While saying this, it is impossible to say that the quality is affected by the environment and the quality characteristics of the genotypes should not be taken into

account. Therefore, while developing high-yielding and high-quality varieties, it should be taken into account that they are less affected by biotic and abiotic stresses as much as possible. In this context, it is important to determine the effects of environmental effects on quality and to consider them while developing high yielding and quality genotypes accordingly to increase breeding success (Burnett and Clarke 2002). In this study, by taking into account the distribution of precipitation, which is one of the important climatic factors, in five different locations, the effect of precipitation on quality was revealed by different statistical methods.

Correlation analysis is important in terms of revealing the result and direction of the relationship between the examined components. Therefore, it is the coefficient showing the positive or negative strength of the relationship between the two components examined in this analysis (Armitage et al., 2002).

Table 2. The correlation between the monthly and annual precipitation values of the locations and the quality components (protein content, MSDS, test weight, thousand seed weight).

	March	**April**	**May**	**June**	**September**	**October**
April	-0,025**ns**					
May	0,040**ns**	0,166**ns**				
June	0,061**ns**	0,015**ns**	0,403**			
September	0,102**ns**	-0,009**ns**	-0,025**ns**	0,070**ns**		
October	-0,190**ns**	0,279*	0,127**ns**	0,325**	0,072**ns**	
November	0,178**ns**	-0,112**ns**	-0,191**ns**	-0,294*	-0,019**ns**	-0,343**
Total Ra.	0,307*	0,457**	0,466**	0,445**	0,176**ns**	0,368**
Test We.	-0,254*	0,266*	0,003**ns**	-0,06**ns**	0,012**ns**	-0,256*
MSDS	-0,035**ns**	-0,141**ns**	-0,136**ns**	-0,087**ns**	-0,013**ns**	-0,027**ns**
Thou. Seed Ve.	-0,061**ns**	0,153**ns**	0,053**ns**	-0,059**ns**	-0,025**ns**	-0,271*
Protein Con.	-0,140**ns**	-0,341**	-0,262*	-0,106**ns**	-0,013**ns**	-0,039**ns**
	November	**Total Ra.**	**Test We.**	**MSDS**	**Thou. Seed Ve.**	
Total Ra.	0,098**ns**					
Test We.	-0,258*	-0,033**ns**				
MSDS	0,126**ns**	-0,306*	-0,326**			
Thou. Seed Ve.	-0,055**ns**	-0,002**ns**	0,674**	-0,300*		
Protein Con.	0,298*	-0,351**	-0,447**	0,649**	-0,385**	

Correlation coefficient takes a value between -1 and +1; the positive coefficient indicates the positive relationship between the two relationships, and the negative coefficient indicates the inverse relationship between the two relationships (Sing and Chaudhary 1977; Sheskin, 2011). The correlation relationship between the climatic factors and protein content, MSDS, test weight and thousand seed weight examined in our study is given in Table 2. Here, the months of September, October, November, March, April, May, June and the annual total precipitation are taken into account. Considering the table, a positive and significant relationship was determined between the protein content and the month of November and June. Again, a negative relationship was determined between protein and precipitation in April, precipitation in May and total precipitation. While determining the positive and significant relationship between protein content and MSDS; a negative and significant relationship was determined between protein content and test weight and thousand seed weight. While determining the negative and significant relationship between MSDS and total precipitation, thousand seed weight and test weight; a positive and significant relationship was determined between MSDS and protein content. While a negative and significant relationship was determined between thousand seed weight and October precipitation, MSDS and protein content; a positive and significant relationship was found between thousand seed weight and test weight. While a negative and significant relationship was determined between test weight and March precipitation, October precipitation, November precipitation, MSDS and protein content; a positive and significant relationship was determined between test weight and April precipitation.

Here, the relationship between protein, MSDS and test weight, thousand seed weight was reversed. This means that, depending on the increasing amount of precipitation, there is a decrease in the protein content and MSDS ratio, while an increase in thousand seed weight and test weight occurs. This means that with increasing precipitation, there is an increase in plant growth and, accordingly, in dry matter production and carbohydrate production, and this increase rate is higher than protein accumulation and related MSDS accumulation. Therefore, the accumulation of protein and MSDS gives the appearance of decreasing relative to the increasing precipitation, that is, it does not increase, but decreases depending on the precipitation. This situation clearly shows the increasing or decreasing relationship between precipitation and quality components. As a matter of fact, it has been determined in studies that nitrogen does not increase that much in wheat compared to increased carbohydrate accumulation and it decreases relatively (Plenet and Lemaire, 2000). Depending on the increase in yield and carbohydrate accumulation due to falling precipitation, although thousand seed and test weights increase, protein and MSDS increase. As a result of the correlation analysis, it was determined that the precipitation in October, April, May and the total precipitation had a negative effect on the protein content and MSDS, while it caused a significant increase in terms of thousand seed weight and test weight. Therefore, the precipitations in October, April, May and the total precipitation were determined as the precipitations that had a significant impact on the quality.

Principle Component (PCA) analysis is an effective form of analysis in defining, classifying and evaluating data, and it is

a method that reduces the large number of data sets and enables to reveal the active elements without losing their properties. The basic logic of PCA analysis is to evaluate the multidimensional data set in terms of basic features and to express it by transforming it into fewer factors (Armitage et al., 2002; Caussinis et al., 2003; Poudel et. al. 2017). In our study, the PCA analysis showing the interaction between monthly and annual precipitation and quality components and the biplot graph accordingly are given in Table 3. As could be seen in Table 3, the explicability of the interaction between monthly precipitation values and quality components was revealed at the PC5 level. As a result of the analysis, protein content, MSDS, test weight and thousand seed weight were determined as important factors such as March precipitation, April precipitation, June precipitation, September precipitation, October precipitation, and November precipitation. In addition, when the biplot graph is examined, it is possible to reach the following results. There is a positive relationship between the total precipitation in April, May, June and the thousand and test weight, that is, the increase in the total precipitation in these months creates an increase in both quality components. Uşak and Kütahya provinces give better results in terms of thousand seed and test weight. In addition, protein content and MSDS decrease with increasing precipitation. The provinces of Eskişehir and Konya, which receive less precipitation, are the provinces with better results in terms of higher protein and MSDS. In addition, the rains falling in November allow better protein and MSDS values to be obtained. As a result of PCA and biplot analysis, April, May, June and annual total precipitation lead to better thousand seed and test weights, and the decrease in precipitation in these months causes protein and MSDS to decrease. While Uşak and Kütahya provinces are superior regions in terms of thousand seed weight and test weight, Eskişehir and Konya provinces are provinces with better MSDS and protein contents. In addition, March precipitation, April precipitation, June precipitation, September precipitation, October precipitation, and November precipitation were determined as important factors on protein content, MSDS, test weight and thousand seed weight. While the strength of the relationship between the two components is correlation, the shape of the relationship is expressed as regression. Therefore, the relationship between the two components can be positive or negative, linear, polynomial, logarithmic; Regression analysis is a successful method that explains the relationship between the components examined. While applying the regression analysis, the process of choosing the most appropriate variables among the independent variables that are effective on the determined dependent variable is called stepwise regression.

In other words, in this analysis, the most suitable components among all independent variables are selected, and this method is called stepwise regression analysis, since this selection is made step by step (Tibshirani, 1996; Buhlmann and Yu, 2003; Efron et al., 2004; Chen et al., 2011). In this analysis, unnecessary elements are removed from the model, the removed elements are added to the model again, and this time both processes are applied together and the result is reached. This uncomplicated and simple system is used as an effective form of analysis for regression analysis. The stepwise regression analysis showing the effect of monthly and total precipitation on the protein content, one of the quality component, is given in Table 4, considering the protein content, one of the quality components examined in the study, as the dependent variable.

Table 3. PCA analysis and biplot plot showing the interaction between monthly and annual precipitation and quality factors.

	PC_1				PC_2	PC_3	PC_4		PC_5
Eigenvalue	2,940				2,211	1,523	1,078		1,021
Proportion	0,245				0,184	0,127	0,09		0,085
Cumulative	0,245				0,429	0,556	0,646		0,731
Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**Variable**	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
March	0,025	-0,155	**-0,616**	0,165	-0,059	**November**	-0,237	0,028	**-0,539**	-0,272	0,184
April	0,323	-0,049	0,022	**-0,665**	0,164	**Total Ra.**	0,368	-0,359	-0,303	-0,187	0,05
May	0,297	-0,247	-0,021	0,337	0,302	**Test We.**	**0,306**	**0,466**	0,138	-0,023	-0,094
June	0,239	-0,368	0,122	**0,439**	-0,024	**MSDS**	**-0,381**	-0,15	0,146	-0,079	0,044
September	0,051	-0,107	-0,133	-0,07	**-0,907**	**Thou.Seed We.**	0,267	**0,448**	-0,063	0,076	-0,004
October	0,158	**-0,415**	0,384	-0,31	-0,082	**Protein Con.**	**-0,469**	-0,152	0,116	-0,043	0,01

Table 4. Stepwise regression analysis showing the effect of monthly and total precipitation on the protein content of the quality components by considering the protein content as the dependent variable in the quality components examined.

Model		Sum of Squares	df	Mean Square	F	Significance
1	Regression	20,722	1	20,722	8,881	0,004[b]
	Residual	147,008	63	2,333		
	Total	167,731	64			
2	Regression	39,438	2	19,719	9,530	0,000[c]
	Residual	128,293	62	2,069		
	Total	167,731	64			
3	Regression	50,516	3	16,839	8,763	0,000[d]
	Residual	117,215	61	1,922		
	Total	167,731	64			

a: Dependent component, Protein content, **b.** Predictors: (Constant), Total rainfall, **c.** Predictors: (Constant), Total rainfall, April rainfall, **d.** Predictors: (Constant), Total rainfall, April rainfall, October rainfall

		Unstandardized Coef.		Standardized Coef.		
		B	Standard Er.	Beta	t	Significance
1	(Constant)	14,120	0,691		20,445	0,000
R^2: 0,351	Total rainfall	-0,004	0,001	-0,351	-2,980	0,004
	(Constant)	13,567	0,676		20,074	0,000
2	Total rain.	-0,005	0,001	-0,384	-3,443	0,001
R^2: 0,485	April rain.	0,020	0,007	0,336	3,007	0,004
	(Constant)	13,190	0,670		19,685	0,000
3	Total rainfall	-0,006	0,001	-0,507	-4,258	0,000
R^2: 0,549	April rain.	0,027	0,007	0,451	3,830	0,000
	October rain.	0,018	0,007	0,303	2,401	0,019

a. Dependent component: Protein

As can be seen from the table, when the parameters affect the quality are considered step by step; especially the three effective parameters, total precipitation, April rain and October precipitations are effective on protein content, and their effects are so negative and significant.

While the total precipitation on protein content was approximately 35%, the effects of April and October precipitations were approximately 13% and 16%. Therefore, the effect of all three components on protein was determined as 54,9% in total. The stepwise regression analysis showing the effect of monthly and total precipitation on the MSDS, which is one of the quality components, is given in Table 5.

Table 5. Stepwise regression analysis showing the effect of monthly and total precipitation on the MSDS, one of the quality components, by considering the MSDS as the dependent variable.

Model		Sum of Squares	df	Mean Square	F	Significance
1	Regression	419,812	1	419,812	6,492	,013[b]
	Residual	4073,950	63	64,666		
	Total	4493,762	64			

a: Dependent component, MSDS., **b.** Predictors: (Constant), Total rainfall,

		Unstandardized Coef.		Standardized Coef.		
		B	Standard Er.	Beta	t	Significance
1	(Constant)	44,706	3,636		12,296	0,000
R^2:0,306	Total rainfall	-0,019	0,007	-0,306	-2,548	0,013

a. Dependent component: MSDS

As seen from the table, the total precipitation has a significant negative effect on the MSDS and the degree of this effect is 30,6%. The stepwise regression analysis showing the effect of monthly and total precipitation on thousand seed weight, one of the quality components, is given in Table 6.

Table 6. Stepwise regression analysis showing the effect of monthly and total precipitation on thousand seed weight, one of the quality components, by considering the thousand seed weight as the dependent variable.

Model		Sum of Squares	df	Mean Square	F	Significance
1	Regression	44,521	1	44,521	4,997	0,029[b]
	Residual	561,293	63	8,909		
	Total	605,814	64			
2	Regression	78,953	2	39,476	4,646	0,013[c]
	Residual	526,861	62	8,498		
	Total	605,814	64			

a: Dependent component, Thousand seed weight., **b.** Predictors: (Constant), October rainfall, **c.** Predictors: (Constant), October rainfall, April rainfall

		Unstandardized Coef.		Standardized Coef.		
		B	Standard Er.	Beta	t	Significance
1	(Constant)	36,450	0,722		50,456	0,000
R^2: 0,271	October rain.	-0,030	0,013	0,271	2,235	0,029
2	(Constant)	35,414	0,873		40,548	0,000
R^2: 0,361	October rain.	-0,038	0,014	0,340	2,760	0,008
	April rain.	0,033	0,016	0,248	2,013	0,048

a. Dependent component: Thousand seed weight

In the analysis, the October precipitation and the April precipitation positively contributed to the thousand seed weight. Monthly precipitations caused an increase in thousand seed weight in contrast to protein and MSDS. While the effect degree of October on the thousand seed weight was determined as approximately 27%, the effect degree of the precipitation in April was determined as approximately 9%. The total effect of both months was determined as 36,1.

Therefore, it has been determined that the precipitations that are effective on the thousand seed weight are the precipitations of October and April. The stepwise regression analysis showing the effect of monthly and total precipitation on test weight, one of the quality components, by considering test weight as a dependent variable, is given in Table 7. As can be seen from the table, monthly precipitations that have a significant effect on the test weight are revealed as precipitation in April, precipitation in October, precipitation in November and precipitation in March. The precipitation falling in these months has a positive effect on the test weight. With increasing precipitation, the test weight also increases. The highest contribution to the increase in test weight was determined as April with approximately 26%, October precipitation with approximately 15%, November precipitation with approximately 12%, and March precipitation with approximately 6%.

Table 6. Stepwise regression analysis showing the effect of monthly and total precipitation on test weight, one of the quality components, by considering test weight as a dependent variable.

Model		Sum of Squares	df	Mean Square	F	Significance
1	Regression	20,605	1	20,605	4,807	0,032[b]
	Residual	270,069	63	4,287		
	Total	290,674	64			
2	Regression	49,429	2	24,714	6,352	0,003[c]
	Residual	241,245	62	3,891		
	Total	290,674	64			
3	Regression	85,428	3	28,476	8,463	0,000[d]
	Residual	205,245	61	3,365		
	Total	290,674	64			
4	Regression	106,829	4	26,707	8,716	0,000[e]
	Residual	183,845	60	3,064		
	Total	290,674	64			

a: Dependent component, Test weight, **b.** Predictors: (Constant), April rainfall, **c.** Predictors: (Constant), April rainfall, October rainfall, **d.** Predictors: (Constant), April rainfall, October rainfall, November rainfall, **e.** Predictors: (Constant), April rainfall, October rainfall, November rainfall, March rainfall

		Unstandardized Coef.		Standardized Coef.		
		B	Standard Er.	Beta	t	Significance
1 R^2: 0,266	(Constant)	77,963	0,537		145,254	0,000
	April rain.	0,024	0,011	0,266	2,192	0,032
2 R^2: 0,412	(Constant)	78,770	0,591		133,282	0,000
	April rain.	0,033	0,011	0,358	2,970	0,004
	October rain.	-0,025	0,009	0,328	2,722	0,008
3 R^2: 0,542	(Constant)	80,327	0,727		110,461	0,000
	April rain.	0,032	0,010	0,351	3,134	0,003
	October rain.	-0,035	0,009	0,454	3,834	0,000
	November rain.	-0,029	0,009	0,375	3,271	0,002
4 R^2: 0,606	(Constant)	81,641	0,854		95,652	0,000
	Total rainfall	0,033	0,010	0,360	3,367	0,001
	April rain.	-0,038	0,009	0,498	4,353	0,000
	November rain.	-0,026	0,009	0,339	3,076	0,003
	March rain.	-0,031	0,012	0,279	2,643	0,010

a. Dependent component: Test weight

Therefore, the main actors of the high test weight are the precipitation in April and October. The effect degree of the test weight of the precipitation received in all four months was determined as 60.6%. As a result of the stepwise analysis, the precipitation in April, the precipitation in October and the total precipitation were effective on the quality. The rainfall in these months causes a significant decrease in protein content and MSDS, while increasing the of thousand seed weight and test weight.

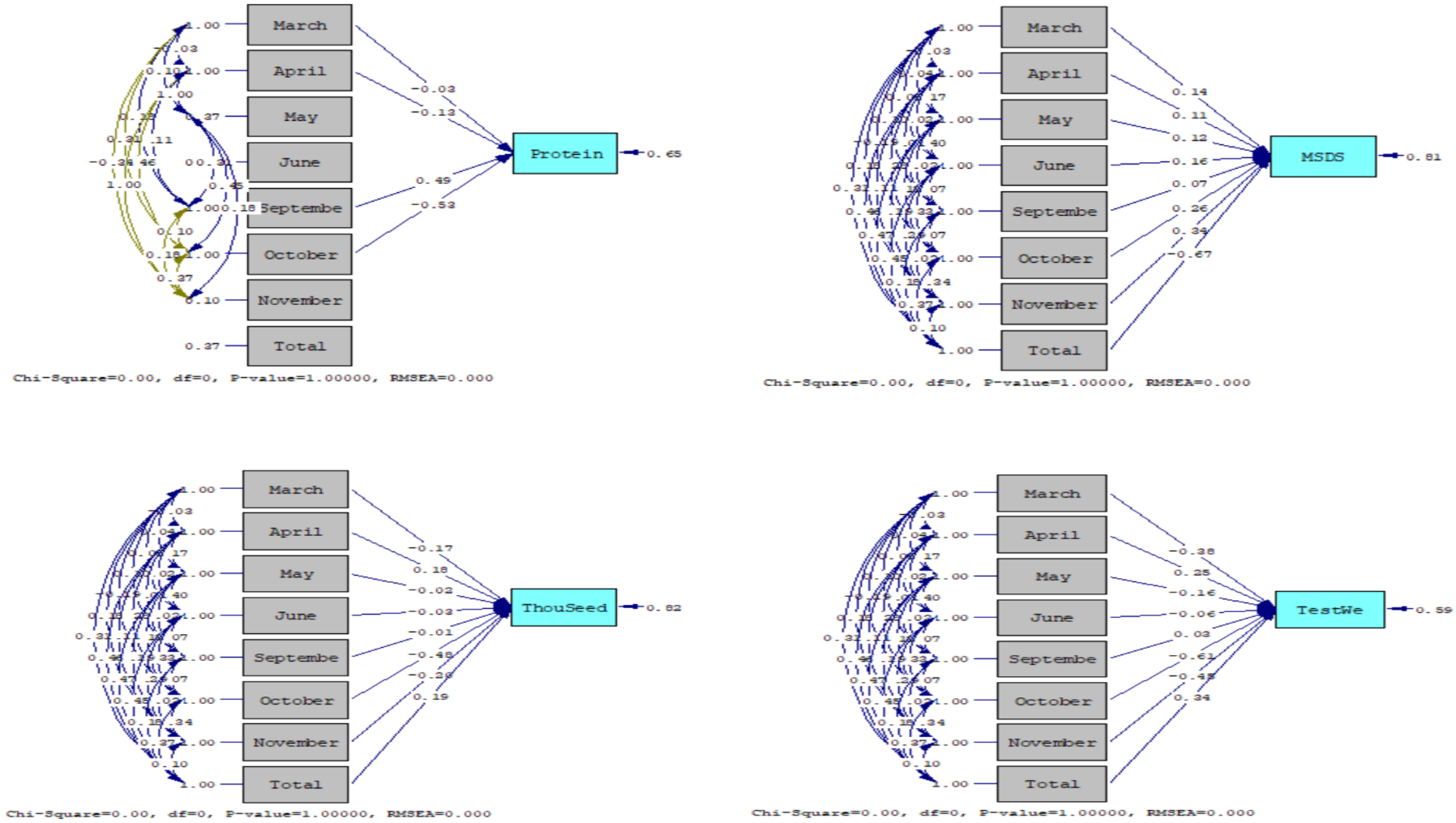
Path analysis has been successfully used to determine the relationship hierarchy among the examined components. Path analysis is successfully used in terms of revealing the direct and indirect effects (effect percentages) of other independent components on the dependent component determined between the components (Scheiner et al., 2000; Kashif and Khaliq, 2004; Anwarmalik et al., 1997; Dalkani et al., 2011). Path analysis is also useful in explaining the variation of the independent components on the dependent, as it effectively shows the direct and indirect effects of the independent components on the dependent. In the path analysis, which can be expressed as the interaction of the correlation relations of the selected independent components with each other and with the dependent component and shown directly and indirectly, the direct effect of each component on each dependent component and the indirect effects on the others are shown, and the effects of the independent components on the dependent component could be successfully

demonstrated (Dewey and Lu, 1959). In this study, the path analysis showing the effects of monthly precipitation and total precipitation on protein content, MSDS, thousand and test weight is given in Figure 1.

The months with the greatest effect on the protein content were determined as April, September, October precipitation and total precipitation. Likewise, the factors that have a significant impact on the MSDS were determined as total precipitation and October precipitation. It has been revealed that the total precipitation and precipitation in March, April, September, October and November have a significant effect on the thousand seed weight. Likewise, it was determined that the total precipitation, October, November, March and April precipitations had a significant effect on the test weight. As a result of the path analysis, total precipitation, autumn September and October precipitation, and spring April precipitation decreased the protein content and MSDS. On the other hand, total precipitation, March, April, May precipitation and September, October and November precipitation had a significant effect. It has been revealed that with increasing precipitation amount and an increase in thousand seed and test weights.

Decision tree, which is one of the analysis methods that has been put forward in recent years and its use is becoming more and more widespread, is an important analysis in terms of revealing the effect hierarchy of the components in question. In this analysis, the main factor in the form of tree branches and sub-factors are determined according to the values of the main factor, and the value of the dependent variable at each factor point is determined theoretically. The decision tree is widely used in the analysis of multiple components and shows the effect of other factors on the determined dependent variable in a hierarchical manner. This method, both visually and scientifically, shows the effect values of the main factors and the side factors under them by affecting the dependent variable and the value that the dependent variable will take in terms of these values (Smith, 1989; Call and Miller, 1990; Pal and Mather, 2003; Danielson et al., 2007; Nie et al., 2009; Phadatare et al., 2014). In our study, although protein content, MSDS, thousand seed and test weight are considered as dependent variables, the effects of monthly precipitation and annual total precipitation on protein content, MSDS, thousand seed and test weight, which are considered as dependent variables, are given in Figure 2. As seen in the figure, the main factor affecting the protein content is the total precipitation, but the protein content was determined as 12,562% when the total precipitation was below 551,250 mm, and 11,124% when the precipitation was higher than 551,250 mm. June precipitation is effective when precipitation is more than 551,250 mm. Protein content is 12,406% when the June precipitation is less than 21,250 mm, and 10,666% when the precipitation is higher than 21,250 mm. Again, if the June precipitation is less than 221,250 mm, the October precipitation is effective. Protein content is determined as 9,527% when the October precipitation is less than 48,595 mm, and 11,121% when it is higher than 48.595 mm. According to the decision tree analysis, total precipitation, June and October oils have a significant effect on the protein content (R^2:0,717). It was determined that the main factor on the MSDS was total precipitation. It has been revealed that the MSDS is 37,870 ml when the total precipitation is less than 551,250 mm, and 30,781 ml when the total precipitation is higher than 551,250 mm. If the total precipitation is less than 551,250 mm, the October precipitation is effective, when the October precipitation is less than 2.8 mm, the MSDS is 32.317 ml, and when it is higher than 2.8 mm, the MSDS is 40.058 ml. Again, when the October precipitation is less than 2.8 mm, the May precipitation is effective, when the May precipitation is less than 40,250 mm, the MSDS is 23,043 ml, and when the May precipitation is higher than 40,250 mm, the MSDS is 35,980 ml. Here, total precipitation and precipitation in October and May are determined as effective on MSDS (R^2:0.669).

Figure 1. Path analysis showing the effects of monthly precipitations and total precipitation of the locations on protein content, MSDS, thousand seed weight and test weight.

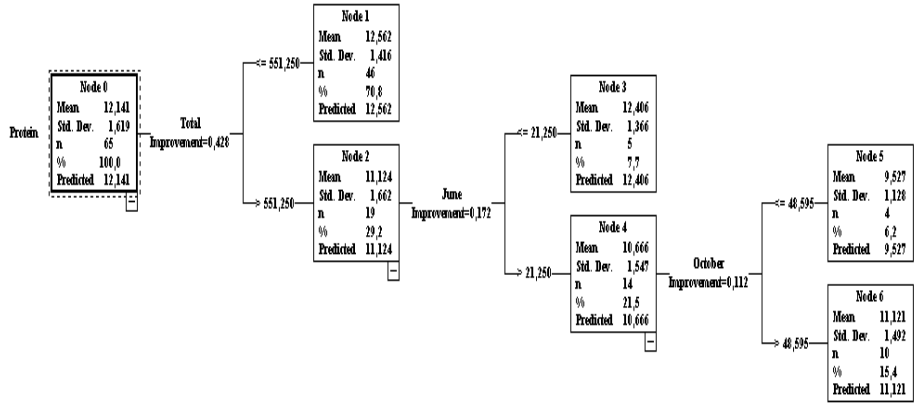


October precipitation mainly affects the thousand seed weight. When the October precipitation is less than 33,750 mm, the thousand seed weight is 36,568 g. If it is more than 33,750 mm, thousand seed weight is 34,183 g. If the October precipitation is less than 33,750 mm, the June precipitation is effective. When the precipitation in June is less than 4,700 mm, a thousand seed weight is 33,197 g. In the case where the precipitation in June is above 4,700 mm, the weight of a thousand seeds is 37,049 g. In the case of April less than 30,150 mm, the thousand seed weight is 34,782 g, and in the case of April precipitation higher than 30,150 mm, the thousand-seed weight is 28,443 gr. October precipitation is effective when April is less than 30,150 mm. When the precipitation in October is less than 25,300 mm, a thousand seed weight is 34,751 g. If the precipitation in October is more than 25,300 mm, thousand seeds weight is 37,585 g. June precipitation is effective when the precipitation in October is less than 25,300 mm.

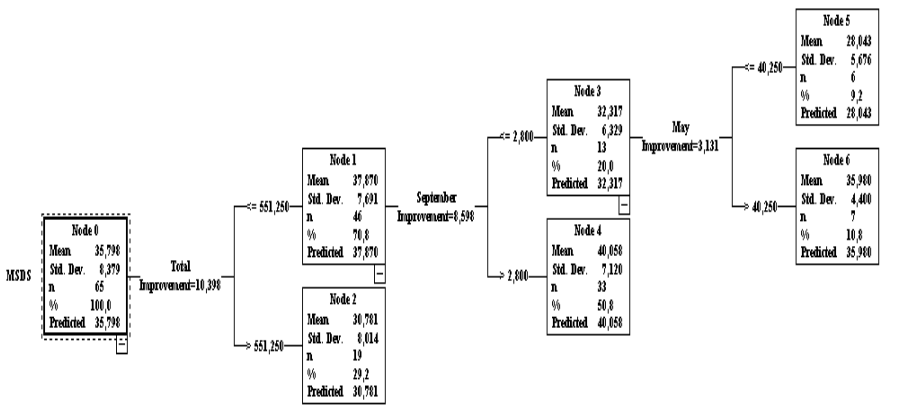
When the June precipitation is less than 17,250 mm, the thousand seed weight is 36,030 g. In case the June precipitation is more than 17,200 mm, the of a thousand seed weight becomes 33,805 g. If the precipitation in October is more than 33,750 mm, November precipitation is effective. When the precipitation in November is less than 62,150 mm, the thousand seed weight is 37,409 g. In case the November precipitation is more than 62,150 mm, the thousand seed weight is 30,313 gr. If the November precipitation is less than 62,150 mm, the April precipitation comes into play. When the precipitation in April is less than 52,100 mm, the thousand seed weight is 33,789 g, and when it is higher than 52,100 mm, it is 35,688 g (R^2: 0.543). Here, the precipitations affecting the thousand seed weight were determined as April, June, October, and November precipitations.

The main precipitation affecting the test weight appears as the precipitation of March. In the case where the precipitation in March is less than 84 mm, the test weight is 79,196 kg/hl, and when precipitation in March is above 84,000 mm, it becomes 72,210 kg/hl. If the precipitation in March is less than 84 mm, the November precipitation comes into play. When the November precipitation is less than 89,550 mm, the test weight is 79,343 kg/hl, and when November precipitation is more than 89,550 mm, test weight is 76.267 kg/hl. October precipitation is effective when November precipitation is less than 87,550 mm, test weight is determined as 80,142 kg/hl when October precipitation is less than 33,750 mm, and test weight is determined as 78,280 kg / hl when October precipition is higher than 33,750 mm.

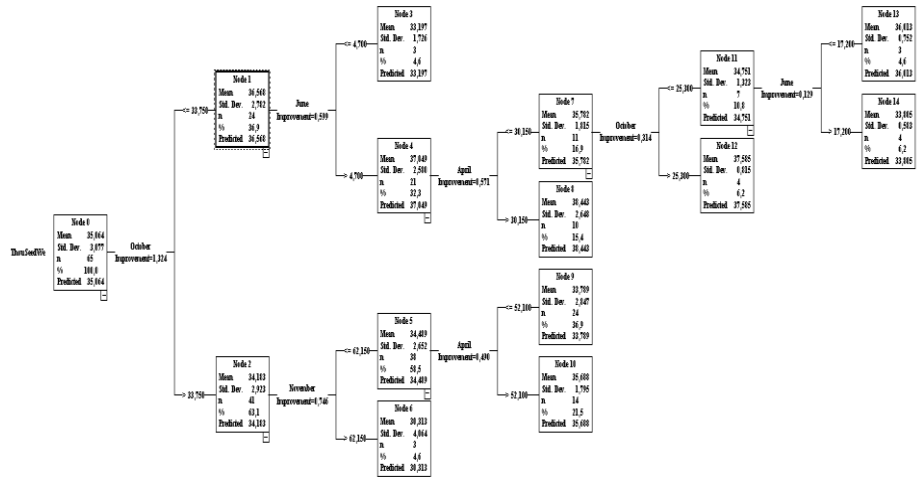
Figure 2. Decision tree analysis showing the effects of monthly precipitations and annual total precipitation on protein content, MSDS, thousand seed and test weight.



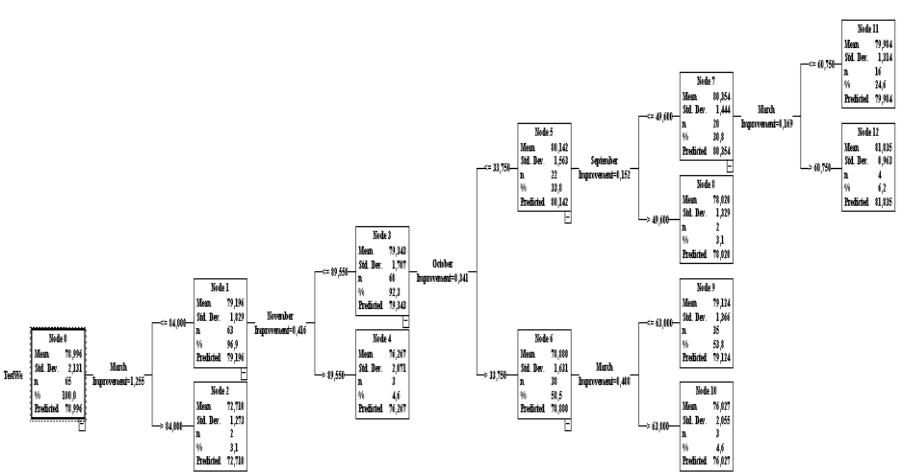
Protein Content, R^2: **Risk Estimation/**δ^2:1,881/2,62: 0,717



MSDS, R^2: 47,007,/70,205: 0,669



Thousand Kernel Weight, R^2: 5,147/9,471: 0,543



Test Weight,R^2:1,731/4,541: 0,381

September precipitation is effective when October precipitation is less than 33,750 mm. If the September precipitation is less than 49,650 mm, the test weight is 80,350 kg/hl, and when the September precipitation is more than 49,600 mm, the test weight is 79,007 kg/hl. When the September precipitation is less than 49,600 mm, the March precipitation comes into play. When the March precipitation is less than 60,750 mm, the test weight is 79,984 kg/hl, and when the March precipitation is more than 60,750 mm, it is 87,835 kg/hl. When the October precipitation is higher than 30,750 mm, the March precipitation comes into play (R2: 0,281). Here, the precipitations that affect the test weight are the precipitations of March, September, October and November. Here, the test weight is mainly affected by the autumn precipitation. As a result of the decision tree analysis, rainfalls in March, April, May, June and October, September, November and total precipitation were determined as effective precipitation on quality.

The quality concept, including the protein content of precipitation, MSDS, thousand seed weight and test weight in bread wheat, is highly affected by the precipitation regime which is, one of the environmental factors. Therefore, although quality is affected at different rates by environmental factors including precipitation; The aim of breeding studies is to develop high yielding and quality, stabile genotypes versus biotic and abiotic stresses (Panozzo and Eagles, 2000; Guarda et al., 2004; Anwarmalik et al. 2007). As a result; March precipitation, April precipitation, June precipitation, October precipitation and total precipitation were determined as significant factors affecting the quality (protein content, MSDS, thousand kernel weight and test weight) in bread wheat. Increasing precipitation, June precipitation, October precipitation and total precipitation cause increase in protein content and MSDS, while they cause a relative decrease in protein content and MSDS. Uşak and Kütahya provinces are superior regions in terms of test weight, thousand seed weight, Eskişehir and Konya provinces were determined as better provinces for MSDS and protein content.

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Author Contributions

Murat OLGUN: Field work, laboratory work, article writing, Savaş BELEN: Field work, article writing, Yaşar KARADUMAN Field work, ,article writing, Zekiye BUDAK BAŞÇİFTÇİ: Field work, laboratory work, article writing, Nazife Gözde AYTER ARPACIOĞLU: Field work, laboratory work, article writing.

Conflict of Interest

The authors are declared that they have no conflict for this research article.

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Fruit morphological and nutritional characteristics of different *Rosa pimpinnelifolia* genotypes

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Abstract

Wild edible fruits have been gained more popularity more recently due to their higher human health related compounds and unique aroma characteristics. The genus *Rosa* includes over 30 species and among them *Rosa pimpinellifolia* has distinct plant and fruit traits and the studies on this specie in literature is very limited. In this study, some fruit morphological and nutritional characteristics of six *R. pimpinellifolia* plants naturally grown in Ilica district of Erzurum province have been studied. Results exhibited that genotypes differed each other for most of the morphological and nutritional characteristics. The six genotypes showed fruit weights and flesh ratio between 1.88-2.21 g and 81.10-90.83%, respectively. Total anthocyanin, vitamin C and total phenolic content of the six *R. pimpinellifolia* genotypes ranged from 3.74-5.02 mg cyanidin-3-glucoside, 37-53 mg per 100 g fresh weight, 1018-1407 mg gallic acid equivalent (GAE) per 100 g fresh weight, respectively. Antioxidant activity was found between 10.11-13.86 µmol Fe (II)/g fresh weight among genotypes. Results indicate potential use of fruits of *R. pimpinellifolia* in food industry in future.

Keywords: *Rosa pimpinellifolia*, morphological and biochemical content, diversity

Introduction

Plants in particular wild edible ones are genetically very diverse group and found different parts of the World and accepted an important part of human history used for nutrients and health care for centuries. They have been using currently mainly as food, herbal medicine and ornamental (aesthetic) purposes (Kaliora and Dedoussis, 2007). The use of wild edible plants including *Rosa pimpinellifolia* have been promoting by World Health Organization due to their local availability, cheapness and effectiveness. During last decade scientific studies have been increased to determine nutritional composition and therapeutic activity of different wild edible plants (Sommano et al., 2013; Alam et al., 2020). The studies showed that consumption of those plants had positive effect on human diet and health indicating wild edible plants rich sources of antioxidant compounds such as phenolics, anthocyanins, ascorbic acid etc. (Sommano et al., 2013; Alam et al., 2020).

Rosa pimpinellifolia is one of the most important rosehip species whose awareness and importance has increased in recent years in Turkey (Ercisli, 2005, Kan, 2021). It is widely found in Eastern and Northeastern Anatolia region in Turkey and had distinct variable black fruit color. It is more populated in particular in area of Bayburt, Erzurum and Gümüşhane in Eastern and Northeastern Anatolia (Ercişli 2005). In these regions *Rosa pimpinellifolia* is known by different local names. For example, it is called "Şilan" in Siirt, "Sarıgül" in Konya, "Kara kuşburnu" in Erzurum and "Koyun gözü" in Bayburt, Gümüşhane and Erzincan provinces (Korkmaz and Ozcelik, 2015). Due to its black color, it is generally known as black rosehip among the people in the other growing areas in Turkey (Macit and Köse, 2015).

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For a long time, fruits and roots of *Rosa pimpinellifolia* have been used in traditional medicine in the treatment of digestive and stomach ailments or bloating, hemorrhoids, cough and urinary infections. Its roots are also used as a natural dye (Ercisli 2005).

Rosa pimpinellifolia differs from the other rosehip species in terms of fruit characteristics and root structure. In general marmalade and jam obtained from fruits of different *Rosa* species but marmalade and jam cannot be obtained from the fruit of this species. Instead of marmalade and jam, its fruits and roots commonly used as herbal tea (Ercişli 2005). Compared to the other *Rosa* species, it spreads in a narrower area and resistant to pest and diseases. The distribution of the species in Turkey is 1200-2750 m (Ercişli 2005; Korkmaz et al. 2013). *Rosa pimpinellifolia* is also an aesthetic *Rosa* species with narrow spread and white flowers.

The aim of this study to determine some important fruit morphological and nutritional characteristics of six *R. pimpinellifolia* plants.

Materials and Methods

Plant material

The plant material naturally found in Ilica district of Erzurum province at the altitude of 1800 m. Fully maturated fruits were harvested at 2018 year from different parts of shrubs in *R. pimpinellifolia*. Harvested fruits were brought to laboratory in cold chain.

Morphological characteristics

Fruit weight and fruit flesh ratio are important fruit morphological characteristics of *Rosa* species. Fruit weight of six genotypes was measured by using 0.01 g electronic balance on 50 fruit per genotype. Fruit flesh ratio was determined on 50 fruits per genotype as well by using fruit weight-seed weight/fruit weight x 100 formula.

Nutritional composition

Sample preparation and extraction

The harvested fruits of six genotypes *R. pimpinellifolia* stored at -80 ºC until further analysis. During the analysis, the frozen fruits were taken and thawed to 24-25 ºC. Later, fruit samples were homogenised and a single extraction procedure (taking 3 g aliquots transferred inside tubes and extracted for 1 hour with 20 mL buffer including acetone, water (deionized), and acetic acid (70:29.5:0.5 v/v) (Singleton and Rossi, 1965).

Extraction of sugars

For individual sugar determination a mix (five grams of samples plus metaphosphoric acid, 2.5%) used. After that homogenates centrifuged at 10000 rpm for 10 min then filtered into HPLC vials. All samples and corresponding standard injection were repeated three times. The sugar content expressed as g/100 g fresh weight.

Total phenolic contents

The total phenolic content (TPC) of the fruit samples of *Rosa pimpinellifolia* was determined by Singleton and Rossi (1965) and results expressed mg of gallic acid equivalents (GAE) per 100 g fresh sample.

Total anthocyanin content

pH differential method of Giusti and Wrolstad (2005) was used to determine total anthocyanin content. And results expressed as mg of cyanidin-3-glucoside equivalent in 100 g of fresh sample.

Ferric Reducing Antioxidant Power Assay

FRAP (Ferric reducing antioxidant power) method was used for antioxidant capacity analysis according to Benzie and Strain (1996). The FRAP was expressed as µmol Fe (II)/ g fresh weight.

Statistical Analysis

For statistical analysis, SPSS software and procedures were used. For replicate was used each analysis The data subjected to Duncan multiple range tests at the significant level of $p<0.05$. , Data were processed according to principal component analysis (PCA) using SPSS for Windows Version 15.0, SPSS Inc. (Chicago, IL, USA).

RESULTS AND DISCUSSION

Morphological traits

Fruit weight, fruit flesh ratio and external (peel) color of six *R. pimpinellifolia* genotypes are shown in Table 1. As indicated in Table 1, fruit weight and flesh ratio were found to significant at $p<0.05$ level among six *Rosa pimpinellifolia* genotypes. The highest fruit weight was obtained from RP1 genotype as 2.21 g and followed in descending order RP2 (2.10 g)>RP5 (2.07 g)>RP6 (2.02 g)> RP4 (1.94 g)>RP3 (1.88 g), respectively. In addition, RP5 and RP6 genotypes placed same statistical group for fruit weight (Table 1). The fruit weight of selected rose hip genotypes belongs to different species varied between 0.61-4.95 g in different regions of Turkey (Yamankaradeniz, 1983; Balta and Cam, 1996; Kazankaya et al., 2005).

The fruit flesh ratio of six *Rosa pimpinellifolia* genotypes were found between 81.10 % (RP4) and 90.33% (RP1) and RP3 and RP6 genotypes were found at same statistical group. Among six genotypes the majority had black fruit peel color (RP1, RP3, RP4 and RP6) and one genotype had light black and one genotype had dark black fruit peel color (Table 1). Kan (2021) indicated that average fruit weight of *R. pimpinellifolia* is 1.77 g which is close value with our samples. Fruit weight of *Rosa* is specie and genotype dependent and ecological and geographical conditions are also effects fruit weight values of the rose hips (Yamankaradeniz, 1983; Yoruk, 2006; Ercisli, 2007). Ercisli (1996) reported a wide variation among *Rosa* species for fruit flesh ratio which ranged from 54.88% to 91.86% with average of 67.30%. In another study, Karakuş ve Bostan (2017) found fruit flesh ratio of a large number of *Rosa* genotypes between 63.89-75.01%. Özen (2013) found fruit flesh ratio between 61.69% and 82.83% among *Rosa* genotypes. According to those results it is possible to say that *R. pimpinellifolia* had higher fruit flesh ratio than the other *Rosa* species due to lower number of seeds.

Table 1. Fruit weight, flesh ratio and peel color of *Rosa pimpinellifolia* genotypes

Genotypes	Fruit weight (g)	Flesh ratio (%)	Peel color
RP1	2.21a	90.33a	Black
RP2	2.10b	87.06b	Light Black
RP3	1.88cd	85.80bc	Black
RP4	1.94c	81.10cd	Black
RP5	2.07bc	83.38c	Dark Black
RP6	2.02bc	85.40bc	Black

*Different letters indicate the statistical difference within the same column among genotypes and species at 5% level.

Ascorbic Acid (Vitamin C), Total phenolic, total anthocyanin and total antioxidant capacity

Vitamin C (Ascorbic acid), total phenol, total anthocyanin content, and total antioxidant capacity of the six *R. pimpinellifolia* genotypes are given in Table 2. As seen in Table 2, there were statistically significant differences (p<0.05) among genotypes in terms of vitamin C, total phenol, total anthocyanin and antioxidant capacity.

Vitamin C content were found between 37-53 mg per 100 g fresh fruit. Kan (2021) showed that *R. pimpinellifolia* had 59 mg vitamin C in 100 g fresh fruit. Previous studies indicated that *Rosa* species exhibits variable vitamin C content. In Romania, Roman et al. (2013) reported that *Rosa* genotypes had vitamin C between 112-360 mg per 100 g of fresh rose hips. Celik et al. (2009) showed vitamin C content in fruits of different *Rosa* species between 604 and 1032 mg/100 g. Vitamin C content of rose hips also varies with geographical and climatic conditions (Ercisli, 2005). The results indicated that fruits of *Rosa pimpinellifolia* had the lowest vitamin C content among *Rosa* species.

Total phenol content of *R. pimpinellifolia* genotypes ranged from 1018-1407 mg GAE per 100 g. This result revealed that *R. pimpinellifolia* had high total phenolic content among *Rosa* species. Montazeri et al. (2011) reported average 424 mg GAE/100 g total phenol content in *Rosa canina* fruits. Phenolic compounds are mainly responsible for total antioxidant capacity in plants (Javanmardi et al. 2003). Koczka et al. (2018) found that fruits of *Rosa pimpinellifolia* (*R. spinosissima*) grown in Hungary had higher total phenol content than the other *Rosa* species and total phenolic content were in decreasing order *Rosa pimpinellifolia* > *R. canina* > *R. rugosa* > *R. gallica*. The total phenol differences among genotypes could be explained by genetic background and also growing and cultivated conditions.

Total anthocyanin content of *R. pimpinellifolia* genotypes were in range of 3.74-5.02 mg cyanidin-3-glucoside equivalent per 100 g of fresh fruit and the genotypes statistically differed each other for total anthocyanin content (p<0.05) (Table 2). Kan (2021) found that *R. pimpinellifolia*, *R. canina* and *R. villosa* had total anthocyanin content 3.72 mg, 2.75 mg and 2.80 mg cyanidin-3-glucoside equivalent per 100 g fresh weight, respectively. The present results implied that *R. pimpinellifolia* had the highest total anthocyanin content than the other *Rosa* species. Previous study showed that fruits of *Rosa* species mainly had cyanidin-3-glucoside (Guimaraes et al., 2013) and cyanidin-3-glucoside was reported to have the highest oxygen radical scavenging effect (Wang et al., 1997).

The antioxidant capacity of six *R. pimpinellifolia* genotypes determined by FRAP assay has been shown in Table 2. Statistically significant differences (p<0.05) were evident among six genotypes in the total antioxidant capacities. FRAP values ranged from 10.11 μmol Fe (II)/g (RP2) to 13.86 μmol Fe (II)/g (RP5) (Table 2). Previously Demir et al. (2014) reported differences on total antioxidant capacity of *Rosa* species. Cunja et al. (2015) reported that the highest antioxidant capacity was observed in *R. canina* fruit. Koczka et al. (2018) in their study conducted in Hungary determined that the antioxidant activity of *Rosa* species and the fruits of the *Rosa pimpinellifolia* (*R. spinosissima*) was found to be higher antioxidant capacity than the other *Rosa* species. They found a direct high correlation between the total phenol content and antioxidant activity. These results reveal that the fruits of *R. pimpinellifolia* can be used as a natural antioxidant source.

Table 2. Vitamin C, total phenol, total anthocyanin and antioxidant capacity of *Rosa pimpinellifolia* genotypes

Genotypes	Vitamin C (mg/100 g)	Total phenol (mg GAE/100 g)	Total anthocyanin (mg cy-3-gluc/100 g)	FRAP (μmol Fe (II)/g)
RP1	37b	1090ab	4.33bc	10.96cd
RP2	48ab	1018cd	3.74c	10.11d
RP3	53a	1164b	4.12bc	11.60c
RP4	50ab	1333c	4.78ab	12.44b
RP5	44b	1407a	5.02a	13.86a
RP6	40bc	1227c	4.55b	11.89bc

*Different letters indicate the statistical difference within the same column among genotypes and species at 5% level

Individual sugars

Sugar content in fruits of six *R. pimpinellifolia* genotypes is shown in Table 3. Sugar and organic acid contents are in general determining fruit quality and taste (Kobus et al., 2005). As presented in Table 3, there were significant differences (p<0.05) among genotypes for glucose, sorbitol

and fructose content. For all genotypes glucose was the main sugar (5.74-6.55 g/100 g) and followed by sorbitol (4.67-5.11 g/100 g) and fructose (3.98-4.35 g/100 g). Sucrose content was the lowest (0.18-0.44 g/100 g) and found insignificant among genotypes (Table 3). Kan (2021) reported glucose, sorbitol, fructose and sucrose in *R. pimpinellifolia* fruits as 6.88, 4.82, 4.23 and 0.36 g/100 g fresh weight base. Previous studies indicated that glucose was the major sugar in rose hips, ranged between 7.45–12.94 g/100 g and followed by fructose (Yoruk et al., 2008; Barros et al., 2011; Rosu et al., 2011; Ozrenk et al., 2012; Demir et al., 2014). Ozrenk et al. (2012) reported sucrose content in *Rosa* species between 0.17-0.88%.

Table 3. Specific sugars (g/100 g) in fruits of *Rosa pimpinellifolia* genotypes

Genotypes	Glucose	Sorbitol	Fructose	Sucrose
RP1	6.07c	5.03ab	4.07ab	0.27^{NS}
RP2	6.55a	5.11a	4.35a	0.44
RP3	5.85cd	4.88ab	4.24ab	0.23
RP4	5.97cd	4.95ab	4.18ab	0.30
RP5	5.74d	4.67b	3.98b	0.18
RP6	6.33b	4.80ab	4.02ab	0.35

*Different letters indicate the statistical difference within the same column among genotypes and species at 5% level. NS: Non Significant

Conclusion

Total phenol, anthocyanin content and antioxidant activities of the fruits belonging to the *R. pimpinellifolia* were found to be quite high. Due to the high content of anthocyanins and antioxidants, the fruits of this species could be able to be used as a natural source of anthocyanins and antioxidants. The fruits of this specie have aromatic feature and plants resistance to diseases and pests that could be important for organic agriculture. Considering the limiting feature of the climate and its distribution in a narrow area, it is necessary to ensure the reproduction of the species in order to spread it.

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Advantages of microorganism containing biological fertilizers and evaluation of their use in ornamental plants

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Abstract

In recent years, due to issues such as irregular and unplanned urbanization and industrialization, the existing agricultural areas have been decreasing rapidly, and the decreasing agricultural areas reveal the necessity of increasing the yield obtained from the unit area. This necessity leads to the pollution of agricultural lands and indirectly groundwater due to more chemical inputs day by day. Chemical fertilizers, one of the most common of these inputs, are the leading cause of pollution. Unconscious and excessive use is one of the most important factors of pollution. In recent years, with the increasing awareness and regulations, efforts on safe and healthy food production have increased, and in this context, the use of more environmentally friendly products that are harmless to humans and other living things has become widespread. In this context, some beneficial bacteria and fungal organisms isolated from soil are the most common biological fertilizers known to improve plant and soil properties. The hyphae formed by mycorrhizae containing fungal organisms can cover the plant roots and improve the surface area of the roots and the absorption of elements by extending deep into the soil. They also support plant growth by increasing phytohormone production. PGPRs (Plant growth-promoting rhizobacteria) containing beneficial bacteria, on the other hand, enable plants to absorb nitrogen and other elements in the atmosphere more easily, and they can also contribute to the development of plants by synthesizing growth-promoting substances. Ornamental plants cultivation, which is one of the most important subjects of agriculture and is carried out in large areas around the world, is one of the sectors where agricultural inputs are used intensively. In this study, more detailed information about biological fertilizers containing microorganisms, which are environmentally friendly fertilizers, will be given and the possibilities of their use in the field of ornamental plants and the studies that have been made will be tried to be examined.

Keywords: Mycorrhiza, *Trichoderma*, Bio Fertilizer, Ornamental Plants

Introduction

Decrease in biodiversity, contamination of surface and groundwater with nitrogen and pesticides, eutrophication of surface waters, ammonia evaporation at varying rates depending on the amount of fertilization, which are the undesirable consequences of intensive agriculture, are some of the biggest threats facing modern humanity. Worldwide, this threat is getting bigger due to reasons such as increasing infrastructure activities, urbanization and the resulting waste problem reaching uncontrollable dimensions and improper forest management. Initially, this system, which is based on the philosophy of using intensive chemical inputs to increase production and efficiency; has also irreversibly deteriorated soil fertility in many areas. Thus; Soil biology, which is one of the most important components of soil fertility, is an ecosystem of organisms living in the soil and interacting with other components, and has a highly complex and dynamic structure that varies greatly according to conditions (Jakoby et al. 2017).

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Among existing organisms, non-pathogenic bacteria and fungi, which are of particular importance for plant growth and soil health and a sustainable environment, have become even more important in the last 30 years. More importantly, these organisms do not exist alone, they interact, and these interactions affect soil fertility more or less than the individual activities of the organism. Besides the soil matrix, the chemical and physical properties of soils, such as the quality and amount of soil organic matter, pH and redox conditions, have a marked effect on the dynamics of microbial community structure and function in soils (Lombard et al. 2011). Therefore, a healthy soil is the net result of ongoing conservation and degradation processes, largely dependent on the biological component of the soil ecosystem. It also affects plant health, environmental health, food safety and quality (Nielsen and Winding 2002). Soil microorganisms have been divided into three groups by scientists as having beneficial, harmful and neutral effects (Whipps 2001; Bais et al. 2006). Beneficial soil microorganisms are involved in fixing nitrogen in the atmosphere, decomposing organic waste, and residues, eliminating the harmful effects of pesticides, suppressing plant diseases and soil-borne pathogens, producing bioactive substances such as vitamins, hormones, and enzymes that increase plant growth, improving soil properties and maintaining natural balance. (Higa 1994; Fuente-Ramirez and Caballero-Mellado 2005; Gupta 2012). The future of agriculture needs to protect the soil microorganisms whose populations are reduced or depleted due to the deterioration of the ecological balance in agricultural practices or to restore the destroyed ones.

In developed countries, producer or consumer groups, who become conscious with increasing education and income levels, give more importance to the production branches called biological, ecological or organic agriculture, and prefer practices that harm the natural balance less, pollute the environment less, and are harmless to the health of other living things (Özyazıcı et al. 2010; Kodaş 2011; Çakmakçı et al. 2010; Dursun et al. 2019). In these production lines, researches are carried out on the use of many inputs in order to improve the soil structure and ensure its sustainability, and materials containing microorganisms are the leading ones.

Ornamental plants sector is an important production category with high added value in plant production. The ornamental plants sector, which is grouped as cut flowers, indoor plants, outdoor plants, and bulbs, emerges as an agricultural activity area where interest is increasing day by day due to its worldwide export potential. The production of ornamental plants, which started to gain value at the beginning of the 20th century, is made in more than 50 countries around the world and on a total area of 749.200 hectares. In this production field, where a substantial agricultural area is used, it is important to research, use and disseminate the application of environmentally friendly inputs, as in other production lines (Anonymous 2020).

In this evaluation; microbial fertilizers' types, properties and capabilities will be mentioned and information about the studies in the field of ornamental plants will be gathered under one roof and the possibilities of using these fertilizers in this field will be discussed.

Effects Of Microorganism Fertilizers On Ornamental Plants Growıng

The soil mass around the root, called the rhizosphere, is a very common environment for microorganisms. Although microorganisms have numerous tasks, they have an important place in terms of plant development, yield and soil fertility (İmriz et al. 2014, Koç et al. 2015). Rhizobacteria, AMFs and Tricodermas are the leading microorganisms that increase plant growth.

Rhizobacteria, named PGPR (plant growth-promoting rhizobacteria) by Kloepper and Scroth and discovered in 1978, colonize the rhizosphere and phyllosphere of plants and provide many benefits to plants (Ram et al. 2013). PGPRs directly support plant growth by promoting the production of plant growth regulators, facilitating the uptake of soil nutrients, contributing to disease control and increasing nitrogen fixation (Alagawadi and Gaur 1992, Zhang et al. 1996, De-Ming and Alexander 1998; Zahir and Arshad 2004, Bashan and de-Bashan 2005; Antoun and Prevost 2006; Podile et al. 2006; Çakmakçı et al. 2010). AMFs, on the other hand, are beneficial fungi species that live in symbiosis with all terrestrial plants and occur in the root zone of 80%-90% of land plants (Newman and Reddell 1987; Abdel Latef and Chaoxing 2011a,b; 2014). The hyphae formed by the arbuscular mycorrhizal fungi in harmony with the plant roots increase the surface area of the roots and improve the mineral and nutrient uptake of the plants from the soil, thus encouraging better development of the plants. It is reported that thanks to the hyphae formed by AMFs, they help the uptake of water and nutrients from the points that the plant roots cannot reach, and provide resistance to environmental stresses such as soil salinity, heavy metal pollution, nutrient deficiency and adverse soil pH conditions (Balla et al. 2008; Turkmen et al. 2008). Arbuscular mycorrhizal fungi, which have an important place in terms of sustainability, are in important interaction with many families in horticultural plants especially ornamental plants and herbal products such as basil, thyme, rosemary, etc. (Smith and Read 2008). Trichoderma, which are composed of nearly two hundred species, are also beneficial fungal species that play an important role in plant growth and development, such as AMFs, and increase the tolerance of plants to environmental stresses (salinity, drought). Trichoderma species are also used in seed and seedling production to provide tolerance to some root diseases (Chang et al. 2008; Hermosa et al. 2012; Atanasova et al. 2013; Studholme et al. 2013; Bitterlich et al. 2018).

The effects of beneficial microorganisms have been studied on different species, but studies in the field of ornamental plants have been limited. The idea of making the production of ornamental plants, which have an important place in the world, in a more practical and faster way, as well as in a more environmentally friendly way, has been one of the main reasons for the studies. With the help of fertilizers containing microorganisms, it is normal for the success to be obtained from cultivation in plant and root development, flower yield and stress conditions to differ according to the strain used and the genotypic effect of the applied plant (Abdel-Rahman and El-Naggar 2014). For this reason, diversification of applications (bacteria and fungi species) and plant species to be applied is a

matter directly related to the adequacy of the knowledge to be obtained on this subject.

It has been reported that microorganism-containing (PGPR, AMF and T-22) fertilizers can provide positive effects in many stages of plant development and growth in both perennial woody and annual herbaceous plant species such as Strawberry (Aslantaş et al. 2009; Ertürk et al. 2012; Koç et al. 2016; Balcı et al., 2021), raspberry (Orhan et al. 2006; Balcı et al. 2020), kiwi (Ertürk et al. 2010), cherry (Eşitken et al. 2006; Akça and Ercişli 2010), apple (Pırlak et al. 2007), tea (Ertürk et al. 2008; 2013, Çakmakçı et al. 2010, 2013; 2015; 2017; Bhattacharyya et al. 2020, Ertürk et al., 2021), rosehip (Ercişli et al. 2004), hazelnut (Bassil et al. 1991; Ertürk et al. 2011), pistachio (Orhan et al. 2007), plum (Karakurt et al. 2010), apricot (Eşitken et al. 2003), banana (Kavino et al. 2010), corn (Dobbelaere et al. 2002; Yazdani et al. 2009), wheat (Turan et al. 2010), sorghum (Baghiae and Aghilizefeei 2019), mint (Kaymak et al. 2008), okra, spinach and tomatoes (Adesemoye et al. 2008; Öztekin et al. 2015). Similarly, in the field of ornamental plants, a study was conducted to determine the effects of mycorrhizal fungi on factors such as the number of buds, the number of flowers and their development, and the N, PK concentrations accumulated in the shoots of the gum geranium (*Pelargonium peltatum*). In the study, compost at two different rates, 20% and 40%, and 3 different AMF applications were made. It was reported that a statistically significant increase was observed in the number of buds, the number of flowers and development, and the accumulation of P and K in the shoots when compared to the control group in all AMF applications. However, dry matter accumulation and N concentration increase in shoots were not found to be statistically significant (Perner et al. 2007).

In another study, the effects of Trichoderma (T-22) and mycorrhizal fungus on the growth characteristics of willow (*Salix fragilis* L.) plant, shoot length, shoot and root formation and increase in plant biomass parameters were investigated. It was determined that T22 and mycorrhizal applications increased in all parameters compared to the control group, and T-22 application encouraged 20% longer shoot and root formation compared to mycorrhizal fungus application and 40% longer than the control application in terms of shoot length. In addition, when the biomass of the plant was examined, it was determined that the T-22 application produced more than 50% extra biomass compared to the mycorrhiza application and more than twice that of the control. As a result of the study, it was reported that T-22 and mycorrhiza application were beneficial for plant growth in willow (*Salix fragilis* L.) plant, but T-22 application performed better than mycorrhiza application in individual evaluation (Adams et al. 2007).

PGPRs can have a positive effect on the rooting properties of plants by promoting the production of plant growth regulators and at the same time supporting the uptake of nutrients (Antoun and Prevost 2006). Mycorrhizal fungi, on the other hand, facilitate nutrient and water uptake by forming hyphae on the roots and can help root development (Türkmen et al. 2008; Abdel Latef and Chaoxing 2011a,b). In the light of this information, Scagel (2001) investigated the effect of AMF application on rooting amount and quality of rooted cuttings at the time of cutting of 5 different miniature rose (*Rosa* spp.) varieties. In the study, it was reported that an increase in the number of rooted cuttings was observed in two varieties that took longer to root four weeks after the cuttings were planted. It was also determined that the combined use of growth regulators (IBA and NAA) and AMF increased the number of rooted cuttings and the number of roots per cutting compared to hormone application alone.

Also, Sezen et al. (2014) investigated the effects of PGPRs on the propagation and rooting ability of cuttings taken from *Ficus benjamina* L., which is an important ornamental plant and known as Benjamin Flower. In the study where *Agrobacterium rubi* (A1 and A18), *Pseudomonas putida* (BA-8) and *Bacillius subtilus* (BA-142) bacteria were used as rooting agents, the rooting percentage was reported as 100% in all applications. The lowest rooting percentage was found in the control group with 86.7%. In the research, in which root length, fresh root weight and new leaf number parameters were examined besides rooting percentages, BA-142 strain gave high results in all parameters and according to these results, it was reported that BA-142 strain containing *Bacillus subtilis* had a high potential for propagation and rooting studies for *Ficus benjamina*. In another study, it was reported that *Bacillus subtilis* (MA-2 strain) and *Pseudomonas fluorescens* (MA-4 strain) applications had a positive effect on vegetative growth parameters of Geranium (Mishra et al. 2010). In a similar study, Rahman et al. (2014) investigated the effect of rooting-promoting hormones together with PGPR and AMF applications on the rooting of cuttings in Bougainvilleas species. As a result of their study, they determined that the combination of PGPR and AMF together with IBA, which is used as a hormone, significantly increased rooting compared to the use of IBA alone. Researchers have reported that this may have occurred as a result of stimulation of growth hormone production (such as IAA) by PGPR and AMF.

In another study, Aalipour et al. (2021) examined the effect of used arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria against to Cd-contaminated soil in Arizona cypress. As a result of the research, they reported that either co-inoculation with AMF and P. fluorescens or individual inoculation with AMF could potentially ameliorate harmful effects of Cd on Arizona cypress growth.

Poinsettia is an ornamental plant that has been extensively studied with different PGPR isolates. As a matter of fact, FCA-8, FCA-60 and FCA-56 isolates belonging to the Pseudomonas putida species showed positive effects on development and growth parameters compared to the control (Silva and Iveth 2011). The published information on the ability of fertilizers with microbial content to help plants take in nutrients has led to the idea of reducing the use of artificial fertilizers, which is the most important chemical input in plant cultivation, through the effective use of microbial fertilizers. For this purpose; In a study conducted with 3 different *Pseudomonas putida* isolates and mixtures on the poinsettia plant; It has been reported that applications increase plant growth, have positive effects on anthocyanin pigmentation, and provide positive effects on development criteria such as leaf number and leaf area compared to control (Zulueta Rodriguez et al. 2014). In another study of the same cv, the effects of 4 different bacterial formulations (BI, BII, BIII and BIV) and chemical fertilization (KG) application,

as well as the combined use of chemical fertilizers (50% reduced) and bacterial combinations on growth parameters were investigated. In the study, in which parameters such as plant height, main stem diameter, root number, root length and diameter, fresh and dry weight of the plant were examined, bacterial formulations and half of the chemical fertilizer dose were used. As a result of the study, it was determined that BIV+KG, BIII+KG, BIV and BII applications were applications that had a significant positive effect on plant growth parameters. As a result of the research; It has been reported that bacterial formulations can be used in the production of poinsettia, thus reducing the use of chemical fertilizers and producing quality flowers at a lower cost (Parlakova and Dursun 2019). In studies on the same species; It has been determined that different strains contribute to the formation of balance within the scope of plant characteristic nutrients, improve color formation, and increase heavy metal and boron accumulation depending on the characteristics of the isolates (Parlakova and Dursun 2020a,b). In another similar study, Meenakshi et al. (2014) evaluated the effect of three different bio-fertilizers such as *Azotobacter*, KSB and PSB and various levels of inorganic fertilizers (applied both alone and in combination) . In the application of 1/2 N, P and K + Azotobacter + PSB + KSB, the maximum number of flowers opened per spike and the available P content in the soil were reported. Maximum fresh and dry spike weight was determined in applications containing 3 / 4th N, P and K + *Azotobacter* + KSB. Among all applications, the longest vase life was observed in 3 / 4th N, P and K + PSB + STM applications.

Azotobacter and *Azosprillum* species have positive effects on development and flowering in gladiolus (Dalve et al. 2009), some isolates of *Pseudomonas* and *Bacillus* genera in chrysanthemum plants improve growth and chemical composition compared to control (Arab et al., 2015), different bacterial strains in cyclamen have similarly been reported to make positive contributions to developmental parameters (Girgin 2019). In the rooting study carried out in lavenders; It has been reported that *Azosprillum brasilence* Sp245 isolate applications have positive effects on root growth parameters (Zulfitri, 2012). In another study, PGPR's used with zeolitites for optimised fertilisation and this study shown that PGPR's can significantly improve the agronomic and physiological quality of buttercup plants. Zeolites can improve the uptake of water and fertilizer by the roots and the study shown that PGPR's can increase the properties of the zeolites by working together (Domenico, 2020).

Plants infected with mycorrhizal fungi are affected by drought (Reid and Bowen, 1979; Auge, 2001), nutrient deficiencies (McArthur and Knowles, 1993; Moora and Zobel 1998), heavy metal (Schutzendubel and Polle 2002), and low temperature (Zak et al. 1998) can show higher resistance to abiotic stress conditions. It is reported that this effect is due to the better nutrient uptake capacity of the plants in which mycorrhizal fungi colonize their roots (Sylvia et al. 1993; Subramanian and Charest 1999). In ornamental plants, some studies have been carried out to optimize aquaculture performance under stress conditions. Zuccarini and Okurowska (2008) investigated the effects of mycorrhizal fungus (AMF) inoculation and fertilization at different levels under salt stress conditions in a greenhouse study on Sweet Basil (Ocimum basilicum L.), an important ornamental plant with aromatic properties that can be used for different purposes. In their study, eight different applications were compared with the combination of Glomus intraradices Schenck and Smith's inoculum and two different fertilization doses, two salinity levels of irrigation water, and the formation or absence of mycorrhizal colonization in the study, as well as all factors. Salt stress applied in the study significantly reduced plant growth and fluorescence levels, resulting in higher sodium (Na) and chloride (Cl) content in both roots and shoots, while potassium (K) decreased. However, both mycorrhizal inoculation and high fertilization had positive effects on plant growth and fluorescence, and symptoms caused by salt stress were reduced. Inoculation with mycorrhiza showed more pronounced effects than fertilizer application, but co-administration of colonization and high fertilization provided a higher tolerance to salinity stress than a single factor. As a result of the study, it was concluded that fertilization with mycorrhizal fungus applications had positive effects against salt therapy, whereas the colonization rate was significantly reduced with both saline irrigation and high fertilization.

In a study on the plant velvet (*Tagetes erecta* L.), the effects of three arbuscular mycorrhizal fungi, including *Glomus intraradices*, *Glomus constrictum* and *Glomus mosseae*, on growth, root colonization and Cd (cadmium) accumulation under Cd stress were investigated. In the study, physiological properties of *Tagetes erecta* L. such as chlorophyll content, soluble sugar content and antioxidant enzyme activity were evaluated. Under Cd stress, the symbiotic relationship between plants and mycorrhizal fungi is well established, and shoot and root biomass in velvet plants are significantly higher, 15.2-47.5% and 47.8-130.1%, respectively, compared to ungrafted velvet plants. In addition, it was determined that antioxidant enzyme activities were generally higher in plants inoculated with three AMFs under Cd stress than in plants that were not inoculated. In this study, the researchers concluded that antioxidant enzymes have a significant effect on plant biomass and increase the scavenging capacity of reactive oxygen species (ROS) of AMFs, thus helping to reduce Cd concentrations in plants under Cd stress (Ling-Zhi et al. 2011). In another study, which was established under water stress conditions, researchers evaluated the effects of mycorrhizal fungi (AMF) on plant growth, nutrient uptake, flower yield, water relations, chlorophyll contents and water use efficiency in two groups of snapdragon (*Anthirhinum majus*) well-watered and water-stressed. The applied water stress significantly decreased the growth parameters, nutrient contents, flower yield, water relations and chlorophyll pigment contents compared to the normally irrigated group, and increased electrolyte leakage from the cells. However, despite water stress, snapdragon plants inoculated with mycorrhizal fungi in both well-watered and water-stressed groups had higher shoot and root dry weight, water use efficiency, flower yield, nutrient content, (P, N, K, Mg and Ca) and chlorophyll content compared to the group without mycorrhizal inoculation. At the same time, it was determined that water stress increased the accumulation of proline in plant leaves, this increase was significantly higher in the group without mycorrhizal fungus application, therefore, AMF

colonization improved tolerance to water stress in the host plant, snapdragon (Asrar et al. 2012). In another study of clove (*Dianthus caryophyllus* L.), the effects of different *Glomus strains* on growth, quality and mineral concentrations of plants grown under salt stress (1, 3 and 6 dS m-1) were investigated. A moderate dose of salt stress (3 dS m-1) in irrigation water and inoculation with *G. intraradices* have been reported to produce the best clove plant quality. As a result of the research, it was observed that the salt tolerance in cloves increased with the colonization of plant roots by *G. intraradices*. In addition, an increase was observed in the growth of the clove plant, the number and size of flowers, leaves and flower color. Although it was reported that the best results were obtained from moderate salt stress and *G. intraradices* inoculation, it was found that mycorrhizal inoculation provided positive results against salt stress in all doses and plants compared to control (Navarro et al. 2012).

Conclusion

The use of chemical inputs, which initially contributed significantly to the growth of plants, over time seriously threatens both soil biology and human life through the food chain. In particular, the increase in soil and environmental pollution contributed by conventional agricultural activities, deterioration of soil microbiota and changes in climatic conditions appear as a problem in a significant part of agricultural lands. The introduction of microorganisms at this stage will be an important step in re-establishing the soil ecosystem, using lower chemical inputs, and environmentally friendly sustainable agricultural production. In studies carried out in many annual and perennial plant species for the last thirty years, it has been determined that beneficial microorganisms interact with each other and with the plant, they can have different effects depending on the strain and the host, and their activities may vary depending on the soil ecology.

In this context; For ornamental plants, which are less studied than other plant species, determination of appropriate microorganismic associations according to species, and the creation of commercial biological fertilizer formulations suitable for different purposes and species can be important target points for this cultivation. Also; Studies on the biochemical and genetic mechanisms of the effects of microorganisms on the growth and quality criteria of ornamental plants may be the most interesting areas in this subject.

Conflict of Interest

The authors are declared that they have no conflict for this research article.

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Agronomical and technological researches on oregano (*Origanum onites* l.) in Diyarbakir ecological conditions

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Abstract

Origanum onites L. is one of the most important medicinal and aromatic plant grown in the Mediterranean area and extensively using in many traditional medicinal and culinary since ancient times. This study aims to determine the agronomical and technological characteristics of oregano at four different plant densities (70x20, 70x30, 70x40, 70x50 cm) and two harvests. The effects of this application was noticed on the fifteen flowing parameters; plant height, fresh and dry herb yield, dry leaf yield, canopy diameter, essential oil content and essential oil composition etc. According to the results; significant differences were found statistically between harvests, in all properties being studied; except dry herb yield. Fresh and dry herb yield varied from 11.1 to 13.7 t ha^{-1} and from 3.8 to 5.0 t ha^{-1} per harvest, respectively in a year. Dry leaf yield varied between 1.7–2.9 t ha^{-1} per harvest in a year. Carvacrol, 1,8 cineole and γ-terpinene were found as major components of oreganos. Mean Carvacrol percentage determined as 53.9%, 1,8 cineole 9.7% and γ-terpinene as 5.7% respectively. The experimental was randomized complete block design with four replications. The extraction of the samples were performed using a Clevenger-type apparatus for steam distillation. The essential oil components of Oregano were determined by gas chromatography (GC). According to the results of this study, differences between two cuttings except drog yield were found to be significant for all properties studied. As a conclusion, Oregano cultivation can be practicable in Diyarbakır ecological conditions in terms of drog herb and leaf yields and essential oil constitutions.

Key Words: Oregano, Plant Density, Harvest Time, Essential oil

Introduction

In recent years, demand for natural products from aromatic and medicinal plants has increased considerably as substitutes for artificial products, in terms of pharmacological properties (Atanasov et al. 2015). Among the various natural products, essential oils have gained great popularity in different industries, including the cosmetics, food, and pharmaceutical industries, due to their valuable characteristics such as unique colors, strong odor, and high volatility (Carvalho et al. 2016; Maggio et al. 2016). Particularly, essential oils have an important role in the health care due to their remarkable biological features (Raut and Karuppayil 2014).

Turkey is the most exporting country, among countries the thyme exporting. In terms of essential oil and exporting product, *Origanum onites* L. has an important medicinal and aromatic plant. There are about 30 species of *Origanum onites* L. Twenty-one of them exist in Turkey. They are grown naturally in stony, rocky and slope areas (Ietswaart, 1980; Baytop, 1984; Ceylan, 1997; Padulosi, 1996).

The thyme herb includes from 2% to 8% essential oil (Başer, 2001). This oil has antibacterial and antifungal properties (Özgüven et al. 1987, Kızıl and Uyar, 2005). After extracting essential oil, remaining as known hydrosol (thyme's water) has been selling. Recently, consumption of hydrosol is become widespread. It is claimed that hydrosol has beneficial properties for treatment of stomach and intestine ailments (Aydın, 1996, Yılmaz et al., 2017).

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When thyme used as tea, it has a good effect in digesting and as an antioxidant (Başer, 2001). The results of some investigations showed that thyme's products could affect insect (Kesdek et al., 2013) and funguses (Özgüven andnokay, 1986; Ünlü, 1995, Kordali et al., 2008), and overuse of the thyme did not cause toxicity. It is scientifically approved that thyme mostly used as expectorant and its product (carvacrol) has also strong effect as analgesic and curative (Aydın, 1996, Chishti et al., 2013). Thyme contains plentiful pollen for bees and useful grass for animals. Therefore, bees fed with thyme produce more quality honey and animals fed on thyme gave beter quality milks and milk's products (Ortiz and Fernandez, 1992). Moreover, It was reported that thyme and thyme products was being used as food preservative (Er, 1994).

Studies on oregano species are inadequate, especially in our region. There are no standard on products collected from wild-area in Turkey. Importing countries bring a standard for any medicinal plants such as esential oil content should be at least 3%, carvacrol and thymol content should be high. The objective of this study was to determine technological and agronomical properties of *Origanum onites* L. at four densities and two harvests in a year from 2002 to 2004, under Diyarbakır ecological conditions.

Materials and Methods

Field trial was carried out between 2002 and 2004 growing season at Southeast Anatolia Agricultural Research Institute (latitude 37º 53 N longitudes 40º 16 E, 680 m above sea level). The soil of research place is zonal soil being generally red-brown and included in the big soil group having a clayish nature, flat or about-to-be flat, and having very small erosion and deep or medium deep. The region climate belongs to Mediterranean climate. Generally, summer is hot and drought; but winter is cold and rainfall. Long year's climatically findings showed that there were 454 mm total rainfall and average temperature was 15.8 ˚C every year (Meteorology Directorship's 2003–04 data of Diyarbakir)

Cuttings of *Origanum onites* L. were taken from healthy plants, obtained from Department of Field Crops, Agriculture Faculty of Çukurova University, Adana, Turkey, at 12 December 2001. Cuttings planted in sandy pool at green house for forming root. When plants reached at 10–15 cm plant height, they were transplanted to field on May 2002. Field trial was conducted according to randomized four density design with four reapplications. Each block area was 3m x 2.8m (8.4 m^2). The interval between two rows was 70 cm for four densities. On the other hand; the spacing between two plants on the same row was 20 cm for first density, 30 cm for second density, 40 cm for third denisty, 50 cm for fourth denisty; respectively. During the vegetation period, the plots were irrigated and weeded when required. No harvesting was done during the first year, however, the second and third year harvest was done two times; first harvest was done on May and second was done on November. Harvest times were determined according to beginning of blooming for each harvest in two years.

Each year, a basal dose of 60 kg ha^{-1} N and 60 kg ha^{-1} phosphor (20–20–0 compose) and upper dose of 60 kg ha^{-1} N (form of CAN) were applied. Basal dose of fertilizer was applied at October and November, and upper doses applied after each harvest as 30 kg ha^{-1}.

Plants, after removing border effects, were cut at height of 10 cm above soil and weighed to determine fresh herb yield, whereas dry herb yield was determined by drying fresh herb samples from each plot in a shadow and airy place during one-two week. Dry leaf yields were determined after separating the leaves and steam in the dry herb samples. Essential oil content was measured volumetrically, by hydro distillation using a Clevenger apparatus, in 20 g samples taken from each plot (mL/100g-v/w).

Data were analyzed statistically, using TOTEMSTAT and JMP ha^{-1}computer program, and means were grouped, using LSD values at significance level of 5%. The essential oil were analyzed by GC (Hewlett Packard 6890 Gas Chromatograph) Chromatographic separations were accomplished with a CAT capillary column (0,32 mm i.d.x30 m film thickness 1 µm) with injections in the spilt mode. The temperature was set to 50 ºC for 5 min initially, and increased to 180 ºC at a rate of 4 ºC per minute, then again increased 260 ºC. Nitrogen was used as a carrier gas at a flow rate of 30 mL/min. Each sample was analyzed four times every year. The identification of components was based on comparison of their relative RF values with those of authentic standards. Authentic standards were purchased from Sigma-Aldrich chemises (USA).

RESULT AND DISCUSSION

Agronomical Characteristics

Analysis of variance and average values for all agronomical characteristics (plant height, plant canopy diameter, fresh and dry herb yield, dry leaf yield and dry leaf/stem rate) are shown in Table 1. The results shows that all investigated characteristics were influenced statistically by harvests done in one year, except dry herb yield. Different plant densities affected none of characteristics significantly. Plant density x harvest interaction was found important significantly only on plant canopy diameter in 2004.

The plant height obtained from first harvests (47.9-47.4 cm) was higher than second harvests (21.0-30.8 cm) for both years. Our results of plant height are in agreement with Kırman (1993), who reported plant height as 29.7 cm for first harvest and 22.7 cm for second harvest in a year. It is estimated that the differences between harvests is due to harvest time and environmental factors of that time.

The plant canopy diameter from second harvest (50.9 cm) was higher than first harvest (38.8 cm) in 2003. But the plant canopy diameter from first harvests (54.1 cm) was higher than second harvest (43.9 cm) in 2004.Our findings are supported by Kırıcı and İnan (2001), who reported as number of plant's branchs. They reported 14.3 branch/plant for first harvest and 26.3 branch/plant for second harvest in a first year. On the other hand, they reported 23.1 branch/plant for first harvest, and 22.8 branch/plant for second harvest in a second year. The greatest plant canopy diameter (57.1 cm) was determined from 70x20 cm plant density and first harvest in 2004. The least interaction values (40.5, 43.0 and 44.1 cm) were obtained from 70x20; 70x30 and 70x40 cm plant densities and second harvest in 2004.

The fresh herb yield of first harvest (15.2 t ha^{-1}) was determined higher than second harvest (11.3 t ha^{-1}) in 2004. On the other hand, the dry herb yield obtained from first harvests (4.8, 4.7 t ha^{-1}) was higher than second harvests (4.3, 4.3 t ha^{-1}), in both 2003 and 2004. This might be due to the effects of environmental conditions. Similar findings were

reported by Kıryaman (1988), who reported fresh herba as 4.4 t $ha^{-1}$for first harvest and 3.4 t $ha^{-1}$for second harvest. And, He reported dry herb as 1.5 t ha^{-1} for firs harvest and 1.0 t ha^{-1} for second harvest in a year.

The dry leaf yield of second harvest (3.4 t ha^{-1}) was higher than the first harvest (1.8 t ha^{-1}) in 2003. Also, the dry leaf/the stem rate from second harvests (80%, 66%) were higher than the first harvests (39%, 56%) respectively in both years. This variation might be because of the differences in the percentage of dry matter of plants and the enviromental conditions. The results obtained from the study are in agreement with Kırman (1993), who reported dry leaf as 1.4 t ha^{-1} for second and 0.6 t ha^{-1} for the first harvest, but they are in conflict with Kıryaman (1988), who reported dry leaf yield as 1.1 t ha^{-1} for the first harvest and 0.8 t ha^{-1} for the second harvest. The variation might be resulted from the differences of environmental conditions and agronomical applications.

Technological Characteristics

Analysis of variance and average values for all technological characteristics (essential oil content, α-pinene, β-pinene, α-terpinene, 1,8 cineole, γ-terpinene, linalool, borneol and carvacrol constituents) are shown in Table 2. According to the results, between two harvests in a year for all characteristics was determined statistically significant. Plant densities affected none of technological characteristics, except 1,8 cineole and γ-terpinene, Plant density x harvest interaction was determined statistically significant only on carvacrol component.

The essential oil content of oregano was found statistically significant between harvests. The oil content of the first harvests was higher than the second harvests' in both years. Oil content of the first year and the second year at the first and second harvest were determined as 1.8%, 1.4%, and 1.9, 1.7%, respectively. Our findings' relation with essential oil content is in agreement with Kırman (1993), who reported essential oil content of the first harvest higher (2.0%) than the second harvest (1.8%).

β-Pinene, α-terpinene, γ-terpinene, linalool and carvacrol contents from the first harvest (1.6, 1.8, 6.5, 3.0 and 61.7%, respectively) were found higher than that of the second harvest (1.3, 1.3, 4.6, 2.5 and 42.4%, respectively) in the first year. Hovewer, the α-pinene (1.0%-1.1%) and 1,8 cineole (14.7%-12.1%) contents obtained from the second harvest were higher than those of the first harvest (0,7%,0,5%-7,7%, 4.4%) respectively) in both years. Our results are in agreement with Kıryaman (1988) and Kırman (1993). Kıryaman (1988), who reported α-pinene content as 6.5% for second harvest and 4.0% for first harvest; cineol content as 8.0% for the second harvest, 7.7% for the first harvest, terpinene contrnt as 4.0% for first harvest and as 3.5% for second harvest, as borneol content 12.1% for first harvest and as 11.6% for second harvest, carvacrol content as 24.9% for first harvest and as 20.6% for second harvest. Kırman (1993), Who reported β-Pinene content as 3.7% for first harvest, 3.0% for second harvest, α-terpinene content as 9.1% for firs harvest, 6.6% for second harvest, linalool content as 8.8% for fist harvest, 8.5% for second harvest.

The borneol content (1.6%) of second harvest was higher than first harvest (1.2%), only in 2004. The results obtained from our study are in agreement with Kırman (1993), who reported borneol content as 2.3% for the second harvest, 2.0% for the first harvest. It is thought that environmental affects are the reason of this case.

γ-Terpinene content from the first harvest (6.5%) was higher than that of the second harvest (4.6%) for 2003 year. But in 2004, γ-terpinene content obtained from second harvest (7.3%) was higher than the first harvest (4.5%). It is also estimated that this contradiction resulted from the plant age, environmental conditions and plant ability to adapt to environmental conditions.

The highest carvacrol contents (65,0%; 63,3%; 63,0%) were obtained from 70x40, 70x50 and 70x30 cm plant densities at first harvest. But the lowest carvacrol contents (40.1%, 40.3%, 44.4% and 44.7%) were obtained from 40x70, 70x50, 70x20 and 70x30 applications at the second harvest, respectively in 2003. The applications mentioned above are also affective and give positive results on fresh and dry herb. Therefore, it is understandable that the environmental conditions and growing applications are very important parameters on carvacrol content (Naghdi Badi et al. 2004).

Table 1. Effect Of Plant Densities And Harvests On Plant Height, Plant Diameter, Fresh And Dry Yield And Drug Leaf Yield And Dry Leaf/Stem Rate

Density (cm)	Plant height (cm)						Plant Canopy dimater (cm)						Fresh herbage yield (t ha-1)					
	2003			2004			2003			2004			2003			2004		
	Fir. h.	sec.h.	Mean	Fir. h.	sec.h.	Mean	Fir. h.	sec. h.	Mean	Fir. h.	sec. h.	Mean	Fir. h.	sec. h.	Mean	Fir. h.	sec. h.	Mean
70x20	46.1	21.6	33.8	45.9	31.1	38.5	38.1	51.0	22.3	57.1a	40.5e	48.8	12.7	13.2	12.9	14.9	11.1	13.0
70x30	47.6	20.9	34.3	47.1	30.7	38.9	38.5	51.1	22.4	51.8 bc	43.0e	47.4	12. 0	13.2	12.6	15.7	11.5	13.6
70x40	49.7	22.1	35.9	47.2	31.0	39.1	39.9	50.8	22.7	54.4 ab	44.1de	49.2	13.7	13.3	13.5	15.3	11.4	13.4
70x50	48.3	19.8	34.0	49.7	30.8	40.2	38.9	50.9	22.5	53.2 ab	48.4 cd	50.8	11.4	11.1	11.2	15.0	11.1	13.0
Mean	47.9 a	21.0 b	34.5	47.4 a	30.8 b	39.2	38.8 b	50.9a	22.4	54.1a	43.9b	49.0	12.4	12.7	12.6	15.2 a	11.3 b	13.2
LSD(%5)	Harvest: 3.0**			Harvest: 1.9**			Harvest: 4.0**			Int.: 4.7*			n.s.			Harvest: 2.2**		
Density (cm)	Dry herbage yield (t ha-1)						Dry leaf yield (t ha-1)						Dry leaf/ stem rate (%)					
	2003			2004			2003			2004			2003			2004		
	Fir. h.	sec.h.	Mean	fir.h.	sec.h.	Mean	fir.h.	sec. h	Mean	Fir. h.	sec. h.	Mean	fir. h.	sec.h.	ave.	fir. h.	sec. h.	ave.
70x20	5.0	4.5	4.8	4.6	4.3	4.5	2.0	3.5	2.8	2.7	2.9	2.8	40	79	59	58	67	62
70x30	4.3	4.5	4.4	5.0	4.6	4.8	1.7	3.7	2.7	2.7	3.1	2.9	39	82	60	56	67	61
70x40	5.2	4.3	4.8	4.8	4.4	4.6	1.9	3.5	2.7	2.6	2.9	2.8	36	83	59	55	63	59
70x50	4.4	3.8	4.1	4.5	3.8	4.2	1.8	2.9	2.3	2.4	2.6	2.5	39	76	57	54	67	61
Mean	4.8	4.3	4.5	4.7	4.3	4.5	1.8b	3.4a	2.6	2.6	2.9	2.7	39 b	80a	59	56 b	66 a	61
LSD (5%)	ns			ns			Harvest: 0.4**			ns			Harvest: 2.8**			Harvest:3.7**		

*p>0.05, **p>0.01, Int: İnteraction, ns: No significant, Fir.: First, Sec.: Second, h.: Harvest

Table 2. Effect of Plant Densities and Harvest On *α–Pinene, β–Pinene, α-Terpinene, 1,8 cineole, γ-Terpinene, Linalool, Borneol*, and *Carvacrol* Components of *Origanum onites* L.

Density (cm)	Essential oil content (%)						α-pinene content (%)						β-pinene content (%)					
	2003			2004			2003			2004			2003			2004		
	fir.h.	**sec. h.**	**Mean**	**Fir. h.**	**sec. h.**	**Mean**	**fir.h.**	**sec. h.**	**Mean**	**fir.h c.**	**sec. h.**	**Mean**	**Fir h.**	**sec. h.**	**Mean**	**fir. h.**	**sec.h c.**	**Mean**
70x20	1.9	1.4	1.7	1.8	1.7	1.8	1.0	1.2	1.1	0.5	1.1	0.8	1.8	1.4	1.6	1.5	1.4	1.4
70x30	2.0	1.3	1.6	2.1	1.9	1.9	0.7	1.2	1.0	0.6	1.1	0.9	1.7	1.2	1.4	1.7	1.5	1.6
70x40	1.7	1.6	1.6	2.1	1.8	1.9	0.7	1.1	0.9	0.7	1.1	0.9	1.4	1.4	1.4	1.8	1.4	1.6
70x50	1.7	1.4	1.5	1.9	1.7	1.8	0.7	0.8	0.8	0.5	1.1	0.8	1.7	1.1	1.4	1.6	1.4	1.5
Mean	1.8a	1.4b	1.62	1.9a	1.7b	1.9	0.7b	1.0a	0.92	0.5b	1.1a	0.84	1.6a	1.3b	1.45	1.6a	1.4b	1.53
LSd (%5)	Harvest: 0.2**			Harvesr: 0.1*			Harvest: 0.2**			Harvest: 02**			Harvest: 0.3**			Harvest: 0.3**		
	α-Terpinene (%)						1,8 Cineole (%)						γ-Terpinene (%)					
Density (cm)	2003			2004			2003			2004			2003			2004		
	Fir h.	sec.hc.	Mean	fir.hc.	sec. h.	Mean	Fir. h.	sec. h.	Mean	Fir. h.	sec. h.	ave.	Fir. h.	sec. h.	Mean	fir. h.	sec.hc.	Mean
70x20	2.1	1.3	1.7	1.3	1.3	1.3	9.2	14.5	11.9a	4	11.4	7.7	8	4.8	6.4a	4.4	7.2	5.8
70x30	1.7	1.2	1.5	1.3	1.4	1.4	7.9	15.8	11.9a	4.4	12.1	8.3	6.5	4.8	5.6ab	4.5	7.2	5.8
70x40	1.9	1.5	1.7	1.3	1.5	1.4	6	13.1	9.5b	4.6	12.1	8.3	5.8	4.6	5.2b	4.5	7.5	6
70x50	1.7	1.3	1.5	1.3	1.4	1.4	7.4	15.2	11.3a	4.6	12.7	8.6	5.8	4.2	5.0b	4.4	7.2	5.8
Mean	1.8a	1.3b	1.6	1.3	1.4	1.4	7.7b	14.7a	11.2	4.4b	12.10a	8.2	6.5a	4.6b	5.6	4.5b	7.3a	5.85
LSd (%5)	Harvest: 0.3**			ns			Harvest:1.1**		D:1.6**	Harvest: 1.0**			Harvest: 0.6**		D: 0.9*	Harvest: **0.7		
	Linalool (%)						Borneol (%)						Carvacrol (%)					
Density (cm)	2003			2004			2003			2004			2003			2004		
	fir. h.	sec. h.	Mean	fir. h.	sec. h.	Mean	fir. h.	sec. h.	Mean	fir. h.	sec. h.	Mean	fir. h.	sec. h.	Mean	fir. h.	sec. h.	Mean
70x20	3.0	2.5	2.8	4.1	2	3.1	1.9	2.5	2.2	1.1	1.5	1.3	55.5b	44.4c.	50	67.5	41.9	54.7
70x30	3.0	2.4	2.7	4.8	2.4	3.6	1.8	1.9	1.8	1.1	1.6	1.4	63.0a	44.7c.	53.8	58.7	50.2	54.5
70x40	2.6	2.4	2.5	5	2.3	3.6	2.1	2	2.1	1.2	1.7	1.4	65.0a	40.1c.	52.6	62.1	50	56.1
70x50	3.3	2.6	3.0	5.2	2.4	3.8	2.3	2.4	2.3	1.3	1.7	1.5	63.3a	40.3c.	51.8	66.7	49.4	58.0
Mean	3.0a	2.5b	2.7	4.8a	2.3b	3.5	2.0	2.2	2.1	1.2b	1.6a	1.4	61.7a	42.4b	52.0	63.8a	47.9b	55.8
LSd (%5)	Harvest: 0.3**			Harvest: 0.4**			ns			Harvest: 0.1**			Int: 7.0*			Harvest:5.7**		

*p>0.05, **p>0.01, Int: İnteraction, ns: No significant, D: Density, Fir.: First, Sec.: Second, h.: Harvest

Conclusions

According to the results of these study suggestions can be as following;

1. If the first year of experiment was establishment year of oregano and there was not any harvest, 70x30 cm plant density can be suggested in terms of herb yield. This plant density is also its suitable for mechanization.
2. In the terms of essential oil, the first harvest is more suitable than the second harvest. As result, the first harvest can be suggested for obtaining the essential oil, but the second harvest is offered for obtaining high dry leaf yield.
3. As the components of essential oil; β-pinene, α-terpinene, linelool and carvacrol are rich in the first harvest, but α-pinene, 1,8 cineole and borneol are rich in second harvest. γ-terpinene is rich in both harvests in a year.
4. For fresh and dry herb yields, the first harvest is more suitable. On the other hand, the second harvest is more suitable for dry leaf yield.
5. As a conclusion, the ecological conditions of Diyarbakır are suitable for agriculture of oregano for production of essential oil and herb yield.

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Purchasing attitudes for agro-food products and changing financial status during Covid-19 outbreak in Turkey

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Abstract

The COVID-19 pandemic, which was underestimated at the beginning, had become worldwide in 2020 and affected lives in a devastating way. Thus, it turned out to be the new corner point for the era we live in. Many challenges have become visible due to replicated or prolonged social distancing measures or lock-downs within 2020. Due to periodic medical information, many people changed their preferences from a consumption point o f view and reducing physical activities have been a factor affecting the change. In this study, 499 individuals were surveyed online in July 2020 in Turkey to determine the change in their consumption preferences within the pandemic process. The linear relationship between changing amounts of fresh fruits and vegetables (FFVs) and animal products purchased and household income were compared due to COVID-19 encounter. The results indicated that not the level of income but the declination in income affected purchases and consumption of all varieties. Besides, the share of the budget allocated to FFVs and meat and dairy products were assessed as well in relation to amounts consumed. Herewith, the amount of meat and dairy products purchased and consumed by the audience were found to be related to the budget allocated.

Keywords: purchasing, preference, COVID-19, income, FFVs, meat, dairies, Turkey

JEL Codes: C12, D12, E21

Introduction

Adaptation to changing circumstances is hard in terms of lifestyles. However, some situations enforce masses to change the way that they live. The unexpected and extremely contagious novel Coronavirus (SARS-COV 2 or COVID -19) has been in effect around the world since the beginning of 2020 (Cranfield, 2020). It has been 1,5 years and the disease has not been taken under control. It was understood by the mid of 2020, short after its announcement as a pandemic by the WHO, that its effects will be persistent as it is today. In addition to mass losses and socio-economic devastation, the pandemic has been changing lifestyles specifically of the middle class and above around the world (Rizoua et al., 2020). The change in way of living due to COVID-19 and its economic effects have become similar when compared with to previous epidemics (Ceylan et al., 2020). The evolution is not limited with lock-downs, social contraction, inbound and outbound travel restrictions. Economic downsizing is related to these unfortunate challenges as well (Gornicka et al., 2020; Sidor and Rzymski, 2020). Many productive sectors, as well as services that highly incorporate youth labour, have been depressed in supply and demand terms.

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Generally, when supply and demand dynamics are considered due to changing circumstances, daily consumption mainly referring to compulsory food and agricultural products are kept apart. The reasoning behind is related to importance of nutrition demand of the society (Ceylan et al., 2021; Goddard, 2020). However, under limited social contact conditions of the COVID-19 era, reaching products that are essential has also become harder everywhere. The lock-downs and at least temporary unemployment lead rising budgetary concerns and shift to diets that include more carbohydrates, as it has been the case in Mexico (Espinoza-Ortega et al., 2021). In addition to the hardship in reaching the products, the demand structure has been changing drastically.

One of the observations on changing consumption attitudes is rising online shopping tendency (Goddard, 2020; Wilson, 2020). While online shopping was around 8 % across Canada in 2018 (Brown, 2018), the share has risen to 15 % by the mid of 2020 (Goddard, 2020). Prior to this pandemic, there has been a rising tendency for online purchases of durable products or online orders of ready food via specific delivery sites in many countries. This sort of purchasing schemes has become widespread in Turkey as well. However, as a challenge online supermarket and even grocery purchases became an alternative for consumers having specifically moderate or higher income levels within the pandemic process.

However, not only the purchasing attitudes, but also amounts of products demanded and purchased have changed in all product lines. As an instance, calorie intake has become a concern for Polish consumers within the lock-down process. While 43 % of more than 2.000 survey attendants declared they reduced physical activity, 34 % said that their food consumption have risen (Gornicka et al., 2020). The demands for groceries, meat and dairies, packed products have been affected within the process. The change was both related to changing or varying income and affected by socio-economic characteristics of the society almost everywhere in the world. However, it was intended within this study to measure the changing shopping or purchasing attitudes and demand structures of individuals in Turkey. A randomly selected sample of 499 individuals was surveyed online and the findings were evaluated due to the level of association between changing demand and socio-demographic characteristics of the audience within the COVID-19 process. The main target was to measure and evaluate changing demand patterns referring to 2020 conditions and assess contemporary reflections to the market.

Material and Method

The main objective was to evaluate linear correlation and association between indicators demonstrating the features of the audience and their changing purchasing and consumption attitudes. Chi-Square testing was used to measure the differences between observed and expected values of specific datasets and detect the linear association between discrete or categorised variables. Pearson's Chi-Square statistic tests independence of referred criteria (H_0) against their dependence to each other (H_A). Therefore, utilising this test for 499 observations via SPSS statistical package, the following association hypotheses were tested.

- There is no relationship between level of income & Changing amounts of FFVs and meat/dairy products purchased/consumed
- There is no relationship between reduction of income & Changing amounts of FFVs and meat/dairy products purchased/consumed
- There is no relationship between changing amount spent for FFVs purchases & Changing amounts of FFVs purchased/consumed
- There is no relationship between changing amount spent for animal products & Changing amounts meat/dairy products purchased/consumed

These are the main hypotheses set forward. However, the analyses incorporated additional categorised variables depending on the nature of the relationship.

Results

Socio – Demographic Outlay

Prior to assessment of the linear association between variables, it is beneficial to provide some information on socio-demographic features of the random sample surveyed online. 60 % of the sample was composed of female correspondents (301) and 40 % was male (198). The average age of the participants was 39. This age figure infers information on adaptability of participants both to conventional and modern marketing approaches and tools. To be more precise, almost all individuals are experienced in face to face shopping in markets and relevant stores. However, they are also used to maintain purchases online as they have adapted to changing marketing tools.

Keeping in mind that the survey was implemented online, some findings as level of education or employment status might not be reflecting the overall characteristics of the society. This is due to the eligibility of attendee to undertake the online survey and his/her confidence to share the relevant personal data. As an instance, the sample was composed of highly educated individuals. 84,33 % of the sample seemed to have tertiary and above degrees, of which 54 % were Bachelor's graduates. Besides, 60,12 % (300) of the audience was employed on full time basis. There were also people holding part-time jobs and some were non-employed or student. The sample seemed to demonstrate average characteristics for a consumption study.

In addition, level of income, experienced change or variance in income, and employment status of the participant should be indicated in order to enable accurate assessment in the scope of a correlation analysis. The aggregate income distribution was demonstrated in Table 1. As it can be said, almost 90 % of the audience seemed to receive income above minimum wage

depending on 2020 figures[1]. Considering the average per capita income of $ 716,58 (Anonymous, 2021), the relatively well paid sample was also the reflection of online reach to an educated audience. More than 70 % of the audience seemed to have more than $ 640. Yet, this figure represented household income.

Table 1. Distribution of Household Income

Level of Income	Number of people	%
Below minimum wage	21	4,21
Minimum wage	29	5,81
$ 326,99 - $ 639,78	95	19,04
$ 639,79 - $ 924,12	92	18,44
Above $ 924,12	262	52,51
Total	**499**	**100**

A socio-economic factor that would be related to changing purchasing and consumption preferences was the employment status. There have been people in the sample that at least partly lost their jobs/salaries. The total number of unemployed due to the pandemic was 75 and 40 individuals out of these people were still unemployed at the time of the survey. Considering the financial situation experienced during this unemployment phase, 60 people declared that they did not receive unemployment or compensation wage. The number of survey participants was 6 that declared receipt of salary at most for 3 months. They also indicated that the salary was cut afterwards. Besides, the variation in the household income was also significant. Among these 75 unemployed individuals, 53 declared more than $ 142,17 (1.000 TL) monthly income loss and only 9 of these confirmed no change in the income.

The survey participants were asked to indicate their average monthly spending on agro-food products and meat/dairy products to understand their average preferences and consumption tendencies. The average financial allotments of individuals appeared as $ 238,14 for agricultural and food products and as $ 102,22 for animal products. However, there has been reduction and variation in these rates within the COVID-19 process as well. This also affected the purchasing and consumption attitudes.

The changing attitudes can be evaluated from shopping venues preferred within the pandemic process. Most of the society, loosely related to the place of inhabitancy, generally prefers to shop on district markets of groceries to buy FFVs. However, the declination in this preference is significant among the sample. As an instance, 29 % of the audience declared that they have stopped to visit district markets and 36 % said that they stopped to visit green grocers. However, this can only be valued after overviewing the changing situation with regards to online shopping preferences. While 73 % of the audience (365 households) declared no online shopping experience for agro-food products before the pandemic, the share of this group reduced to 58 % (290 households). When read inversely, 42 % (209 households) of the audience seemed to foster online agro-food purchases. Accompanied with total 305 online shoppers (61 %), it can be said that number of people giving online orders within the process has demonstrated a rising tendency for FFVs and durable products.

Test Results for Association of Variables

The linear association between variables were tested with X^2 – Chi Square and Likelihood Ratio G^2 statistics following Chi Square distribution. G^2 statistic is not used for small samples in general due to lack of its statistical reliability (Cochran, 1952). The numbers of categories of each variable are multiplied to determine the statistical inference base. Yet, as the sample size is large enough for the current study, G^2 statistic was also used as a complementary tool (Haberman, 1977).

Firstly, the relationship between income level per se and changing amount of products purchased and consumed in different categories were evaluated. Hereby it is important to define the categories of the variables. Level of income for the sample was categorized as indicated in Table 1 from 'below minimum wage' to 'above $ 942,12' (6.500 TL). The changing purchases for all considered products during COVID-19 pandemic were categorized as 'risen – 1, not changed – 2 and declined – 3'. Therefore, degrees of freedom for statistical testing appeared as 3*5= 15 for this part of the analysis. Referring to this statistical base, it was considered as beneficial to infer about changing amount of purchases and consumption of these four categories.

Checking out the figures in Table 2, it can be said that the share of individuals declaring rising, reducing, and stable purchases were similar for all product groups of FFVs and meat and dairies. Yet, the highest declination was observed in fruit and lowest in dairies consumption. This is not surprising considering the share of the products in the budget of consumers. The interviewed audience seemed to allocate more to FFVs before and higher declination in purchases and consumption of FFVs is expected accordingly.

After this classification, the linear relationships between income attributes and preference alterations were demonstrated and discussed consecutively. Firstly, the changing preferences were found not to be related directly to existing or recorded income level of consumers as demonstrated in Table 3. Therefore, we failed to reject lack of relationship hypotheses and we can infer that with rising income the demand for all product categories seemed to remain on the same level. In other words, the variation in demand for relevant product categories were found as non-related to the variation of households' income. Yet, it is important to keep in mind that this finding do not impose inexistence of a relationship. The statistics only infer unavailability of a linear relationship.

Despite the level of income, the relationship between declination in consumers' income and quantity of products bought is significant in all categories. The statistical findings were demonstrated in Table 4. The declination of spendable income

[1] Financial variables in Turkish Liras were converted into US Dollars by using the 2020 average TL/$ rate of Central Bank of Turkey: 1 $= 7,0337 TL

seemed to be related to amounts demanded/purchased due to test statistics used to detect linear relationship. However, the direction of the relationships for four product categories were detected via Pearson correlation coefficients that were demonstrated in Table 4. All linear relationship components have negative correlations that were significant as well. Therefore, the declination in product demand in all categories rises as higher as the amount of income lost by the participants within the process. In other words, for individuals that experiences more reduction in income, demand and purchases of products have declined as well within our sample.

Following general income level and variation in the income assessment, share of budgetary allotment was associated with the changing purchases. First the relationship between amount spent on FFVs and amount of fruits and vegetables bought were analysed. It was followed with spending on animal products and purchased amounts. The variation in the income devoted for FFVs were neither correlated with the amount of fruits purchased, nor with the vegetables. This finding has been confirmation of the level of income and purchasing amount changes. However, significance of the relationship for animal products and amounts of those products disable us to generalise this finding. This relationship is significant as demonstrated in Table 6. Following this identification, the direction of the relation was checked. With strongly significant positive coefficients, it is not hard to confirm that there is a positive relation between rising allocated budget and quantity purchased within the process. This was also an awaited situation.

Therefore, it can be briefly noted that, not the level of income but declination in the household income is related to the variation in purchases and consumption of FFVs, meat and dairies. In addition, with the rising share of animal products in the household income, the tendency to buy more meat and dairies rose as well within the sample and for the COVID-19 process. However, there detected no uprising or declining relationship for budget allotted to and purchases of FFVs. This is also an indicator for the socio-economic status of the audience interviewed online. Mostly middle-class individuals seemed to reach FFVs approximately on a desired and acceptable level. Their tendency to increase income that is used for FFVs purchases is lower than that the tendency for meat and dairies. Thus, it can be said that these individuals have been reaching adequate amount of FFVs during the survey time. The relationship of this tendency and COVID-19 needs to be considered as well as demand for protein - based consumption seemed to direct more attention within a linear analysis perspective.

Table 2. Changing Purchases of Main Categories during Covid-19 pandemic

	Rose	**Not changed**	**Declined**
Vegetables	159 (31,86 %)	304 (60,92 %)	36 (7,21 %)
Fruits	157 (31,46 %)	295 (59,12 %)	47 (9,42 %)
Meat Products	135 (27,05 %)	323 (64,73 %)	41 (8,22 %)
Dairies	159 (31,86 %)	316 (63,33 %)	24 (4,81 %)

Table 3. Income & Changing Purchases for FFVs and meat and dairy products

Income Level	**Vegetables**	**Fruits**	**Meat Products**	**Dairies**
X^2 (p)	2,69 (0,95)	5,98 (0,64)	8,56 (0,38)	6,33 (0,61)
Likelihood Ratio (G^2)	3,03 (0,93)	6,66 (0,58)	8,53 (0,38)	6,86 (0,55)

Table 4. Reduction in Income & Changing Purchases for FFVs, meat and dairy products

Income Reduction	**Vegetables**	**Fruits**	**Meat Products**	**Dairies**
X^2 (p)	15,86 (0,044)**	20,77 (0,007)***	40,88 (0,00)***	31,54 (0,00)***
Likelihood Ratio (G^2)	14,77 (0,064)*	19,38 (0,012)**	27,61 (0,00)	18,13 (0,02)**
Pearson C.C. (p)	-0,12 (0,009)***	-0,16 (0,00)***	-0,14 (0,00)***	-0,09 (0,03)**

C.I. * 90 %, **95 %, ***99%

Table 5. Budget Devoted for FFVs & Changing Purchases for FFVs

Income devoted for FFVs	**Vegetables**	**Fruits**
X^2 (p)	3,94 (0,41)	5,45 (0,24)
Likelihood Ratio (G^2)	3,85 (0,42)	5,25 (0,26)

Table 6. Budget Devoted for Animal Products & Changing Purchases for meat and dairy products

Income devoted for Animal Products	**Meat Products**	**Dairies**
X^2 (p)	12,34 (0,015)**	16,46 (0,003)***
Likelihood Ratio (G^2)	12,93 (0,011)**	16,59 (0,002)***
Pearson C.C. (p)	0,13 (0,003)***	0,12 (0,005)***

Discussion and Conclusion

This study aimed to detect existence of a relationship between consumers' changing level of income and budget share devoted to vegetative and animal products in accordance with tendency of households to purchase FFVs and animal products. It is inevitable to indicate main economic and social expectations from a group of people involve rising demand of any normal good in response to rising income. The presumed relationships were evaluated referring to the survey data from Turkey. However, under unexpected conditions, as it is the case for COVID-19, the assumed relationships might differ.

Therefore, survey data retrieved from 499 individuals was analysed within this research. As a result of the brief association tests, level of income seemed to have no association with the variation in quantity demanded for any product groups. The classified groups were FFVs (fruits and vegetables) and animal products (red/white meat and dairies). Under COVID-19 conditions, few consumers experienced declining demand and limited purchases of all product groups. Yet, demand for all categories seemed to rise by around 30 % during the process, while reduction was much limited. However, demand for fruits (9,42 %), meat products (8,22 %), vegetables (7,21 %) and dairies (4,81 %) declined relatively. While the declination and rising tendency were steady, the relationships were tested anyway.

In contrast to existing income level, variation of the income seemed to be correlated with measured demand. It was understood that, with declining income, the demand for all product groups had declined as well. A research indicated that especially meat and dairy consumption demand is also related with the concerns regarding that 'the pandemic stemmed from meat intake' (Poudel et al., 2020). However, it was indicated for the USA that 861 online participants tended to consume more meat products within the process to protect themselves with higher protein content (Chenarides at al., 2021). The interest on meat and dairy products remained at its previous levels or even increased despite rising prices and reducing supplies in the USA again within a different setting (Tonsor et al., 2021). Therefore, the tendency to purchase or consume meat and dairy products seemed to be varied. In addition to meat products, some researchers focused on health management and/or nutrition specialists who suggested protein-based diets including dairies as milk, yoghurt, butter and their derivatives (Muscogiuri et al., 2020). From a sales view, it was noted from a study undertook in Italy that consumers' tendency to buy products with longer shelf life has increased and packed milk was enlisted under this category as well (Bracale and Vaccaro, 2020).

When FFVs were considered, a study undertaken in Ethiopia with 3.245 participants via face to face survey in 2019 and phone surveys in 2020 indicated that households' fruit purchases have risen within the process, while there observed a declination in vegetable consumption. Part of the variation within the COVID-19 period was attributed to income loss, while protection and prevention appeared as another reason (Hirvonen et al., 2021). This study also confirmed the rising tendency to consume meat and dairy products.

This background research also confirmed the changing tendencies and relevance to household income and share of products in household budget. The findings are mostly in line with expectations. It was specifically understood that consumers in Turkey have tended to reach and consume more animal-based products within the pandemic process. The share their income devoted to these products have risen as well. It can be said that in contrast to many Asian countries close to the source of the virus, China or relevant developing countries, middle class members did not blame meat and dairies for the pandemic. They rather considered that these protein products would help them to empower their metabolism. Therefore, we can say that the significant finding of the study is the linear relation of rising income share for meat and dairies and rising demand for these products. However, there is still a room for further research on consumption level considering the effects of current COVID-19 pandemic and prospective future encounters.

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Author Contributions

Authors participated in preparation and editing of the research findings together and they are equal in terms of property rights. Dr. Ceylan had managed the online survey process earlier.

Conflict of Interest

There is no conflict of interest between co-authors.

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Determination of grain yield and some yield components of some two and six row barley (*Hordeum vulgare* L.) genotypes in Eskisehir ecological terms

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Abstract

This study was carried out to investigate yield and some yield characteristics of two and six row barley genotypes (5 hybrids and 5 standard varieties) in 2012/13 and 2013/14 growing seasons. The study, which was carried out in the experimental and research fields of Eskisehir Osmangazi University Faculty of Agriculture, was established according to the randomized blocks experimental design with 3 replications. In the research; plant height, spike length, grain number spike^{-1}, grain weight per spike^{-1}, spike number per M^{-2}, harvest index and grain yield characteristics were investigated. Genotypes were found to be statistically significant at the level of 1 % in all the traits examined. When the differences between the years were examined, the years were significant in all characteristics except the ear length. The effect of year x genotype interaction was found to be significant in all traits except for the harvest index value. Grain yield values varied between 347.9 kg/da-1 and 537.76 kg/da-1. The highest grain yield and grain weight per spike were obtained from the CLR x PLS (6) six-row hybrid, followed by PLS x KLC (6) with 468.44 kg/da-1 and PLS x CLR (6) (467.03 kg/da-1). These hybrids have been identified as promising.

Keywords: Barley, genotype, grain yield, yield components, hybrid

Introduction

Barley (*Hordeum vulgare L.*), which has the oldest history among cultivated plants, was used as human food together with settled agriculture. Barley, which is the most produced in the world after wheat, rice and corn, is mostly used in animal nutrition and malt production. (Kün, 1996). The yield value of barley, which has 51 731000 Hectares of cultivation area and 159 738 000 tons of production in the world, is 310 Kg/da. In Turkey, barley production area in 2020 is 28 690 715 decares, production amount is 7 600 000 tons and yield value is 268 kg/da (Anonymous, 2021a). In Eskişehir Province, the production amount was realized with 1 019 278 decares of cultivation area and 272 512 tons (Anonymous, 2021b). In addition to being the most selective among the cool climate cereals in terms of climate and soil requirements, it is produced both in dry farming areas and in areas with sufficient precipitation. Most of the barley production in our country takes place in the Central Anatolian Region (Kınacı and Kınacı, 1992). It is the most preferred cereal in terms of animal nutrition due to its high crude protein and digestible nutrient content in its grain (Akkaya and Atken, 1986).

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The aim of breeding studies is to create varieties with high yield and quality, resistant to diseases and pests. Yield performances of the developed cultivars vary according to environmental conditions. In order to obtain high yield per unit area, varieties that adapt to the ecological conditions of the region should be obtained and production should be made with appropriate cultivation techniques. Changes can be made in terms of cultural practices, but no changes can be made regarding cultivar or genotype characteristics. Considering this factor, it is focused on increasing or improving the yield potential of the genotypes considered in breeding studies. Therefore, the characters that can affect the yield should be well known and the mutual effects on each other should be revealed (Yağdı, 2002). The annual precipitation in the Central Anatolia region shows an irregular distribution according to the months. It is necessary to develop stable varieties that can withstand the irregular precipitation and winter conditions of the region (Yüksel et al., 2017). The study was carried out with the aim of determining the morphological characteristics of the two-row and six-row barley cultivar candidates developed as a result of breeding work and the cultivars adapted to the region and cultivated in large areas. For producers, high yield is very important in barley cultivation.

Material and Methods

The research was carried out in Eskişehir Osmangazi University Faculty of Agriculture Application and Research fields in the 2012/13 and 2013/14 production periods in Eskisehir conditions, and was carried out with a total of 10 barley genotypes consisting of five cultivar candidates (hybrids) and five cultivars. The experiment, which was carried out according to the randomized blocks trial design, was carried out in dry conditions with 3 replications. As the material in the study; PLS x CLR (6) (6-row), PLS x CLR (2) (2-row), PLS x KLC (6) (6-row), CLR x PLS (2) (2-row), CLR x PLS (6) (6-row), Kalaycı 97 (2-row), Plaisant (6-row), Cumhuriyet 50 (2-row), İnce-04 (2-row) and Özdemir-05 (2-row) genotypes were used. The soil in which the research was carried out was prepared for sowing by ploughing with a plow with a plow and plow with a combination of crowbar and rake. The plots were 4 m long, 14.5 cm row spacing and 6 rows in the planting made with the parcel seeder on 15.10.2012 and 25.10.2013. In the experiment, the seed amount was applied as 22 kg/da-1 and the fertilizer amount was 6 kg/da-1 P_2O_5 and 6 kg/da-1 N. Weed control was done by mechanical means. In order to eliminate the edge effects, observations, measurements and harvests were made 0.5 m from the beginning and end of the rows and from the remaining parts by removing the side rows. Eskisehir province is under the influence of terrestrial climate. The meteorological data of the production years (2012-2013-2014) and the long-term averages in which the research was conducted are given in Table 1.

Table.1. Meteorological data for many years (1975-2014) and 2012-2013 and 2013-2014 years in the vegetation period in Eskişehir province

	Year	October	November	December	January	February	March	April	May	June	July	Avarege/Tot.
Avarege Temperature (ºC)	2012/13	14,2	7,3	2,2	1,7	4,3	7,1	10,8	17,7	20,0	21,6	10,7
	2013/14	9,8	6,7	1,7	3,6	6,0	6,2	11,3	16,4	19,9	23,7	10,5
	Long-Term (1975-2014)	12,4	6,5	3,2	0,5	2,9	6,0	10,6	15,4	19,8	22,7	10,0
Total Precipitation (mm)	2012/13	16,1	14,5	73,2	18,5	36,5	33,2	37,8	9,5	14	0,8	254,1
	2013/14	65,0	15,0	1,5	21,0	7,0	27,1	23,2	53,8	70,5	20,4	304,5
	Long-Term (1975-2014)	26,1	29,8	46,1	38,2	32,5	33,4	35,2	43,3	28,6	13,5	326,7

In the application and research fields of Eskisehir Osmangazi University Faculty of Agriculture, where the experiment was carried out, the soils of the trial area contain 1.68 % organic matter and 4.38 % lime. It is loamy and slightly alkaline (pH 7.6–8.2). Some physical properties of the trial site are given in Table 2.

Table 2. Some physical and chemical characteristics of soil for research area

Soil depth (cm)	Total salt (%)	Organic Matter (%)	Lime (%)	Phosphorus P_2O_5 (kg/da)	Potassium K_2O (kg/da)	Structure	pH
0-30	0,050	1,68	4,35	3,84	215,2	Loamy	7,98

For each genotype in the experiment, plant height, spike length, number of grains per spike, grain weight per spike, number of plants per square meter, harvest index and grain yield measurements were made. Statistical analyzes were held trough SAS and and Jump 7 package program. Comparisons between mean values are given using the LSD Test.

Results and Discussion

Plant height, spike length, number of grains per spike, grain weight per spike, number of spikes per m2, harvest index and grain yield parameters were investigated in our study to reveal the performance and yield values of some cultivars and cultivar candidates in Eskisehir conditions. The combined variance analysis results of the genotypes used in the experiment are given in Table 3.

Table 3. Variance Analysis Results of the Parameters Evaluated in the Experiment

		Mean Square						
Source of Variation	**D.F.**	**Plant Height**	**Spike Length**	**Grain Number Spike^{-1}**	**Grain Weight per Spike^{-1}**	**Spike numbers M^{-2}**	**Harvest Index**	**Grain Yield**
Replication	2	1,007 ns	0,077 ns	1,433 ns	0,00*	17,617 ns	4,046 ns	49,822 ns
Year	1	812.544**	0.028	232.460**	0.490**	43578.150**	73.549*	10965.531**
Error	2	1.897	0.210	0.769	0.000	18.650	2.936	5.498
Genotype	9	14.346**	2.201**	808.376**	0.371**	4179.831**	18.365ns	18643.649**
Year x Genotype	9	45.872**	1.915**	29.802**	0.097**	1796.780**	5.031ns	3609.078**
Error	36	0.486	0.076	0.322	0.000	46.244	10.151	43.599
General	59	23.353	0.684	132.069	0.080	1679.745	11.246	3608.818
CV %		5.23	10.85	35.83	20.92	10.03	9.14	14.24

*P≤ 0.05,** P≤ 0.01 ns: Not significant.

The mean values of the genotypes and the classification according to the LSD test are given in Table 4. In terms of plant height, the interaction of year, genotype and year x genotype was found to be statistically significant at the 1% level. Average plant height values among genotypes varied between 91.05 cm and 95.33 cm. Kalaycı 97 (95.33 cm) variety got the highest value, while 2-row PLS x CLR (2) (91.05 cm) hybrid, 6-row CLR x PLS (6) (91.05 cm) hybrid and Cumhuriyet 50 (91. 07 cm) cultivar and took place in the same group statistically.

Table 4. Two-year average values of the parameters examined in barley cultivars and cultivar candidates

Genotypes	**Plant Height**	**Spike Length**	**Grain Number Spike^{-1}**	**Grain Weight per Spike^{-1}**	**Spike numbers M^{-2}**	**Harvest Index**	**Grain Yield**
PLS x CLR (6)	93,93b	6,94f	46,10b	1,67b	439,67a	36,07bc	467,03b
PLS x CLR (2)	91,05e	8,52a	23,15ef	1,26d	423,00b	35,73bc	383,86ef
PLS x KLC (6)	92,27cd	7,53cd	47,83a	1,52c	348,67h	35,92bc	468,44b
CLR x PLS (2)	94,35ab	7,95bc	22,85ef	1,21e	405,50ef	35,50bc	430,73c
CLR x PLS (6)	91,05e	7,01ef	45,35b	1,79a	409,33ef	34,69c	537,76a
Kalaycı 97	95,33a	7,42de	22,58ef	1,11h	420,50cd	38,79ab	347,94h
Plaisant	92,22cd	7,03ef	42,55c	1,50c	390,00g	37,19abc	410,84d
Cumhuriyet 50	91,07e	7,96b	24,65d	1,19f	410,83de	37,50abc	378,62f
İnce-04	91,23de	8,59a	23,32e	1,15g	438,67a	40,31a	390,87e
Özdemir-05	92,37c	7,31def	22,33f	1,10h	398,67fg	35,32bc	402,29d
Mean	92,49	7,62	32,07	1,35	408,48	36,70	421,84
LSD Year	3,53	0,51	2,25	0,01	11,07	1,90	6,01
LSD Genotype	1,10	0	0,89	0,02	10,68	3,73	10,37
LSD Year x Genotype	1,55	0,61	1,26	0,03	15,10	5,28	14,66

The average plant height values of the years are given in Table 5. The plant height value was higher in the 2013-2014 production season (96.17 cm). The high amount of precipitation in April and May, when the vegetative growth of the plants is high, was effective in the high plant height.

Table 5. Average values of parameters examined in barley cultivars and cultivar candidates by years

Years	**Plant Height**	**Spike Length**	**Grain Number Spike^{-1}**	**Grain Weight per Spike^{-1}**	**Spike numbers M^{-2}**	**Harvest Index**	**Grain Yield**
2012/2013	88.81b	7,60a	30,10b	1,26b	381,53b	35,59b	408,32b
2013/2014	96.17a	7,65a	34,04a	1,44a	435,43a	37,81a	435,36a
Mean	92,49	7,62	32,07	1,35	408,48	36,70	421,84

Plant height is a feature that changes according to the effect of genotype and environment and is mostly affected by genotype (Whitman et al., 1985; Yılmaz and Dokuyucu, 1994; Kendal et al. 2010; Sirat and Sezer, 2017a). While some researchers emphasized that shorter varieties should be developed in order to increase lodging resistance and yield (Anderson and Reinbergs, 1985), some researchers (Şener et al., 2020) emphasized the development of tall varieties in order to close the roughage deficit. In the study conducted by Sönmez and Yüksel (2019) in Eskisehir conditions, the plant height values were found to be between 70 cm and 101.3 cm in dry conditions, and Yüksel et al. (2017) reported that it varies between 92.5 cm and 129.5 cm. The findings obtained in the study are similar to the findings of other researchers.

The interaction of genotype and genotype x years in terms of spike length was found to be significant at the level of 1% (Table 3). The highest spike length among the cultivars was the Ince 04 (8.59 cm) cultivar, the PLS x CLR (2) (8.52 cm) two-row hybrid was found in the same group statistically. PLS x CLR (6) (6.94 cm), a six-row hybrid, had the lowest spike length value (Table

4). According to the average spike length values; the 2012-2013 production season had a lower value (Table 5). It is stated that the length of the spike is mostly affected by genetic factors as well as by the time of earing, sowing frequency, precipitation and other environmental factors (Puri et al., 1982; Çölkesen et al. 2002; Kaydan and Yağmur, 2007). In studies conducted by some researchers in similar and different ecologies, the spike length values are 5.8-9.4 (Çölkesen et al. 2002; Sirat and Sezer, 2016; Yüksel et al., 2017; Şener et al., 2020; Çelik, 2020) detected among them.

The difference between year, genotype and year x genotype interaction in terms of grain number spike^{-1} was found to be significant at the level of 1% (Table 3). When the average values of the number of grains per spike of the plants were examined, the six-row PLSxKLC (6) and PLS x CLR (6) hybrids (47.83; 46.10 units) had the highest values. Özdemir-05 variety and Kalaycı-97 variety had the lowest grain count values with 22.33 pieces per spike (Table 4). According to the averages of the years, the 2013-14 production season has a higher value than the 2012-13 production season with 34.04 grains per spike (Table 5). The number of grains per spike is among the yield elements. The number of grains per spike is among the yield elements. It varies depending on the number of flowers in the spikelet and the grain setting ratio of the flowers, and it is also under the influence of environmental factors (Sirat and Sezer, 2013; Kaydan and Yağmur, 2007). The high amount of precipitation in April and May of the 2013/2014 production season affected this feature positively and caused it to have a higher value compared to the first year. The values obtained as a result of this study with six-row and two-row barley genotypes Yüksel et al. (2017) is similar to the obtained values. The number of grains per spike of six-row barley is higher than that of two-row barley. In studies conducted by many researchers in different ecologies in two- and six-row genotypes, the grain count values per spike were reported to be between 19 and 83 (Sirat and Sezer, 2013; Çöken and Akman, 2016; Sirat and Sezer, 2016; Şener et al., 2020).

In the two-row and six-row barley genotypes included in the experiment, the interaction of year, genotype and year x genotype was found to be significant at the level of 1% in terms of grain weight per ear (Table 3).The average of the grain weight values per ear of the two years was determined as 1.35 g. In the 2013/14 production season, the average grain weight per ear was 1.44 g, which was higher than the first year and was consistent with the grain number and yield values per ear (Table 5). CLR x PLS (6) and 1.67 g, PLS x CLR (6) hybrids had the highest values with 1.79 g and Özdemir-05 and 1, respectively, the lowest values with 1.10 g, according to the number of grains per spike in which the two years were evaluated together. Kalaycı 97 cultivars were obtained with .11 g (Table 4). Sirat and Sezer (2013), in their study, average grain weight per ear is 1.25 g in the first year and 1.29 g in the second year, Yüksel et al. (2017) reported that they found it to be 0.86 g in two years. Grain weight per spike is one of the most important features affecting grain yield per unit area (Akdamar et. al., 2002, Sirat and Sezer, 2017a). Spike length is affected by the number of grains per spike, the number of rows of the genotype and other genotypic effects. In addition, the planting frequency is very important in the precipitation it receives during the grain filling period in the growing season (Kenar and Şehirali, 2001; Kaydan and Yağmur, 2007). In the study, it is estimated that the high amount of precipitation in the second year caused the weight to be higher.

The differences between year, genotype and year x genotype interaction in terms of the number of spike number M^{-2} were found to be statistically significant (Table 3). According to the average of two years, the number of spikes per square meter of genotypes varied between 348.67 and 439.67. While PLS x CLR (6) six-row hybrid showed the highest value with 439.67 units, İnci-04 variety was in the second place with 438.67 units and were included in the same group. The lowest value was PLS x KLC (6) six-row hybrid (348.67 units). Sirat and Sezer (2013); while stating that the amount of precipitation after planting takes place and the amount of precipitation during the vegetation period affects the number of ears per square meter, Walker and Matthews (1991) stated that the number of fertile ears may be lower in years with harsh winters. In the first year of our study, precipitation amounts in October and November, and in April and May were lower than in the second year, and it is estimated that this may be the reason for the lower value compared to the second year. In some studies conducted in different ecologies, the number of spikes per square meter varied between 397.57 - 516.73 (Sönmez et al., 1996; Sirat and Sezer 2013; Sirat and Sezer 2017a). The findings of our study are in agreement with the findings of the researchers.

According to the results of analysis of variance in terms of harvest index, statistically significant ($P \leq 0.05$) differences were found between years, while the interaction of genotype and year x genotype was found to be insignificant (Table 3). While the average of the harvest index values of the genotypes was determined as 36.70% in the experiment, the second year was found to be 37.81% higher than the first year (35.59%) (Table 5). The highest harvest index was the Ince-04 variety with 40.31%, while the lowest harvest index was the CLR x PLS (6) hybrid with 34.69 % (Table 4). Sirat and Sezer (2017a) expressed the harvest index as the efficiency of conversion of dry matter into yield. Harvest index varies according to variety, sowing time and frequency, number and weight of grains per spike, number and weight of stems (Kenar and Şehirali, 2001; Akdamar et. al., 2002). In the study conducted by Sirat and Sezer (2017b) on some barley varieties in the Bafra Plain, the average harvest index values were found to be between 36.11-42.92 % and this is consistent with our study. It was determined as 15.50-30.17 % and did not comply with our study.

According to the variance analysis results in terms of grain yield per decare, the differences between genotypes, year and year x genotype interaction were found to be statistically significant at the $P \leq 0.01$ level (Table 3). According to the average of two years, the highest grain yield was obtained with 537.76 kg/da-1 from CLR x PLS (6) 6-row hybrids. This was followed by PLS x KLC (6) 6-row barley cross with 468.44 kg/da-1 and PLS x CLR (6) 6-row barley cross with 467.03 kg/da-1. The lowest average yield was determined from Kalaycı 97 (347.94 kg/da-1) and Cumhuriyet 50 (378.62 kg/da-1) varieties (Table 4). High precipitation amount received in the second year of the study affected the total yield and higher grain yield was obtained compared to the first year. In the experiment, 408.32 kg da-1 in the first year, 435.36 kg/da-1 in the second year and 421.84

kg/da-1 in the average of two years were determined (Table 5). Grain yield is a parameter that occurs as a result of genotype characteristics reflecting cultivar characteristics and environmental factors (Feil, 1992; Poehlman and Sleper, 1995; Paunovic et al. 2006). Genotypic features are stated as morphological features including tillering, ear length, grain number and size, hectoliter weight (Kandemir N. 2004; Kaydan et al. 2007). Varieties can show different yield values according to the ecological structure of the environment and the cultural processes applied (Kalaycı et al. 1991; Karadoğan et al., 1999; Sirat and Sezer, 2017b). The most effective factors among these are the amount of precipitation and its distribution, and temperature (Kalaycı et al. 1991; Hay and Porter, 2006). In the experiment, the total amount of precipitation in the second year was higher than the first year, which caused the plant height, number of grains and weight, the number of spikes per square meter and the harvest index values to be higher. Some researchers stated with their findings that yield values in grain differ according to years and varieties. Çöken and Akman (2016), who stated that the grain yield changed between 169.67-363 kg/da-1 in Isparta ecological conditions and Zeynelağa and Bolayır cultivars had the highest value, reported that the grain yield of some barley lines and cultivars was 471.4-697 in Eskişehir ecological conditions. Indicating it as kg/da-1, Yüksel et al. (2017)'s findings show parallelism with the findings in our study. Similar results have been obtained in many studies conducted in different climates and locations (Sirat and Sezer 2013; Yüksel et al. 2017; Sönmez and Yüksel, 2019; Şener et al. 2020).

Conclusion

According to the findings obtained from a total of ten genotypes consisting of 5 cultivar candidates (hybrids) and 5 cultivars; yield and yield components of genotypes were investigated. According to the results obtained, the interaction of genotypes, year and year x genotype was found to be statistically significant. According to the results of the research, the highest grain yield is CLR x PLS (6) (537.76 kg/da-1), PLS x KLC (6) (468.44 kg/da-1) and PLS x CLR (6) (467, 03 kg/da-1) were determined from 6-row hybrids. Among the cultivars, Plaisant and Özdemir-05 had the highest grain yield. The highest plant height is from Kalaycı 97 variety and CLR x PLS (6) and PLS x CLR (6) hybrids; highest spike length from the Slim 04 variety and the PLS x CLR (2) hybrid; highest grain number per spike from PLS x KLC (6), PLS x CLR (6) and CLR x PLS (6) hybrids; The highest grain weight per ear was obtained from CLR x PLS (6) and PLS x CLR (6) hybrids, and the highest yield index with the highest number of ears per square meter was obtained from İnce-04 variety. According to the yield results obtained from the study, CLR x PLS (6), PLS x KLC (6) and PLS x CLR (6) hybrids are more promising in Eskisehir conditions compared to other genotypes. Their performance was better than the cultivars used in the study.

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Author Contributions

Nazife Gözde AYTER ARPACIOĞLU: Field work, ,article writing; Zekiye BUDAK BAŞÇİFTÇİ: Field work, ,article writing.

Conflict of Interest

The authors are declared that they have no conflict for this research article.

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Antioxidant enzymes activities of walnut nursery trees to drought stress progression

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Abstract

This research was carried out to determine the biochemical responses of Chandler and Fernor walnut cultivars saplings grafted on the *Juglans regia* seedling rootstock under water stress in 2020. In accordance with this purpose; three different irrigation levels were applied to the one-year-old seedlings in polyethylene tubes in the greenhouse for three months: 1) full irrigation as control (100% of potted field capacity (PFC)) and 2) two different levels of restricted water application (50 and 25% of PFC). The amount of decreasing water was provided every 5 days. The antioxidant enzyme activities including ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) in the leaves were determined every 15 days. In terms of the properties examined, statistically significant differences were found between the applications in the all analysis periods. In general, as the severity and duration of the water stress increased, the antioxidant enzyme activities gradually increased and while the highest values were determined in the 25% PFC application, the lowest values were determined in the 100% PFC control application.

As a result, the walnut saplings developed antioxidant defense mechanisms against water stress, demonstrating a possible tolerance. This suggests that the tolerance may be due to the activation of antioxidant systems and the reduction of oxidative damage. It has been determined that the antioxidant enzyme activities have different tendencies for both cultivars in response to the oxidative damage. While POD and CAT activities were at the higher levels in the Chandler cultivar; SOD and APX activities were at the higher levels in the Fernor cultivar.

Keywords: *Juglans regia*, Fernor, Chandler, Water stress, Enzyme

Introduction

Abiotic stress factors such as drought, salinity and extreme temperatures are among the main causes of crop yield losses in the world (Oliveira et al., 2013; Shahzad et al., 2016; Soni et al., 2017; Ilyas et al., 2020). As compared to the salt stress, the drought problem is more common and creates bigger economical problems (Vahdati and Lofti, 2013; Soni et al., 2017). Although drought is seen in all climatic zones in the world, the arid and semi-arid areas are more affected by the drought due to the lack of moisture and high variability of precipitation. The drought, which occurs in certain periods, especially in the semi-arid regions, can turn into an important disaster. In addition, it has been reported that the fresh water resources, 70% of which are currently used in the agriculture, will decrease seriously due to the population growth and global warming, and the world's water demand will increase by 55% until 2050 (Smedley, 2017). This situation raises the concerns regarding the drought. For this reason, it is very important to determine the fruit species that are tolerant to severe drought events and recover afterwards and their ability to cope with environmental stresses for the sustainable fruit growing.

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Juglans regia (walnut) species, which is in the *Juglans* genus of the Juglandaceae family, has been an important and strategic product of the daily diet of human beings since ancient times, especially because its fruits can be stored for a long time (Sen, 2011; Vahdati, 2014). Walnut, which is considered as a drought sensitive species, is mostly grown in the arid and semi-arid areas in the world (Vahdati, 2014). Therefore, the increasing danger of drought and severe water scarcity in these areas are the biggest limitations in the walnut cultivation. Although walnut roots go deep, since most of the roots are collected in the topsoil, the water taken from deep roots in the arid conditions is insufficient to prevent stress (Vahdati and Lofti, 2013). As a matter of fact, it has been reported that the walnut plant growth slows down under water stress, and its yield and quality decrease (Cohen et al., 1997; Simsek et al., 2017). Thus, it is economicaly important to know the capacity of walnut species and varieties to withstand drought stress. Liu et al. (2019) have reported that *Juglans mandshurica* Maxim and *Juglans regia* L. cv. Jizhaomian species were more tolerant to the drought stress as compared to the *Juglans nigra* L. species.

When plants are exposed to the water stress, they show many reactions on a morphological, physiological, biochemical and molecular bases (Buyuk et al., 2012; Soni et al., 2017; Liu et al., 2019). The most important biochemical response that they show is the accumulation of reactive oxygen species (ROS) such as H_2O_2. Excessive accumulations of these highly reactive ROSes cause oxidative stress that results in the programmed cell death by rapidly damaging macromolecules (e.g. DNA, protein, lipid and carbohydrate) (Khaleghi et al., 2019). On the other hand, plants have developed many defense and adaptation mechanisms (synthesis of substances such as stress proteins, osmolytes, antioxidants, etc.) that can increase their ability to survive and grow under short or long-term drought stress (Ashraf and Foolad, 2007; Akinci and Lösel; 2012; Anjum et al., 2011; Buyuk et al., 2012; Marcin´ska et al., 2013; Vahdati and Lofti, 2013). Antioxidant enzymes the synthesis of which is probably the main process in the plant tolerance to the environmental stresses play an important role in the scavenging of these reactive oxygen species (Khaleghi et al., 2019). For example, it has been reported that the plants have develped enzymatic (superoxide distumase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) and non-enzymatic (glutathione, β-carotene, ascorbic acid, α-tocopherol) antioxidant defense mechanisms to reduce the oxidative damage (Anjum et al., 2011; Buyuk et al., 2012; Farajzadeh et al., 2017; Popovic et al., 2017; Selmi et al., 2017). In a study conducted on two different types of walnuts, it was determined that drought stress promoted SOD and POD activity and increased the proline content (DaPei et al., 2018). In another study, it was reported that there were significant increases in the POD and APX activities in both leaves and roots of tolerant walnut genotypes starting from the 7th day of drought application (Lofti et al., 2010). As a result, many biochemical reactions are activated in the cell in terms of the tolerance levels of the genotypes under oxidative stress.

In this study, it was aimed to determine the tolerance mechanisms of one year old Chandler and Fernor walnut cultivars grafted on the *J. regia* seedlings against the increasingly limited water stress created by different irrigation levels, by the activity of some antioxidant enzymes.

Material and Method

Plant material and treatment

The research was carried out in the Agricultural Research and Application Center located in the campus of Isparta University of Applied Sciences (ISUBÜ) in 2020, in a plastic greenhouse of approximately 175 m^2 with manual ventilation from the side. The plant material of the study consisted of one-year-old tuberous seedlings of the Chandler and Fernor cultivars grafted on the *Juglans regia* seedlings. One year after grafting the walnut seedlings were planted in 8-liter plastic tubes with holes from the bottom filled with a 1:2:1 mortar containing sand, peat and perlite, and kept outdoors until they were put into the trial. Then the tubed saplings were transplanted into the greenhouse before the treatments were made. The growing medium used in the experiment was neutral, low in lime, salt-free and high content of organic matter, and has a clay loam structure (Anonymous 2020a). Irrigation water (C2S1) was in the class suitable for irrigation (Anonymous 2020b).

Three different irrigation levels were applied to the potted one-year-old saplings in a greenhouse for three months as follows: 1) full irrigation as control (100% of the pot field capacity (PFC)) and 2) two deficit irrigation treatments (50% of the PFC and 25% of the PFC). Irrigation treatments were started in the last week of June (24 June 2020) and ended in the third week of September (17.09.2020). Irrigation was performed once every 5 days to make up for the water missing from the pot capacity. The average temperature and humidity values measured in the greenhouse from July to September during the trial were given in Table 1.

Table 1. Average temperature and humidity values measured in the greenhouse from July to September in 2020, when the experiment was conducted.

Month	Average Maximum Temperature (°C)	Average Minimum Temperature (°C)	Average temperature (°C)	Average Maximum Humidity (%)	Average Minimum Humidity (%)	Average Humidity (%)
July	40.2	19.5	29.9	59.3	16.5	37.9
August	40.5	18.1	29.3	73.1	16.7	44.9
September	38.1	14.6	26.4	70.1	17.0	43.6

Biochemical analyses

Leaf Sampling: Leaf sampling was started 15 days after the first irrigation application and continued every 15 days until the end of the experiment. After washing the leaves with distilled water, they were frozen in liquid nitrogen and stored at -80 °C until the analysis.

Ascorbate peroxidase (APX) enzyme activity: APX enzyme activity analysis was performed according to the method described by Nakano and Asada (1981). For this purpose, 12 ml of 50 mM potassium phosphate buffer (Ph:7.3) containing 1 mM (EDTA), 2 mM DTT and 1 mM ascorbic acid was added to 4 g of leaf sample and the homogenate was centrifuged at 10,000 g and 4°C for 15 minutes. 0.9 ml of 0.05 M sodium phosphate buffer (Ph: 7.0) containing 0.5 mM ascorbate, 0.1 mM EDTA Na_2 and 1.2 mM H_2O_2 was added to the 0.1 ml enzyme extract and the absorbance values were readed at 470 nm. The results were expressed as mol/min/g protein.

Catalase (CAT) enzyme activity: CAT enzyme activity was determined as using the method described by Beers et al. (1952). For this purpose, 10 g sample and 25 ml 50 mM cold sodium phosphate buffer containing 0.5 g polyvinyl polypyrolidine (PVPP) was prepared (Ph: 7.0) and the lysed samples were centrifuged at 27,000 g and 4°C for 50 minutes. Then, 2 ml of sodium phosphate buffer (50 mM and pH: 7.0), 0.5 ml of H_2O_2 (40 mM) and 0.5 ml of enzyme extract were mixed and the absorbance values of the samples were read at 240 nm wavelength. The results were expressed as U/mg protein.

Peroxidase (POD) enzym eactivity: POD enzyme activity was determined using the method described by Jiangetal. (2010). According to this; 10 g of fresh leaf sample and 25 ml of cold 100 mM sodium phosphate buffer containing 0.5 g of polyvinyl polypyrolidine (PVPP) (pH: 6.4) were were homogenized, and then centrifuged at 27,000 g at 4°C for 50 minutes and then the supernatant was collected. After that, 100 mM sodium phosphate buffer (Ph: 6.4) containing 8 mM quaiacol was added to the 0.5 ml enzyme extract and incubated at 30°C for 5 minutes. The absorbance values were read at 460 nm wavelength and the results were expressed as Δ_{A460}/min/mgprotein.

Superoxide dismutase (SOD) enzyme activity: SOD enzyme analysis was performed as described by Jiang et al. (2010). For this purpose, 10 g of fresh leaf sample was added to 25 ml of cold 100 mM sodium phosphate buffer containing 0.5 g of polyvinyl polypyrolidine (PVPP) (pH: 6.4). After the samples were crushed with the help of a homogenizer, they were centrifuged at 27,000 g at 4°C for 50 minutes and the supernatant was collected. The analyses were made according to the method described by Constantine and Stanley (1977). 2.9 ml of 50 mM sodium phosphate buffer (Ph:7,8) containing 13 mM methionine, 75 µM NBT, 10 µM EDTA and 2 µM riboflavin and 0.1 ml of sample were mixed and the absorbance values were read in the spectrophotometer at 560 nm wavelength. The results were defined as the amount of enzyme reducing the amount of SOD by 50% as a result of NBT and expressed as U/mg protein.

Statistical Analysis: The experiment was established according to the completely randomized plots experimental design with 3 replications and 5 plants per replication. The data was subjected to the variance analyses ($p<0.05$) and the differences between the means were evaluated with the Tukey test.

Results and Discussion

The accumulation of ROS'es comes as a biochemical response in the plants exposed to the water stress. However, excessive accumulation of ROS'es inevitably causes oxidative stress by inducing the lipid peroxidation, protein reduction and DNA fragmentation in the cell. Plants have also developed antioxidant defense mechanisms to reduce this oxidative damage (Buyuk et al., 2012; Farajzadeh et al., 2017; Popović, 2017; Selmi et al., 2017). In many studies, it has been determined that the enzyme activities such as SOD, POD, APX, CAT and GR increased in the plants under water stress (Farajzadeh et al., 2017; Popović, 2017; Selmi et al., 2017).

The APX enzyme, which is highly expressed in the plant cells, plays an important role in the defense mechanism by reducing H_2O_2, one of the reactive oxygen species (Buyuk et al., 2012; Sharma 2012). In this study, a significant difference was determined between the treatments in terms of APX activity in all analysis periods, and the APX activity increased linearly as the severity of water stress increased (Table 2, Figure 1). Moreover, as the period progressed in the study, APX activity increased at higher rates in the all treatments until the 60th day analysis, and this increase was realized at the higher levels in the limited water stress applications. However, APX activity remained slightly increased or decreased at similar levels with the 60th day analysis results in the last two analysis periods. In the measurements made at the end of the experiment (90th day), the APX activity increased 1.5 times in the 50% PFC application and 2.1 times in the 25% PFC application as compared to the 100% PFC application. According to the results of the 15th day analysis, at the end of the period, it was 206.0% in 100% PFC, 171.1% in 50% PFC and 182.4% in 25% PFC. These results were similar to the literature. As a matter of fact, it was determined that there was a significant increase in the APX activities in both leaves and roots of tolerant walnut genotypes starting from the 7th day of drought treatment (Lofti et al., 2010). Moreover, similar results were also obtained in the studies conducted on different plants (Gur, 2018; Faaek, 2018; Babalik, 2012).

In the study, the difference between the cultivars was significant in terms of APX enzyme activity (Table 2). Fernor cultivar showed higher activity. These results may indicate that the drought tolerance of cultivars may be closely related to the antioxidant enzyme capacity. Indeed, Lofti et al. (2010) indicated that the tolerant walnut genotypes showed higher APX activity, but Lofti et al. (2019) reported that the APX enzyme increased as the drought severity increased in the seedlings of the Chandler cultivar. Moreover, Vahdati (2014) stated that the differences between the walnut genotypes that show resistance or sensitivity to abiotic stresses could be attributed to the differences in the mechanisms underlying oxidative stress damage and subsequent tolerance to abiotic stress.

Table 2. Effects of water stress treatments on the APX enzyme activity (mol/min/g)

Cultivar	Water Stress Treatments (% PFC)	Analysis Dates					
		15. Day	30. Day	45. Day	60. Day	75. Day	90. Day
Chandler	100 (control)	0.70	0.91 d	1.41 d	1.39	1.66	2.35 d
	50	1.34	1.82 bc	2.75 c	2.84	2.91	3.33 c
	25	1.59	2.55 ab	3.60 b	4.49	5.35	5.32 a
Fernor	100 (control)	0.96	0.85 d	1.39 d	2.62	1.84	2.73 cd
	50	1.50	1.52 cd	2.44 c	3.50	3.41	4.38 b
	25	2.16	3.22 a	4.71 a	5.72	5.73	5.31 a
Treatment Average	100 (control)	0.83 c	0.88 c	1.40 c	2.00 c	1.75 c	2.54 c
	50	1.42 b	1.67 b	2.59 b	3.17 b	3.16 b	3.85 b
	25	1.88 a	2.89 a	4.16 a	5.11 a	5.54 a	5.31 a
Cultivar Average	Chandler	1.21 b	1.76	2.59 b	2.91 b	3.31 b	3.66 b
	Fernor	1.54 a	1.87	2.84 a	3.95 a	3.66 a	4.14 a

PFC: Pot Field Capacity; *: There is no statistically significant difference between the means shown with the same letter in the columns ($p<0.05$).

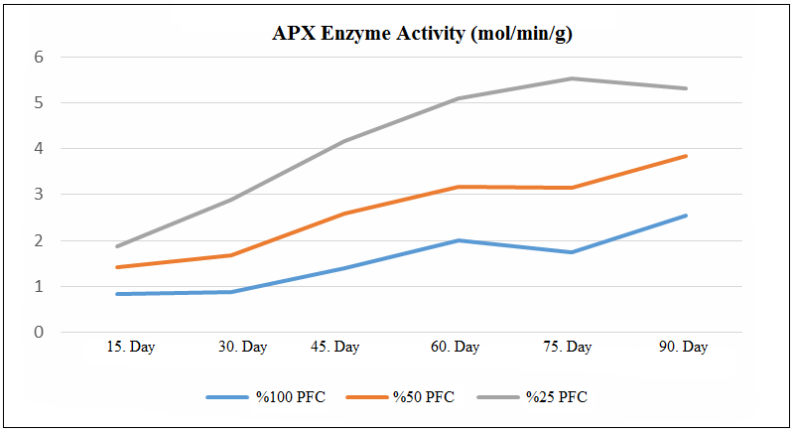

Figure 1. Variation of APX activity according to the periods in different water stress applications

CAT is one of the important antioxidant enzymes that play a role in the decomposition of harmful H_2O_2 into H_2O and O_2, which occurs under stress conditions (Buyuk et al., 2012). In this study, a significant difference was found between the treatments in all analysis periods in terms of CAT activity. As the severity of water stress increased, CAT enzyme activity increased and the highest CAT values were determined in 25% PFC treatment, while the lowest values were determined in 100% PFC control treatment (Table 3 and Figure 2). Moreover, in 100% PFC and 50% PFC treatments, CAT activity increased at high rates until the 45th day analysis, while it followed a slight increase or decrease in similar levels with the 45th day analysis results in the following periods. On the other hand, there was a significant increase in the CAT activity in the 15th day analysis of 25% PFC application and this increase continued as the period progressed. In the measurements made at the end of the experiment (90th day), CAT activity increased 1.7 folds in 50% PFC application and 2.4 folds in 25% PFC application as compared to the 100% PFC treatment. According to the results of the 15th day analysis, it was observed as 40.9% for 100% PFC, 109.0% for 50% PFC and 103.2% for 25% PFC at the end of the period. These results clearly show that CAT enzyme activity plays a role under the drought stress in walnuts. This was particularly evident under the severe stress conditions. Similar results have also been reported in the studies performed on the other plants (Bolat et al., 2014; Hassan et al. 2018; Tiryaki, 2018).

Table 3. Effects of water stress treatments on the CAT enzyme activity (U/mg)

Cultivar	Water Stress Treatments (% PFC)	Analysis Dates					
		15. Day	30. Day	45. Day	60. Day	75. Day	90. Day
Chandler	100 (Control)	22.03	27.47 cd	20.18 d	30.86 cd	28.79 c	25.22
	50	20.91	45.96 ab	36.09 b	42.00 b	46.92 b	44.29
	25	29.66	53.70 a	57.62 a	52.52 a	60.69 a	58.00
Fernor	100 (Control)	12.21	23.25 d	21.94 cd	24.68 d	21.07 d	23.03
	50	18.31	34.25 c	26.41 c	39.52 bc	26.27 cd	37.67
	25	27.81	36.05 bc	37.50 b	58.14 a	55.15 a	58.75
Treatment Average	100 (Control)	17.12 b	25.36 b	21.06 c	27.77 c	24.93 c	24.12 c
	50	19.61 b	40.10 a	31.25 b	40.76 b	36.59 b	40.98 b
	25	28.73 a	44.88 a	47.56 a	55.33 a	57.92 a	58.37 a
Cultivar Average	Chandler	24.20 a	42.38 a	37.96 a	41.79	45.67 a	42.50
	Fernor	19.44 b	31.18 b	28.62 b	40.78	34.16 b	39.81

PFC: Pot Field Capacity; *: There is no statistically significant difference between the means shown with the same letter in the columns ($p<0.05$).

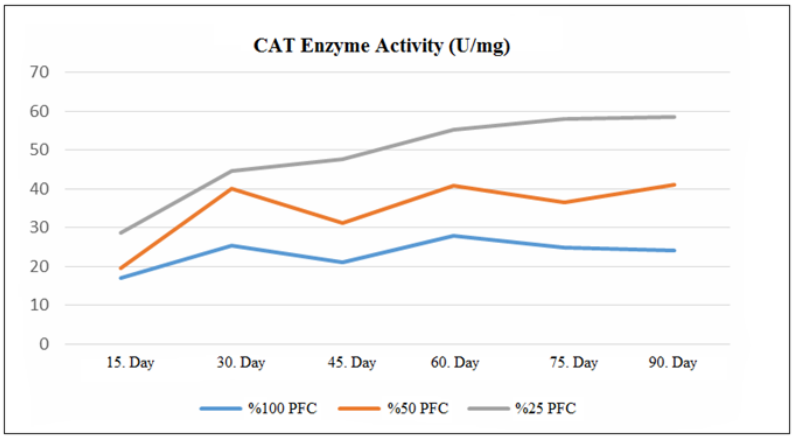

Figure 2. Variation of CAT activity according to the periods in different water stress treatments

Significant differences were found between the varieties in terms of CAT activity. Accordingly, Chandler cultivar had the higher CAT enzyme activity in all analysis periods. Similarly, Gur (2018) reported the differences in the CAT activities in the different pear rootstocks under drought stress.

SOD, a metalloprotein, is the first enzyme to play a role in the cellular defense mechanisms against reactive oxygen species (Buyuk et al., 2012; Khaleghi et al., 2019). SOD catalyzes (produces H_2O_2) and eliminates the superoxide (O_2-) radical (Khaleghi et al., 2019). H_2O_2 is converted to H_2O and O_2 by CAT (Zhu et al., 2020). In this study, a significant difference was found between the treatments in terms of the SOD activity in all analysis periods, and the SOD activity increased linearly as the severity of water stress increased (Table 4 and Figure 3). In the study, there was no increase in SOD activity in the first two months in the control application (100% PFC), but an increase was found in the analyzes of the 75th day with the progression of the period. On the other hand, as the period progressed in the 50% PFC and 25% PFC treatments, the SOD activity values gradually increased. In the measurements made at the end of the experiment (90th day), the SOD activity increased 2.3 times in the 50% PFC treatment and 3.2 times in the 25% PFC treatment as compared to the control. According to the results of the 15th day analysis, at the end of the period, it was 14.2% for 100% PFCs, 70.0% for 50% PFCs and 71.7% for 25% PFCs. This increase in the SOD activity in walnut leaves with water stress may be associated with the mechanism of protection against oxidative stress-related damage. In the studies conducted on walnuts, it was also determined that the SOD enzyme activity increased under drought stress (JiMing et al., 2012; DaPei et al., 2018). Similar results have also been reported in the studies performed on the other plants (Tiryaki, 2018; Khaleghi et al., 2019).

In terms of SOD activity, a significant difference was found between the cultivars in the all period analyses except the 15th day and 45th day analyses. Accordingly, Fernor cultivar had the higher SOD enzyme activity in all analysis periods (Table 4). Zhu et al. (2020) also reported that there were differences between the Cassava cultivars that they examined in terms of SOD content, and one cultivar showed the higher SOD activity under severe drought. Similarly, Gur (2018) reported that there were significant differences in the SOD activity among the pear rootstocks that they examined.

Table 4. Effects of the water stress treatments on the SOD enzyme activity (U/mg)

Cultivar	Water stress Treatments (% PFC)	Analysing Dates					
		15. Day	30. Day	45. Day	60. Day	75. Day	90. Day
Chandler	100 (Contol)	1.28	1.13	1.02	1.12	1.33 f	1.74 d
	50	1.85	2.30	2.54	2.55	2.83 d	3.39 c
	25	2.75	3.42	4.42	4.42	4.87 b	4.30 b
Fernor	100	1.40	1.13	1.32	1.10	1.93 e	3.32 d
	50	2.30	2.54	2.90	2.79	3.89 c	3.64 c
	25	2.90	3.72	4.51	4.59	5.59 a	5.41 a
Treatment Average	100 (Control)	1.34 c	1.13 c	1.17 c	1.11 c	1.63 c	1.53 c
	50	2.07 b	2.42 b	2.72 b	2.67 b	3.36 b	3.52 b
	25	2.83 a	3.57 a	4.47 a	4.50 a	5.23 a	4.86 a
Cultivar Average	Chandler	1.96	2.28	2.66 b	2.70 b	3.01 b	3.14 b
	Fernor	2.20	2.46	2.91 a	2.82 a	3.80 a	3.46 a

PFC: Pot Field Capacity; *: There is no statistically significant difference between the means shown with the same letter in the columns ($p<0.05$).

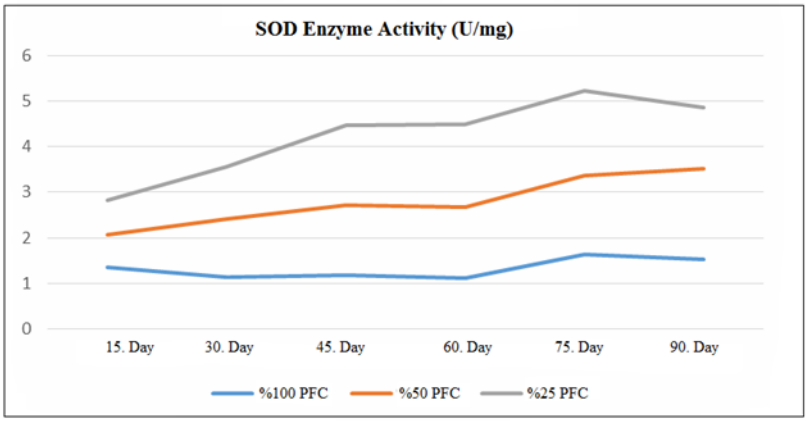

Figure 3. Variation of SOD activity according to the periods in different water stress treatments

Referring to Asada and Takahashi (1987), another important enzyme involved in the scavenging of H_2O_2 produced under stress is POD (Gokmen, 2011). Moreover, Li et al. (2017) reported that POD plays a role in the protective mechanism under water stress. In this study, POD activity linearly increased with the increasing water stress severity and stress duration (Table 5 and Figure 4). In the analyses performed at the end of the experiment (90th day), POD activity increased 2.1 times in the 50% PFC application and 2.6 times in the 25% PFC application as compared to the control. It was determined that the POD activity increased as the period progressed in all treatments. According to the results of the 15th day analysis, the increases at the end of the period were 53.4% for 100% PFCs, 109.3% for 50% PFCs, and 106.9% for 25% PFCs. In the previous studies on walnuts, POD enzyme activity was found to increase under drought stress (Lofti et al., 2010; JiMing et al., 2012; DaPei et al., 2018; Lofti et al., 2019). This increase in the POD activity in walnut leaves with water stress may be associated with the mechanism of protection against oxidative stress-related damage. However, different results have been reported in the literature regarding the POD activity. Renju et al. (2017) reported that the POD activity increased in a moderate water stress application, whereas in moderate and severe drought applications, it first increased and then decreased in the later stages of drought. Similarly, Hui et al. (2016) reported that POD activity increased in the drought-resistant potato variety but decreased in the non-durable potato variety. Tiryaki (2018) reported that the limited water applications in oil rose reduced POD activity. Zhu et al. (2020) also reported that restricted water applications significantly reduced POD activity in Cassava plant. These results indicate that water stress affects POD activity differently in different species. This suggests that POD activity can be explained by whether it is suppressed or not by the transcription factors (Zhu et al., 2020).

Significant differences were found between cultivars in terms of POD activity. Accordingly, the Fernor cultivar provided higher POD activity in the 15th day and 45th day analyses, but the Chandler cultivar performed higher POD enzyme activity in the other periods (Table 5). Lofti et al. (2019) also reported that the POD enzyme activity increased with increasing drought severity in the seedlings of Chandler cultivar. Moreover, Zhu et al. (2020) reported that there were differences between the Cassava cultivars that they examined in terms of POD contents and the two cultivars showed lower POD activity.

Table 5. Effects of water stress treatments on the POD enzyme activity (Δ_{A460}/min/mg)

Cultivar	Water stress treatments (% PFC)	Analyses Dates					
		15. Day	30. Day	45. Day	60. Day	75. Day	90. Day
Chandler	100 (ontrol)	76.13	79.91 c	91.63 d	96.30 c	96.90 d	128.08 c
	50	125.06	135.70 b	207.81 b	169.78 b	231.87 b	308.00 a
	25	153.31	153.85 b	250.36 a	275.33 a	345.26 a	341.32 a
Fernor	100	90.88	60.23 c	93.08 d	99.91 c	142.41 c	128.15 c
	50	131.0	134.46 b	134.00 c	140.84 bc	166.71 c	227.94 b
	25	167.97	203.86 a	208.45 b	193.85 b	224.13 b	323.39 a
Treatment Average	100 (Control)	83.51 c	70.07 c	92.35 c	98.11 c	119.65 c	128.11 c
	50	128.03 b	135.08 b	170.90 b	155.31 b	199.29 b	267.97 b
	25	160.64 a	178.85 a	229.41 a	234.59 a	284.69 a	332.35 a
Cultivar Average	Chandler	118.17 b	123.15	183.26 a	180.47 a	224.67 a	259.13 a
	Fernor	129.95 a	132.85	145.18 b	144.87 b	177.75 b	226.49 b

PFC: Pot Field Capacity; *: There is no statistically significant difference between the means shown with the same letter in the columns ($p<0.05$).

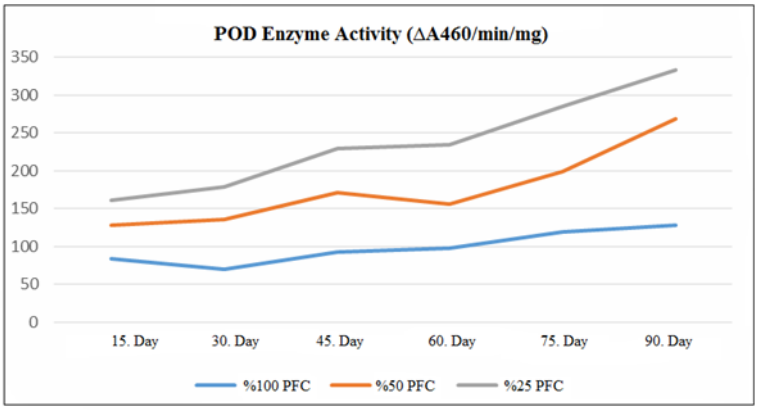

Figure 4. Variation of POD activity according to the periods in different water stress treatments

Conclusion

In the study, the responses of one-year-old tubedsaplings of commercially important walnut cultivars to the different levels of drought stress were investigated on the basis of some antioxidant enzymes. As a result, walnut saplings developed antioxidant defense mechanisms against water stress, demonstrating a possible tolerance. This tolerance suggests that it may be related to the activation of antioxidant systems and the reduction of oxidative damage. Antioxidant enzymes were found to have different affinities for both cultivars in response to the oxidative damage. While POD and CAT activities were at higher levels in the Chandler cultivar; SOD and APX activities were at higher levels in the Fernor cultivar. In addition, it was determined that especially the APX enzyme activity increased at a high level as the period progressed under the controlled conditions.

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Conflict of Interest

There is no conflict of interest between co-authors.

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