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Research Article

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Using The Response Surface Method to Determine Optimum Temperature and Gam Usage in Egg Storage

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ABSTRACT

Response surface method (RSM) is a form of optimization based on the creation of an empirical model for evaluating the relationship between factor levels and the responses obtained therefrom. RSM is a multivariate analysis created by applying multiple regression and space geometry together. This optimization method can also be used as a preliminary stage of factorial experiments, since factor levels determine the optimum points before a factorial experiment. In this way, since the difference between the factor levels will be less, it provides more healthy results. In other words, optimization is used to increase the significance and sensitivity of factor levels.In this study, 130 table chicken eggs were divided into 9 groups according to their storage temperatures and percentages of coating gam arabic matter. Weight losses during the 28-day storage period of eggs were calculated. The eggs were weighed on the 7th day, the 14th day, the 21st day, and the 28th day. After the study was completed, the differences of the weights on the first day and 28th day were calculated. While applying RSM, Central Composite Design trial pattern was used. As a result of the analysis, optimum storage temperature and gam arabic composition were determined for egg storage with RSM. According to the results of the statistical analysis, at the end of the 4th week, it was determined that the optimum storage temperature and gum substance composition for the minimum egg weight loss (1.58 g) were 7.64-8.24 ^{o}C and 15%. When the results of the study and the results obtained from the analysis are compared, it is thought that RSM has obtained an intermediate dose estimation for the minimum egg weight loss in optimization of egg preservation conditions and this may be beneficial in the field of animal breeding.

1. Introduction

Foods of animal origin are more important than foods of vegetable origin in meeting the energy, protein, vitamins and minerals that the human body needs. Chicken eggs are a relatively inexpensive animal protein source compared to other animal foods. In addition to being a quality food source due to its high protein content and sufficient essential amino acids, chicken eggs and chicken meat are consumed more than red meat by those who have a balanced nutrition awareness. However, since egg storage conditions are not developed in our country, eggs are mostly consumed fresh. Therefore, freshness must be preserved until the chicken eggs reach the consumer. Determination of physical, chemical and microbiological changes depending on different temperature conditions in eggs to be consumed fresh is very important in terms of both preventing economic loss, protecting public health and consumer satisfaction.

Optimization; is a term that enables achieving certain targets (such as reducing costs, increasing profitability, increasing capacity utilization) by using the resources in a system (such as labor, time, process, raw material, equipment) as efficiently as possible (Banga et al., 2003).

In other words, optimization is a tool used for high process efficiency and product quality in processes. It is to choose the best alternative among the alternatives possible under certain conditions. The aim of optimization is to increase efficiency by using limited production resources that will provide minimum cost and maximum profit. Response surface method (RSM) is a

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statistical technique for finding the best result possible (Saguy et al., 1984).

RSM explores the relationships between various explanatory variables and one or more response variables. The method was introduced by Box & Wilson in 1951 (Box & Wilson, 1992). The main idea of RSM is to use a series of experiments designed to obtain an optimal response. To do this, researchers propose a quadratic polynomial model. They accept that this model is only an approach, but they use it, because such a model is easy to guess and apply even if little is known about the process. RSM is applied after determining minor factors that have little or no effect on the response (Anonymous, 2018). In the first stage, it is necessary to determine the factors that are thought to have an effect on the response variable. The levels of these factors should then be determined.

In this study, by using RSM, it is aimed to determine the best preservation temperature and gum arabic percentages that will give the least weight loss during the 28-day storage period.

2. Materials and Methods

2.1. Material

One hundred thirty uniform chicken eggs from a local egg supplier were used as research material.

2.2. Method

Eggs were stored at 4 °C, 14 °C and 24 °C, covered with 3%, 9% and 15% gum arabic coater. After the eggs covered with the gum arabic substance have dried out, they are divided into groups according to different temperature and gum arabic mixture percentages as in Table 1. The stored eggs were weighed and weight losses during the storage period were determined. Face-Central Composite Design was used for analysis.

Table 1
Egg groups according to their temperatures and gum arabic percentages

Gum Arabic(%)	Storage Temperature (°C)		
	4	14	24
3	10	10	10
9	10	50	10
15	10	10	10

2.3. Response Surface Method

The Response Surface Method (RSM) is a statistical method that is useful for modeling and analyzing factors that affect a response by several variables, and the aim is to optimize this response (Teja &Muneiah, 2018). RSM was originally established to model experimental responses and then started to be used to model numerical experiments (Box & Draper, 1987). The difference is in the error type generated by the response. In physical experiments, inaccuracies may be due to reasons such as measurement errors or various errors made by the observer, while in computer experiments numerical noise is a result of discrete representation of iterative processes, rounding errors, or continuous physical events. The errors are assumed to berandom in RSM (Namdev et al., 2014). One of the most important features of RSM is that it optimizes the factors that are thought to affect the response variable effectively by using appropriate mathematical and statistical methods (Montgomery, 2001).

While all treatment combinations have to be included in the experiment in factorial trials, not all factor combinations are used as RSM will be based on optimum points. The unexamined combinations can be used by researchers since they do not cause any loss of information (Yılmaz, 2002). For example, in a factorial trial with 3 factors and 3 levels, $3^3 = 27$ treatment combinations are required, while $2^n + (2n + 1) = 2 \times 3 + (2 \times 3 + 1) = 15$ treatment combinations is sufficient in RSM (Yılmaz, 2002).

While a model can be created using multivariate regression models in RSM, many variables that affect the response can be examined together, and the response can be defined in the best way by making the least number of experiments. Allowing the determination of the optimum point by taking into account a large number of responses, RSM stands out among other optimization methods. If the relationship between dependent and independent variables is linear, then it makes use of the following linear regression equation (Montgomery, 2001).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n + \varepsilon$$

If the system has curvature, a higher order polynomial should be used, such as a second order model. If the relationship between dependent and independent variables is not linear, that is, if the system has curvature, a higher order polynomial, such as a second order model, should be used. It is calculated with the help of nonlinear regression (quadratic) equation (Montgomery, 2001).

$$Y = \beta_0 + \beta_1 X_1 + \cdots + \beta_n X_n + \beta_{11} X_1 + \beta_{11} X_1^2 + \cdots + \beta_{nn} X_n^2 + \beta_{11} X_1^2 + \cdots + \beta_{12} X_1 X_2 + \cdots + \beta_{n-1,n} X_{n-1} X_n + \varepsilon$$

2.4. Central Composite Design

The system needs a more detailed model to optimize the process and show the relationship between response and the values of the factors (Brereton, 2003). CCD is a very suitable design model for second-order models. Box & Hunter (1957) suggested that the quadratic response surface design should be rotatable. Central Composite Design can be rotated by choosing "α". There may be occasions when the region of interest may sometimes be desired to be cubic rather than global. In such cases, Face-Centered Central Composite Design, which is a derivative of Central Composite Design, is used. In Face-centered Center Composite Design, $\alpha = \pm 1$. Face-Centered Central Composite Design is not rotatable. The Face-Centered Center Composite Design does not require as much center points as the global CCD (Montgomery, 2001).

3. Results and Discussion

For the 4-week preservation of eggs at different temperatures and gum arabic concentrations, the predicted results obtained by the response surface method are shown in Table 2.

Table 2
Top 5 predictions for fourth week results

Results	°C	Gum Arabic (%)	Weight loss (g)	Composite Desirability
1	7.64	15.00	1.58	0.92
2	8.04	15.00	1.58	0.92
3	4.00	14.12	1.73	0.90
4	11.35	9.89	2.04	0.86
5	12.31	4.54	2.52	0.79

When Table 2 is examined, it is determined that the minimum weight loss (1.58 g) in eggs is the conditions where the temperature is 7.64 °C and 8.04, and the gum arabic concentration is 15%. Composite Desirability value was found to be 0.92 for these predictions. The remarkable point here is that the weight loss in the egg increases with the increase of the temperature and the decrease of the gum arabic substance.

It was determined that the residues obtained as a result of preserving the eggs at different temperatures and gum arabic concentrations for 4 weeks showed normal distribution (Figure 1).When the Figure 1 is examined, it is seen that the residuals (the difference between the real values and the predicted values) show a normal distribution.

The figure showing the interaction of different amounts of gum arabic and temperature values is given in Figure 2.

Figure 3 shows the effects of temperature and gum arabic separately on the response. When the temperature and gum are considered separately in the figure, it is seen that the weight loss in the egg is less as the temperature decreases and the amount of gum increases at the end of the fourth week.

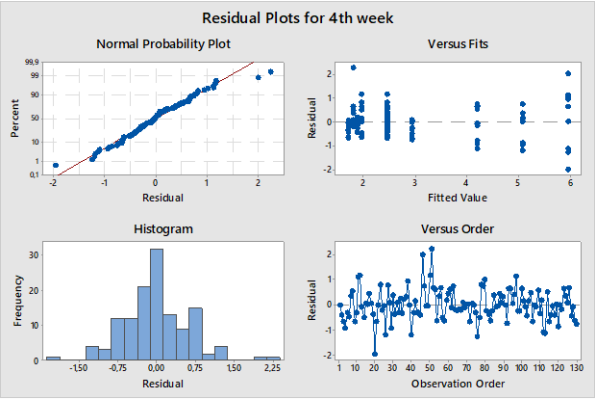

Figure 1
Residual plots for fourth week storage results

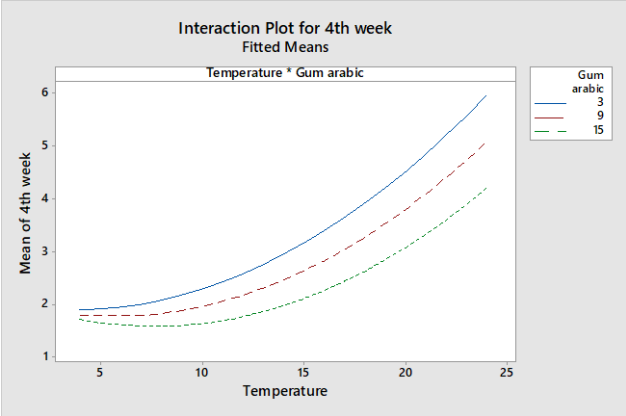

Figure 2
Interaction plot for fourth week results

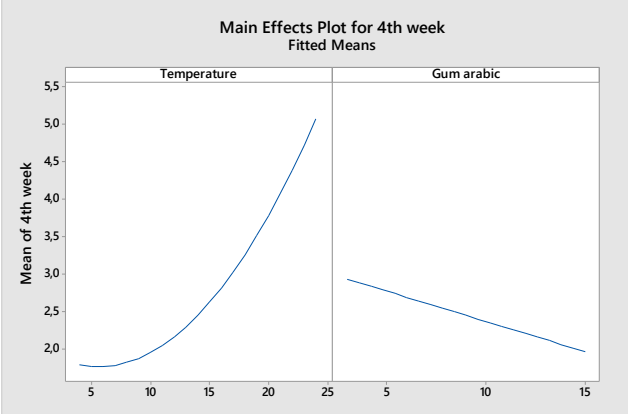

Figure 3
Main effects plot for fourth week

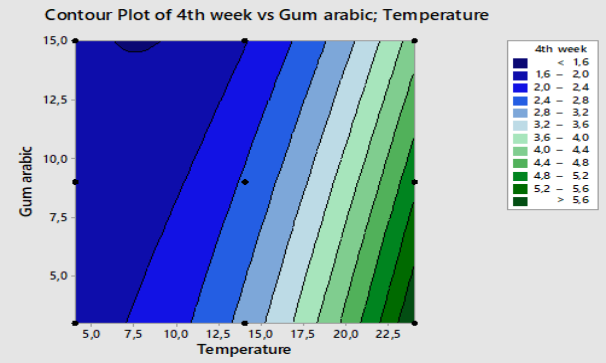

Figure 4
Display of egg weight loss with contour chart for the fourth week

When Figure 4 is examined, the region where egg weight loss is optimum is shown in dark blue (<1.6). It can be seen that this region is approximately at 7-8 °C and 14-15% gum arabic.Figure5 shows the surface graph of the fourth week results, and Figure 6 shows the optimum gum and temperature values for egg weight loss.

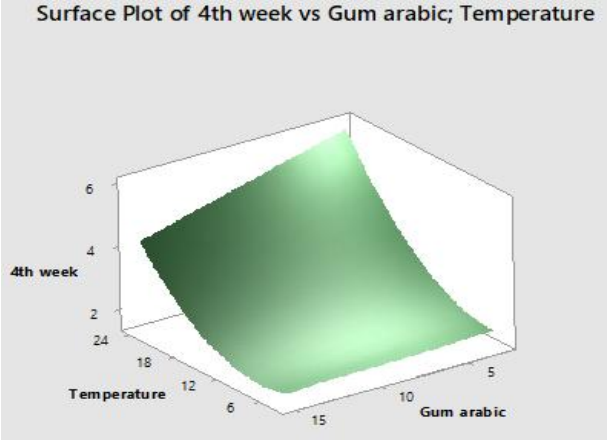

Figure 5
Representation of the results of the fourth week with surface graph

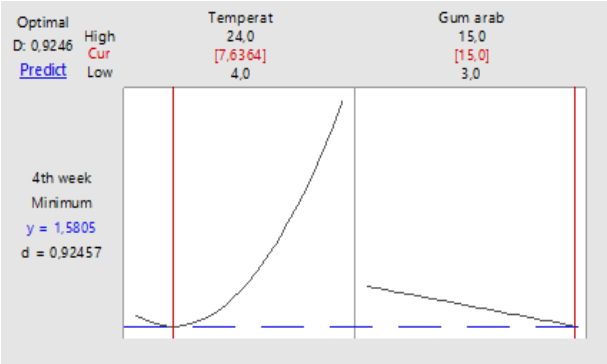

Figure 6
Graph showing the optimization of the fourth week results

When Table 2 is examined, the best predicted value for the 4th week results was determined as the conditions where the temperature was 7.64 °C and the gum arabic concentration of 15% with a weight loss of 1.58 grams. Composite desirability was found to be 0.92 for this estimate.

4. Conclusion and Suggestions

Although the egg can partially protect itself against microbiological deterioration with its natural structure, this is not enough. For this reason, eggs that are not stored in appropriate storage conditions lose their freshness and lose quality. There are some important losses in egg quality in the process that the egg passes from production until it reaches the consumers. Therefore, various storage and preservation methods are applied to increase the resistance against deterioration in the egg. Among these methods, common uses include dipping, preservation in cold stores, cryopreservation, and preservation with preservatives.

The purpose of preserving with preservatives is to reduce the oxygen inflow into the egg and the carbon dioxide output from the egg. For this purpose, paraffin coating and shell coating processes are applied. Since the cost of the gum arabic used in this study is low, easily available and easy to apply, it is recommended to be used as a coating agent.

The optimum weight loss for eggs was predicted to be 1.58 g after four weeks of storage, and the conditions providing this condition were found to be about 7.64 °C and 15% of the gum. Desirability for this result was calculated as 0.92.

When the eggs were stored for 28 days, the optimum point for the temperature factor was calculated as 7.64 °C, an intermediate dose not addressed in the trial, instead of the lowest level of 4 °C. It has been observed that the gum arabic substance should be used from the highest limit discussed in this study. According to RSM, when these conditions are met, the predicted minimum weight loss is calculated as 1.58 g. An egg with an average of 65 g will have an average weight of 63.42 g with the loss of 4 weeks storage. If the condition where the temperature is 4 °C and the gum is 15% as a storage condition is examined, the obtained value is predicted as 1.71 g with 0.90 desirability degree. Both the desirability and weight loss of the response optimized by RSM are better than 4 °C and 15% gum arabic. Considering these results, when an average of 65 g egg is kept in optimum conditions, it is foreseen that it will not change the quality criterias of the class A egg published in the Turkish Food Codex Egg Communique. In case the storage conditions were 24 °C and 3% gamut, the weight loss was predicted as 5.95 g. In this case, an average of 65 g of eggs will fall to 59.05 g and there will be a change in the quality classification. Therefore, determination of optimum storage conditions is an important consideration for both the consumer and the manufacturer. In cases where it is not known how long the storage will take, it will be in the manufacturer's interest to store the eggs with the optimum conditions for each week in any future change in storage period by determining a common optimum point within 4 weeks. The study on this method in the field of zootechnics is limited. The cost of the smallest calculation errors to be made in these sectors can be huge. While mistakes in the ration go to animal losses, mistakes in the food and construction industry can lead to material and life losses extending to human life. The work to be carried out in such risky sectors should be carried out with the utmost care and the highest possible efficiency. The experiments carried out until the optimum point is the most important factor affecting the cost and result.

While preparing poultry and dairy cattle rations that require precise calculation in the livestock field, it may be possible to obtain the highest possible yield from animals by preparing a more optimum feed mixture with fewer experimental materials using RSM. In the field of animal breeding, it can help enables the simultaneous evaluation of the factors and determination and subtraction of the unimportant factors in the optimization of cold storage conditions. It also contributes to the examination of the results with the help of various graphics.

It can be used to help where it is necessary to predict the optimum points with less trial material, optimizing the response from factor levels that are not tested in experiment, making new optimums based on the changing levels of the factors, RSM may be recommended.

In cases where it is necessary to predict the optimum points with less trial material, RSM can be recommended in such cases, by optimizing the response from the factor levels not tested in the experiment and making it possible to make new optimities according to the changing levels of the factors.

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The Effect of Heat Stress on Milk Yield, Milk Fat Rate and Rectal Temperature in Holstein-Friesian Dairy Cattle

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ABSTRACT

Heat stress is an environmental factor that negatively affects the morphological and physiological properties of dairy cattle. The aim of this study is to investigate the relationship between heat stress and milk yield, milk fat ratio and body temperature in Holstein-Friesian dairy cattle. The data of the study was obtained from a private Kurtalan Farm of Siirt province, the Southeastern Anatolia Region of Turkey. Milk yield and other traits of 13 head Holstein-Friesian dairy cattle were recorded in March, April, May, June, July, August and September. In addition, temperature and humidity records were recorded in the farm and in the parlor to be used for calculating the temperature humidity index value. In the analysis of data, correlation and regression methods were used. As a result of the study, the negative correlation ($P<0.01$) was found between milk yield and milk fat ratio and the positive correlation ($P<0.001$) were detected between heat stress and body temperature. In addition, a significant negative relationship was observed between rectal temperature and milk yield ($P<0.01$).

1. Introduction

Animal husbandry is important in terms of adequate and balanced nutrition of people and consumption of animal-derived proteins such as meat, milk and eggs that determine the level of development of countries (Hekimoğlu and Altındeğer, 2008). In order to meet the needs of the increasing world population such as meat and milk, the productivity per animal needs to be increased. Factors affecting the productivity of animals are examined under two main headings as genetics and environmental factors (Tuncel, 1994). Among environmental factors, care, feeding and climate factors come to the fore. Although temperature, humidity and wind come to the fore among the climate factors, it is seen as the most important factor since the temperature factor affects the productivity of the animals negatively (Mutaf and Sönmez, 1994; Öten et al., 2010). When the temperature occurred by the ambient temperature and humidity values (Thom, 1959) exceeds the humidity index threshold values, the animals are exposed to heat stress. Studies have reported that heat stress begins when the temperature humidity index value exceeds 65 and mortality rates increase when it exceeds 80 (Vitali et al., 2009; Collier et al., 2011). Many studies have been conducted to show that the heat stress negatively affects milk yield (West, 1999; West et al., 2003; Brouček et al., 2009; Baumgard et al., 2012; Brown et al., 2015; Al Reyad et al., 2016; Trajchev et al., 2016; Zhu et al., 2016) and milk fat rate (Arieli et al., 2004; Rejeb et al., 2012; Brouček et al., 2009; Ghavi Hossein-Zadeh, 2013). However, studies reporting that heat stress negatively affects the reproductive characteristics of animals have also been conducted (Evans et al., 2010;Khodaei-Motlagh et al., 2011; El-Wishy, 2013).This study aimed to investigate the effect of heat stress on milk yield, milk fat ratio and body temperature in Holstein-Friesian dairy cattle.

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2. Materials and Methods

This study was carried out in a private Agricultural Enterprise located in Gökdoğan village of Kurtalan district of Siirt province. The enterprise is located in the Southeastern Anatolia Region, where the continental climate prevails and the temperatures can rise 42 ^{o}C in August (Figure 1). The animals are housed in semi-open shelters (Figure 2).

Figure 1
Geographical location of Siirt province

Figure 2
Business where the study is carried out

Feeding is done two times a day after morning and evening milking. Milking is done automatically between 05:00-07:00 in the morning and 17:00-19:00 in the evening. 21 kg corn silage, 10 kg concentrate and 3 kg wheat straw are given per animal. In addition, when corn harvest is made, wheat straw is halved and 1.5 kg corn straw is used instead. The feedstuffs used were analyzed for dry matter (DM), crude protein (CP), crude fat (CF), crude ash (CA), acid detergent cellulose (ADC) and neutral detergent cellulose (NDC) (Table:1)

Table 1
Nutrient content of feeds used for farm

Nutrient contents, %	DM	CP	CF	CA	ADC	NDC
Corn straw	91.18	3.05	1.29	6.57	54.14	75.05
Wheat straw	92.61	2.84	1.40	6.32	57.39	74.23
Corn silage	95.05	4.90	1.61	7.92	34.93	61.21
Concentrated feed	88.61	18.01	4.26	7.94	9.17	27.48

In determining the cattle, the calving date and milk yields of the cows were taken into account. Because there are no farms in Siirt province that uses herd management program or support academic studies, 13 animals were used in the current study. Accordingly, study started with 9 animals giving birth in March and 4 animals giving birth in April. Three of these animals were first, five of these animals were second twoof these animals were third, one of these animals was forth and two ot these animals were seventh lactation. The selected animals were kept with other animals and no different treatment was made. Samples collecting were started in a week after birth in order to lose the effect of colostrum milk and continued from March to September. Milking was performed with an individual milking machine, milk samples were taken and milk yield was recorded. After milking, the rectal temperatures of the animals were measured with a thermometer. During milking, environmental temperature and humidity values were recorded with a temperature and humidity recorder. The milk samples were analyzed immediately on the milk analyzer.The temperature and humidity recorder placed in an area where the animals spend the most time in the shelter and where the sun does not directly contact, the environmental temperature and humidity values were recorded every two hours. In calculating the temperature and humidity index (THI) in the shelter and in the parlorthe Formula (1)

THI = (0.8 * Tdb) + ((RH / 100) * (Tdb-14.4)) + 46.4 (1) was used (Mader et al 2006).

For statistical analysis, milk yield and milk fat ratio were selected as dependent variable, months, lactation number, rectal temperature and temperature humidity index values were evaluated as independent variables. Also rectal temperature was evaluated as dependent variable. Since the measurements are in a longitudinal (repetitive) structure obtained at 7 different time points for each animal, analyzes have been carried out in a linear fixed-effect mixed model structure that takes into account repeated measurements.

2.1. Model

$Y_{ijkl} = \mu + a_i + b_j + b_1 (x_{ijkl} - \bar{x}) + b_2 (x_{ijkl} - \bar{x}) + e_{ijkl}$

Y_{ijkl} = Daily milk yield, milk fat ratio and rectal temperature, μ = average of the population,

a_i = The effect of i. month

b_j = The effect of j. lactation period,

b_1 = regression coefficient of milk yield and milk fat ratio according to temperature humidity index values during milking,

b_2 = regression coefficient of milk yield and milk fat ratio according to rectal temperature,

x_{ijkl} = temperature humidity index value and rectal temperature,

$\bar{x}$ = average temperature humidity index value and rectal temperature,

e_{ijklm} = error.

When the rectal temperature is dependent variable, rectal temperature has not evaluated as a independent varible. The most likelihood method was used to obtain model estimates. However, the mean of the least squares for the temperature humidity index at milking point was obtained for each dependent variable. Pearson's correlation coefficients were used in order to determine the relationships between the variables in the continuous structure used in the study and regression models were created between the features whose relationship was significant. In addition, the mean, standard deviation, minimum and maximum values and 95% confidence limits for the mean variables are given as descriptive statistics. SAS 9.4 software was used for the statistical evaluation of the data. Proc Means, Proc Corr and Proc Mixed commands were used to perform analysis. Also, some regression models were constituted between the milk yield, rectal temperature, temperature humidity index and milk fat ratio.

3. Results and Discussion

Temperature, humidity and temperature humidity index values obtained during milking and in the shelter are given in Table 2. The highest temperature (41 ^{o}C) was observed in the shelter in August and September, while the lowest temperature (6.7 ^{o}C) was detected in April. The highest humidity was experienced in May (90.1%) and the lowest humidity (6.30%) in September. The highest and lowest temperature humidity index values were observed in August (83.36) and April (46.07), respectively. The lowest (11.5 ^{o}C) and the highest (30 ^{o}C) temperatures during milking were observed in March and August, respectively. The lowest (46%) and highest (75%) humidity rates were observed in July and May, respectively. However, the lowest (53.54) and highest (77.97) temperature humidity index values were found to occur in March and August, respectively.

Temperature humidity index values obtained from the shelter were found to be higher than the threshold values reported in some previous studies (Vitali et al 2009; Collier et al 2011; Bouraoui et al 2002; Bernabucci et al 2010; De Rensis et al 2015). Although the temperature humidity index values during milking were found several units higher than inside the shelter, it was observed that the temperature humidity index values reached 83.56, 82.96 etc. in some days in the shelter. It has been determined that dairy cattle are exposed to heat stress from the middle of May to the end of September in the current farm. In other words, the farm experiences about 5.5 months of heat stress within a year. This shows that the region has a warm climate. The use of fan and wetting methods, semi-open shelters and canopies in the farms ensures that the temperature and humidity index values decrease. It is estimated that cattle are exposed to heat stress for a long time and their productivity decreases in the Southeastern Anatolia Region where these methods are used almost negligibly.

In the current study, the rectal temperatures of cattle ranged from 35.6 ^{o}C (May) to the highest between 39.65 ^{o}C (August) between March and September. According to the monthly average values, the rectal temperatures of the cattle were determined the lowest in May (36.89 ^{o}C) and the highest in August (38.48 ^{o}C) (Table 3). A positive correlation was found between temperature humidity index and rectal temperatures of cattle ($P < 0.0001$). Rectal temperatures of cattle reached their highest values in August, when temperatures increased. In the current study, a negative relationship was determined between rectal temperature and milk yield ($P<0.01$). Heat stress causes the rectal temperatures in cattle to increase and thus to decrease milk yield. The negative relationship between rectal temperature and milk yield determined in this study is in agreement with (Nardone et al 2006)

Table 2
Temperature, humidity and temperature-humidity index values in the farm and during milking

Months	Values	MAT	MAH	MATHI	MPT	MPH	MPTHI
March	Mean	13.95±1.91	61.45±6.41	57.27±2.71	11.50	71.00	53.54
	Sample number	6	6	6			
	Minimum	11.80	52.80	54.08			
	Maximum	16.10	67.90	60.39			
April	Mean	14.95±3.90	69.17±13.65	58.64±5.69	21.00	68.00	67.69
	Sample number	360	360	360			
	Minimum	6.70	31.60	46.07			
	Maximum	26.40	89.60	73.89			

Table 2
Temperature, humidity and temperature-humidity index values in the farm and during milking

May	Mean	19.83±4.61	63.33±16.47	65.11±5.78	19.00	75.00	65.05
	Sample number	373	373	373			
	Minimum	9.20	20.80	49.70			
	Maximum	30.60	90.10	76.97			
June	Mean	24.11±6.17	47.63±19.04	69.19±6.21	25.50	47.50	72.07
	Sample number	336	336	336			
	Minimum	11.50	13.80	53.18			
	Maximum	36.60	87.90	79.09			
July	Mean	29.78±6.33	31.56±13.25	74.29±5.34	30.00	46.00	77.58
	Sample number	385	385	385			
	Minimum	17.10	8.70	61.78			
	Maximum	40.30	67.60	82.96			
August	Mean	30.42±6.57	29.20±13.06	74.61±5.37	30.00	48.50	77.97
	Sample number	228	228	228			
	Minimum	18.60	7.40	63.17			
	Maximum	41.00	56.50	83.36			
September	Mean	28.11±6.61	30.03±11.99	72.33±5.96	26.00	52.00	73.23
	Sample number	504	504	504			
	Minimum	14.30	6.30	57.80			
	Maximum	41.00	56.80	82.30			
Total	Mean	24.42±8.00	45.09±21.86	68.91±8.02	23.28±6.65	58.29±12.50	69.59±8.52
	Sample number	2192	2192	2192	7	7	7
	Minimum	6.70	6.30	46.07	11.50	46.00	53.54
	Maximum	41.00	90.10	83.36	30.00	75.00	77.97

MAT: Monthly average temperature, MAH: Monthly average humidity, MATHI: Monthly average temperature-humidity index, MPT: Milking parlor temperature, MPH: Milking parlor humidity, MPTHI: Milking parlor temperature-humidity index

During the study, the highest (41.7 kg) and the lowest (8.4 kg) daily milk yield was obtained in April, while the average milk yield was determined as 24.39 kg. It was determined that milk yield decreased in the summer months from May to September (Table 3). A significant negative correlation was found between milk yield and temperature humidity index ($P < 0.05$) and rectal temperature ($P < 0.01$). In accordance with this study, it has been reported that heat stress reduces milk yield in different studies (Brouček et al 2009; Brown et al 2015; Al Reyad et al 2016; Gaafar et al 2011; Nardone et al 2006).

The lowest (2.40%) and highest (7.35%) milk fat ratio was obtained in September and April, respectively. A significant negative correlation was found between temperature, humidity index value and milk fat ratio ($P < 0.01$). In different studies, it was determined that heat stress caused a decrease in milk fat ratio (Bouraoui et al 2002; Joksimović-Todorovićet al 2011; Gorniak et al, 2014; Tuyttens et al 2015) and it was found similar to this study.

Regression models between temperature humidity index and rectal temperature, milk yield and milk fat ratio and between milk yield and rectal temperature are as follows;

1. RT (Rectal temperature) = 33.480 + (0.063 x THI)
2. MY1 (Milk yield-1) = 43.104 + (-0.263 x THI)
3. MFR (Milk fat ratio) = 7.001 + (-0.044 x THI)
4. MY2 (Milk yield-2) = 132.843 + (-2.855 x RT)

As the temperature humidity index increases, the rectal temperature of the cattle increases, the milk yield and the milk fat ratio decrease. According to the regression models, for example, while milking temperature humidity index values are 65, 69, 73 and 80, the rectal temperatures of the cattle are 37.58 ^{o}C, 37.83 ^{o}C, 38.08 ^{o}C and 38.52 ^{o}C, milk yields 26.01 kg, 24.96 kg, 23.91 kg and 22.06 kg and milk fat rates were found as 4.14%, 3.97%, 3.79% and 3.48%.However, according to the regression model between rectal temperature and milk yield, the rectal temperature was 37.58 ^{o}C, 37.83 ^{o}C, 38.08 ^{o}C and 38.52 ^{o}C, while milk yield was 25.57 kg, 24.85 kg, 24.13 kg and 22.87 kg. In other words, when the temperature humidity index value is less than 71, milk yield is more affected by the increased rectal temperature, and when it is more than 71, it is observed that there is more decrease in milk yield due to the increasing temperature humidity index value. As it continues to rise, it can be interpreted that it is more effective in the beginning when the body temperatures rise faster.

Table 3
Monthly average rectal temperature, milk yield and milk fat ratio values

Months		Rectal temperature (^{o}C)	Milk yield (kg)	Milk fat (%)
March	Mean	37.25±0.71	26.88±8.80	5.09±0.32
	Animal number	5	4	4
	Minimum	36.40	14.70	4.75
	Maximum	37.95	35.40	5.45

Table 3
Monthly average rectal temperature, milk yield and milk fat ratio values

April	Mean	38.35±0.45	24.72±11.21	4.63±1.35
	Animal number	13	13	12
	Minimum	37.15	8.40	2.75
	Maximum	38.80	41.70	7.35
May	Mean	36.89±0.68	28.06±7.47	3.44±0.56
	Animal number	13	13	13
	Minimum	35.60	11.40	2.55
	Maximum	37.90	40.55	4.25
June	Mean	37.90±0.44	26.38±5.56	3.69±0.64
	Animal number	13	13	13
	Minimum	36.85	18.58	2.45
	Maximum	38.55	34.30	4.60
July	Mean	38.35±0.46	24.63±5.30	3.42±0.57
	Animal number	12	12	12
	Minimum	37.65	15.43	2.45
	Maximum	39.20	31.30	4.25
August	Mean	38.48±0.44	21.54±3.49	3.68±0.57
	Animal number	13	12	13
	Minimum	37.80	15.74	2.85
	Maximum	39.65	27.90	4.60
September	Mean	38.23±0.35	20.07±4.22	4.01±0.78
	Animal number	13	13	13
	Minimum	37.75	12.40	2.40
	Maximum	38.80	26.20	5.60
Total	Mean	37.98±0.74	24.39±7.14	3.87±0.89
	Animal number	82	80	80
	Minimum	35.60	8.40	2.40
	Maximum	39.65	41.70	7.35
R^2		-	0.354	0.182

4. Conclusion

As a result, it was determined that the rectal temperature increases with the heat stress and milk yield decreases and cooling systems should be used to eliminate these negativities. It has been determined that cattle are exposed to heat stress from mid-May to mid-September, although the current farm is half open and the farm uses 2 fans to cool during milking. It is understood that the cooling system used here is insufficient. Considering this dairy cattle farm, it is considered that more effective fans and wetting methods should be used for cooling the animals in the dairy cattle farms in Siirt province and Southeast Anatolia region where the temperatures are high. In addition, it is expected that artificial shades and afforestation in pastures will be beneficial in reducing heat stress.

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The Identification of Genetic Variation in Insulin-Like Growth Factors-I (IGF-I) Gene Region in Some Turkish Sheep Breeds**

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ABSRACT

Insulin-like growth factors (IGFs) areknown as peptides with important metabolic effects required for cellular growth and metabolism. IGF-I is synthesized in liver tissue under the control of growth hormone (GH) and released to blood. In the process of GH to accelerate growth, IGF-I occupies a large place. IGF-I clearly showed its important effects on the growth of animal studies. In this study, promoter region of the IGF-I gene were amplified by polymerase chain reaction (PCR) in sheep breeds (Akkaraman, Kivircik, Awassi, Sakiz, Daglıc, Morkaraman, MalyaKarayaka (15 to 20 sheep per breed)) reared in Turkey. Informative restriction fragment length polymorphisms (RFLPs) were obtained with *Hae*II enzyme. The digestion of IGF-I gene with *Hae*II produced two alleles and three genotypes. Genotype frequencies were 59%, 19%and 22% for AA, BB and AB genotypes, respectively. Allele frequencies were 0.70 for A allele and 0.30 for the B allele. This study indicates the genetic profiles of the IGF-1 gene in native Turkish sheep breeds.

1. Introduction

To date, many hormones have been shown to be effective in growth physiology. It was discovered in the middle of the 20th century thatgrowth hormone (GH) enabled growth in mice (Kopchick et al., 2014). It was initially thought that growth hormone alone would provide growth, but studies have shown that it stimulates growth by stimulating peptide cell division called insulin-like growth factor (IGF) (Daughaday, 1997). It is now known that both hormones are effective in growing.

There are 2 forms of IGF; IGF-I and IGF-II, which are in the structure of the single- chain polypeptide (Le Roith et al., 2001). IGF-I is also called a somatomedin C and a basic polypeptide containing seven amino acids. IGF-II is a neutral polypeptide of sixty-seven amino acids. IGF-I amino acid sequence is 43% with proinsulin and IGF-II is 41% similar to proinsulin (Bondy et al., 1994). Polymorphism of IGF-I gene plays an important role in the regulation of IGF-I concentration, growth features (Ge et al., 2001; Yılmaz et al., 2005; Behzadi et al., 2015; Grochowska et al., 2017) and many hormones (He et al., 2012).

Studies conducted up to now have found that IGF alleles are associated with many yield characteristics such as birth weight, live weight gain, milk yield and fertility (Yilmaz et al., 2005; Pereira et al., 2005; Siadkowska et al., 2006; Li et al., 2008; He et al., 2012; Ali et al., 2016; Othman et al., 2016). IGF-I takes place in chromosome 3 in sheep and chromosome5 in cattle and goats; and it contains 5 regions of exon (Alakilli, 2012).

Turkey's natural and economic conditions, agricultural structure and tradition, is suitable for sheep and goat breeding. (Kaymakci andEngindeniz,2010). However, sheep breeding has not shown adequate development. In Turkey, there is a shortage of meat production comparing to the nutritional requirements, and there is an increasing gap between meat products produced domestically and the amount consumed. It has even begun to import meat in recent times. Production improvements can be achieved by using new genetic technologiesandthe selection of heritable traits. IGF-I is acandidate gene for selection programmesthat can be done in terms of the meat production efficiency.

Insulin-like Growth Factor I (IGF-I) is a hormone-like polypeptide related to several economically important traits including growth and reproduction parameters in sheep. Due to the lack of knowledge about the genetic characterization and nucleotide sequence

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** *Short communication*

variations of the IGF-I gene in Turkey's sheep breeds, this study is aimed to detect the genetic polymorphism of IGF-I in different sheep breeds reared in Turkey.

2. Materials and Methods

In this study, eight different sheep breeds' (N=144) blood samples from different parts of Turkey are used. The sampling locations and provinces are given on the map of Turkey (Fig. 1). Genomic DNA was extracted from blood samples by the salting-out procedure (Miller et al., 1988). The purity and concentration of the isolated DNA were determined by electrophoresis in a 1% agarose gel and UV spectrophotometry.

Figure 1
The location of the samples used for PCR-RFLP

The PCR reaction for the amplification of the promoter region of the IGF-I gene (265 bp was given by Yilmaz et al. (2005). The PCR reaction was as follows; 2 µL 10 X PCR buffer, 3 mM $MgCl_2$, 3 mMdNTP, 0.5 µM primer, 0.2 U Taq DNA polymerase. Primer sequences were: forward 5'-ATTACAAAGCTGCCTGCCCC-3' and reverse 5'-TCACATCTGCTAATACACCTTACCCG-3'. The PCR cycleinclude; initial denaturation at 94 °C for 5 min, 30 cycles of94 °C for 30 s, annealing at 62 °C for 30 s and extension at 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min.

In order to investigate the nucleotide sequence variability in the ovine IGF-I gene, restriction enzyme *Hae*II were selected according to their ability to digest theDNA. The PCR product (265 bp) was digested with this enzyme for overnight at 37 °C. After ethidium bromide staining, the gels were photographed under UV light and the DNA bands were evaluated. PopGene 3.1 was used for allele and genotype frequencies.

3. Results and Discussion

We determined three genotypes as the result of the restriction by *Hae*II enzyme; aa (179, 86 bp), bb (265 bp) and ab (265, 179, 86 bp) (Figure 2 and 3). Alleles found in this study were similar to those previously identified and reported by Yilmaz et al. (2005). Yilmaz et al. (2005) identified two single nucleotide polymorphisms; A and T to C transition at position 179.

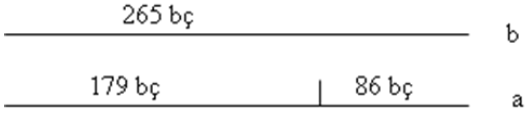

Figure 2
The digestion patterns of IGF-I with *Hae*II restriction enzyme.

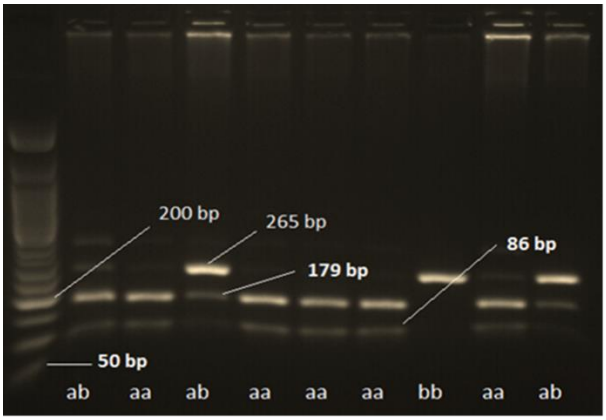

Figure 3
Digestion of the PCR amplified IGF-I gene with *Hae*II, First lane: 50 bp ladder; lane 2-4-10: ab genotype; lane 3-5-6-7-9: aa genotype; lane 8: bb genotyp

Table 1
The allele and genotype frequencies for IGF-I gene as digestedby*Hae*II restriction enzyme

Breed	N	Genotype frequencies(%)			Allelefrequencies(%)		χ^2
		Aa	Ab	bb	a	B	
Karayaka	19	79.00	10.50	10.50	0.84	0.16	8.42**
Daglıc	18	75.00	12.50	12.50	0.81	0.19	4.31*
Awassi	15	23.07	53.86	23.07	0.50	0.50	0.18
Sakiz	17	60.00	33.30	6.67	0.77	0.23	0.16

Table 1
The allele and genotype frequencies for IGF-I gene as digestedby*Hae*II restriction enzyme

Kivircik	16	75.00	12.50	12.50	0.81	0.19	6.80**
Akkaraman	19	68.75	6.25	25.00	0.72	0.28	12.75**
Morkaraman	20	13.30	46.70	40.00	0.37	0.63	0.013
Malya	20	70.59	11.76	17.65	0.76	0.24	8.86**
Allbreeds	144	58.80	22.7	18.50	0.70	0.30	25.51**

*P<0.05; **P<0.01

Allele b obtained from the digestion of IGF-I with *Hae*II was found more frequent in the Morkaraman breed. Allele a wasfound more frequent in all the other breeds. The HW test showed that the studied Awassi, Sakiz and Morkaraman breeds fit the theoretical proportions. However, Karayaka, Daglic, Kivircik, Akkaraman and Malya don't fit the theoretical proportions for the *Hae*II digestions of this gene (P <0.05; P <0.01) (Table 1).

This research is the first investigation of detecting the polymorphism of IGF-I gene with *Hae*IIrestriction enzyme in Turkishnativesheep breeds. Two allells and three genotypes were observed when RFLP markers performed to detect the polymorphisms of promoter regionof IGF-I.

4. Conclusion

Meat production of Turkey cannot meet the demand. Therefore, priority should be given to breeding studies aimed forincreasing the meat yield of the existing animals. The relationship between the meat yield and IGF-I genes in sheep has been demonstrated by many studies in the world. Turkey doesn't have enough information about this gene region. In this study, considering the lack of information on some of the sheep breeds raised in Turkey we havetried to reveal polymorphism of the IGF-I gene. There are very few studies in sheep breeds of Turkey on this subject. Therefore, this study in terms of revealing the genotypes for the IGF-I gene in the Turkish sheep populations is a pioneer study. When the genotypes have been determined, it will be possible to select them by using this gene region in the sheep by revealing the relationship between the sequence studies and the yield.

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Determination of damage Rate of Sunn pest (*Eurygaster* spp.) and Wheat Sting Bug (*Aelia* spp.) in Some Bread Wheat Varieties in Konya, Turkey

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ABSTRACT

This study was carried out in order to determine the damage rate of Sunn pest (*Eurygaster* spp.) and Wheat sting bug (*Aelia* spp.)in bread wheat varieties which brought to Konya Commodity Exchange and grown in 33 neighborhoods of Karatay which is the central district of Konya province of Turkey, during 2014-2018. Samples were taken homogeneously with an automatic probe device in according with the ISO 24333 Sampling Standard. Analysis samples were taken from these samples in according with TS 2974Wheat Standard and were cleaned and sieved from foreign materials. The grains that have been damaged by Sunn pest (*Eurygaster* spp.) and Wheat sting bug (*Aelia* spp.)damage have been determined, selected and weighed, and the damage rate of the grains by mass was calculated. Protein and hectolire values were also determined by measuring the remaining sample on the device working with the Near Infrared Transmission (NIT) principle. As a result of the research; in harvest seasons between 2014 and 2018, the average of Sunnpests damage in bread wheat coming from all districts of Karatay District were 1.1%, 0.8%, 0.9%, 0.9% and 1.1%, respectively. It was observed that the aveage of Sunn pest (*Eurygaster* spp.) and Wheat sting bug (*Aelia* spp.)damage rate ranged between 0.8-1.5%, protein averages ranged between 12.7-14.1% and hectoliter weights between 76.8-79.4 kg in 2014-2018 harvest seasons.

1. Introduction

Agriculture is a strategic sector that has maintained its importance throughout the history of humanity and has been increasing itsimportance day by day. People are involved in agriculture for centuries to meet their nutritional requirements.Cereals constitute one of the most basic foodstuffs that plays a role both in human nutrition and animal nutrition.

Wheat has a special place among the grains, as it has a unique feature unlike other available grains and is the main raw material used in the production of many bakery products, especially bread. Among the cereal crops, wheat ranks 1st in various countries of the world in terms of cultivation area and production and same pattern is present in Turkey as well. It is an important culture plant due to its easy cultivation, its suitability for conversion to a wide variety of foods and its role in nutrition (Anonymous, 2009).

According to the 2018/2019 production reports, Europe shared 19% of the total wheatproductionand gained 1st place in world, followed by China with 18% and India with 14%. Turkey shared 3% and ranked ninth in the world in wheat production (Anonymous, 2019).

Wheat can be grown in every region ofTurkey, however it is widely produced in the Central Anatolia Region. In 2017, the Central Anatolia Region ranked first with 32% share for bread wheat production. This is followed by the Marmara Region with 18% and the Southeastern Anatolia Region with 15%. The regions with the lowest production are Eastern Anatolia and Aegean Regions (Anonymous, 2018).

Konya stand 1st with a total share of10.7% in grain production in Turkey with 1.686.326tons of wheat production in the years leading state at 2020 year in Turkey. Konya alone produce 9.7% of the wheat produced in Turkey. On the basis of higher agricultural potentials, especially Çumra, Altınekin, Karapınar, Karatay, Ereğli, Kulu and Cihanbeylidistricts of Konya, stand out due to their irrigation facilities (Anonymous, 2020a).

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Variety of wheat, climate, soil characteristics, growing techniques and conditions,Sunnpest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage, classification and registration of wheat, are the main factors affecting yield and quality in wheat.Sunn pest and Wheat sting bug, the leading pests of grains in Turkey that negatively affect yield and quality before harvest (Anonymous, 2005; Duman et al., 2008;Mutlu et al., 2014; Mutlu et al., 2016; Kılıç et al., 2018; Mutlu and Karaca, 2019). Since it is impossible to distinguish between the wheat varieties damaged by Sunn pest and Wheat sting bug, the damage of the Sunn pest and Wheat sting bug is evaluated together (Lodos, 1986). The reason, grain damaged of Sunn pest and Wheat sting bugimpairs the bread quality of wheat is the proteolytic and amylolytic enzymes that these Sunn pest and Wheat sting bug leave in the grain while feeding from the wheat. These enzymes pass into flour during wheat processing and break down proteins during dough formation (Lorenz & Meredith, 1988; Karababa&Ozan, 1988; Türker, 2002).

Bilici (2013) reported that the rate of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage was below 1.5% in the wheat varieties most traded in Konya Commodity Exchange in 2011-2013 harvest seasons. Hüdaverdi and Muştu (2018) reported that the average damage rate of Sunn pest and Wheat sting bug were 0.80% in the wheat cultivars grown in Edirne Province in 2017. Özbek and Fidan (2013) reported that among the disease / pest factors affecting the price most for wheat coming to the Konya Commodity Exchange during the harvest season of 2010 was the Sunn pest and Wheat sting bug damage.

Quality and classification of bread wheat are made to evaluate the physical analysis results according to the quality criteria specified in the wheat Purchase Scale published by the Turkish Grain Board. Bread wheat divided into several groups according to the color, hardness and quality of wheat in the TMO Scale. Minimum prices are determined in Turkish lira (TL) per tonne according to these groups and these minimum prices which are determined every year, are published in the scales by the TMO. The price of a bread wheat is according to the determined group and quality criteria determined according to the physical analysis result. Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.)infesting rate, protein value and hectolitre weight have a very important place in terms of affecting the price within these quality criteria. Price formation in sales transactions is also made ac-cording to these determined quality criterias.

The reason for choosing Karatay District (Konya) to determine to wheat damage rates of the Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.)in this study was that it has a high agricultural potential and it has the highest transaction volume in Konya Commodity Exchange.

2. Materials and Methods

In the study, bread wheats were brought to Konya Commodity Exchange from the neighborhoods of Karatay District of Konya province weresampled during in the harvest seasons of 2014-2018.subregions werecreatedfrom 33 neighborhoods (three groups with5 neighborhood sand three other groups with 6 neighborhoods were assembled) according to their proximity to the center and toeach other and information about the groups is given in Table 1. Neighborhoods are listed alphabetically among their groups and the locations map of the neighborhoods that make up the sub regions are given in Figure 1.

Physical analysis of wheat samples was carried out in according with TS 2974 Wheat Standard in order to determinethe Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage ratio and protein and hectolitre weight.

3-kg samples were taken homogeneously from the bread wheat with an automatic probe device in according with ISO 24333 Sampling Standard. Then 1 kg sub-sample was taken homogeneously from the sample of 3 kg with conical sample divider. This 1 kg sample was sieved with the relevant sieves and cleaned from foreign materials and 50 g of wheat samples required for homogeneous analysis were seperated. This 50 g of analysis sample was first spread on a flat surface and all the Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damaged grains were selected by naked eye and separated with the help of analysis forceps, weighed on a sensitive scale and the result was multiplied by 2 and the ratio of damaged grain to mass was determined as percentage. In order to determine the protein and hectolitre weights of the remaining sample, the sample was poured into the receptacle of the measuring device working on the principle of Near Infrared Transmission (NIT), and protein and hectolitre weights were measured. The data obtained as a result of all physical analyzes were recorded and evaluations were made according to these data.

3.Results and Discussion

This study was carried out in Konya Commodity Exchange, in Karatay District (Konya), which has the highest transaction volume covering 2014-2018 harvest seasons, aiming to determine the rate of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage in the bread wheats brought and how this damage affects the protein and hectolitre value of wheat. The results of physical analysis was performed in bread wheat during the 2014-2018 harvest seasons are shown in Tables 2, 3, 4, 5 and 6, respectively.

Table 1
Karatay District Neighborhoods Sub Regions

	Sub Regions					
	I	II	III	IV	V	VI
Neighborhood Name	Bakırtolu	Acıdort	Göçü	Akbaş	Akörenkışla	Başgötüren
	Erler	Çengilti	Hayıroğlu	İpekler	Aksaklı	Köseali
	Sakyatan	Divanlar	İsmil	Karadona	Beşağıl	Obruk
	Saraçoğlu	Karakaya	Ovakavağı	Katrancı	Büyükburnak	Sürüç
	Şatır	Ortakonak	Yarma	Kızören	Yağlıbayat	Yavşankuyu
	Tatlıcak	Zincirli			Yenikent	

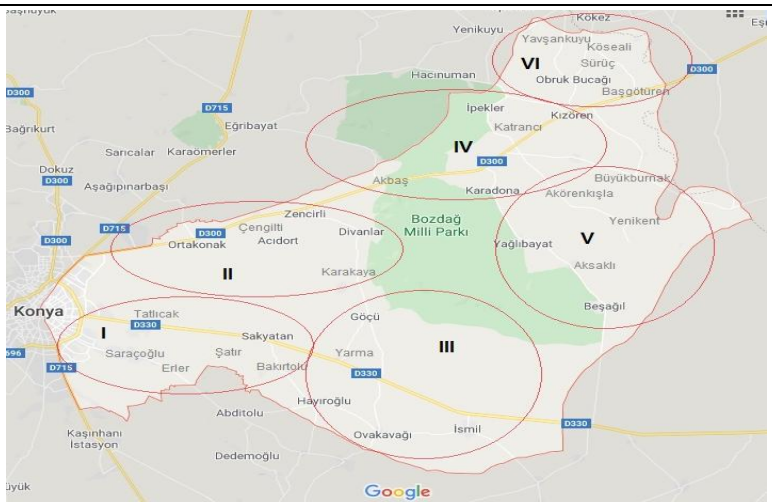

Figure 1.
Karatay District Map Neighborhood Sub Regions (Anonymous, 2020b)

Table 2
Physical analysis results of Wheat samples in Karatay District in 2014 harvest season

Year	Neighborhood Sub Regions	Total Amount (Ton)	Incoming Product (Number)	Sunn pest and Wheat sting bug Loss Rate Average (%)	Protein Value Average (%)	Hectoliter Weight Average (kg)
2014	I. Sub Regions	12.930.163	701	1.11	12.1	80.1
	II. Sub Regions	10.750.497	691	1.34	12.9	79.5
	III. Sub Regions	18.935.107	1.067	1.15	12.4	80.2
	IV. Sub Regions	6.773.730	412	1.04	13.5	78.7
	V. Sub Regions	18.061.815	1.142	0.98	13.5	78.6
	VI. Sub Regions	11.544.482	644	0.93	13.2	78.1
Average				1.09	12.9	79.2

In the year 2014, a sum of 4.657 produces from six sub regions came to the Konya Commodity Exchange. The total amount of incoming products was 78.995.794 tons. The average of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage ratio on wheat was the highest with 1.34% in the II. subregion and the lowest with 0.93% in the VI. Sub Region. The average protein value was the highest with 13.5% in the IV and V. sub regions, the lowest was with 12.1% in the I. sub region. The highest average of hectoliter weightwas with 80.2 kg in the III. subregion, the lowest was with 78.1 kg in the VI. subregion. For whole of the six subregionsin this year, the average of Sunn pest and Wheat sting bug damage ratio was 1.09%, the average protein value was 12.9% and the average hectoliter weight was 79.2 kg.

Table 3
Physical analysis results of Wheat samples in Karatay District in 2015 harvest season

Year	Neighborhood Sub Regions	Total Amount (Ton)	Incoming Product (Number)	Sunn pest and Wheat sting bug Loss Rate Average (%)	Protein Value Average (%)	Hectoliter Weight Average (kg)
2015	I. Sub Regions	15.592.122	792	0.82	11.6	79.3
	II. Sub Regions	19.296.331	1.051	0.84	11.3	79.8
	III. Sub Regions	24.462.756	1.236	0.84	12.0	79.7
	IV. Sub Regions	12.422.154	594	0.79	11.7	79.8
	V. Sub Regions	37.301.919	1.839	0.75	12.0	79.6
	VI. Sub Regions	18.390.843	863	0.72	12.2	78.0
Average				0.79	11.8	79.4

In the year 2015, a sum of 6.375 produces from six sub regions came to the Konya Commodity Exchange. The total amount of incoming products is 127.466.125 tons. The average of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.)damage ratio on wheat was the highest with 0.84% in the II and III. subregions and the lowest with 0.72% in the VI. subregion. The average proteinvalue wasthe highest with12.2% in the VI. subregion, while the lowest was with11.3% in the II. subregion. The highest average hectoliter weight was with79.8 kg in the II and IV. subregions, the lowest waswith78.0 kg in the VI. subregion. Forwhole of the six subregions total for this year, the average Sunn pest and Wheat sting bug damage ratio was 0.79%, the average protein value was11.8% and the average hectoliter weight was 79.4 kg.

Table 4
Physical analysis results of Wheat samples in Karatay District in 2016 harvest season

Year	Neighborhood SubRegions	Total Amount (Ton)	Incoming Product (Number)	Sunn pest and Wheat sting bug Loss Rate Average (%)	Protein Value Average (%)	Hectoliter Weight Average (kg)
2016	I. Sub Regions	10.926.218	608	0.94	14.0	77.4
	II. SubRegions	9.171.325	565	1.06	13.3	77.9
	III. Sub Regions	17.694.220	920	0.87	13.5	78.3
	IV. Sub Regions	5.418.625	315	0.81	15.0	76.5
	V. Sub Regions	21.346.653	1.123	0.78	14.2	77.9
	VI. Sub Regions	10.250.830	520	0.71	14.4	76.6
Average				0.86	14.1	77.4

In the year 2016, a sum of 4.051 produces from six subregions came to the Konya Commodity Exchange. The total amount of incoming products was 74.807.871tons. The average of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage ratio on wheat was the highest with 1.06% in the II. subregion and the lowest with 0.71% in the VI. subregion. The average proteinvalue wasthe highest with15.0% in the IV. subregion, while the lowest was with13.3% in the II. subregion. The highest average hectoliter weight was with78.3 kg in the III. subregion, the lowest waswith76.5 kg in the IV. subregion. Forwhole of the six subregions total for this year, the average Sunn pest and Wheat sting bug damage ratio was 0.86%, the average protein value was14.1% and the average hectoliter weight was 77.4 kg.

Table5
Physical analysis results of Wheat samples in Karatay District in 2017 harvest season

Year	Neighborhood Sub Regions	Total Amount (Ton)	Incoming Product (Number)	Sunn pest and Wheat sting bug Loss Rate Average (%)	Protein Value Average (%)	Hectoliter Weight Average (kg.)
2017	I. Sub Regions	11.658.961	561	0.96	13.1	78.2
	II. Sub Regions	11.548.958	618	1.03	12.6	78.7
	III. Sub Regions	19.159.700	920	0.86	13.5	78.0
	IV. Sub Regions	8.323.648	418	0.74	12.6	78.7
	V. Sub Regions	21.912.554	1.094	0.74	13.1	78.7
	VI. Sub Regions	11.248.507	507	0.73	13.0	77.6
Average				0.84	13.0	78.3

In the year 2017, a sum of 4.118produces from six sub regions came to the Konya Commodity Exchange. The total amount of incoming products was 83.852.328 tons. The average of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage ratio on wheat was the highest with 1.03% in the II. subregionand the lowest with 0.73% in the VI. subregion. The average proteinvalue wasthe highest with13.5% in the III. subregion, while the lowest was with12.6% in the II. and VI. subregions. The highest average hectoliter weight was with 78.7 kg in the II. IV. V. sub regions, the lowest waswith77.6 kg in the VI. sub region. Forwhole of the six subregions total for this year, the averageSunn pest and Wheat sting bug damage ratio was 0.84%, the average protein value was13.0% and the average hectoliter weight was 78.3 kg.

Table 6
Physical analysis results of Wheat samples in Karatay District in 2018 harvest season

Year	Neighborhood Sub Regions	Total Amount (Ton)	Incoming Product (Number)	Sunn pest and Wheat sting bug Loss Rate Avarage (%)	Protein Value Average (%)	Hectoliter Weight Average (kg)
2018	I. Sub Regions	6.835.908	328	1.10	12.6	76.3
	II. Sub Regions	8.940.480	460	1.49	12.5	77.0
	III. Sub Regions	17.062.935	795	1.10	12.8	77.5
	IV. Sub Regions	7.654.070	350	1.03	12.9	77.0
	V. Sub Regions	24.868.084	1160	0.94	12.6	77.1
	VI. Sub Regions	13.070.851	551	0.90	12.9	76.1
	Average			1.09	12.7	76.8

In the year 2018, a sum of 3.644 produces from six subregions came to the Konya Commodity Exchange. The total amount of incoming products was 78.432.328 tons. The average of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage ratio on wheat was the highest with 1.49% in the II. subregion and the lowest with 0.90% in the VI. subregion. The average protein value was the highest with 12.9% in the IV and VI. subregions, while the lowest was with 12.5% in the II. subregion. The highest average hectoliter weight was with 77.5 kg in the III. subregion, the lowest was with 76.1 kg in the VI. subregion. For whole of the six subregions total for this year, the average Sunn pest and Wheat sting bug damage ratio was 1.09%, the average protein value was 12.7% and the average hectoliter weight was 76.8 kg.

In a study conducted by Bilici (2013), during the harvest seasons of 2011-2013in the five districts of Konya province, Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damaged grain ratio, protein and hectolitre weights had been determined and reported. The average Sunn pest and Wheat sting bugdamage on wheat from all districts were 1.12% in 2011, 1.35% in 2012 and 1.13% in 2013. In the 2011-2013 harvest seasons. Bilici (2013) also reported the pest damage intensity was higher in 2012 compared to other years, and in the all wheat varieties selected from all districts average of Sunn pest and Wheat sting bug damage was below 1.50%, protein averages were 11.0% and hectoliter weights above 79.0%. As a result of analyzes carried out in bread wheat varieties in Karatay district, the sucked(damaged?) grain ratio of Sunn pest and Wheat sting bug reported to vary between 0.93% and 2.09%.

In the study of Özbek and Fidan (2013), according to the results of the data obtained from Konya Commodity Exchange reported that among the disease / pest factorsthe Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage was the most affecting factors determining the price in wheat varieties harvested in 2010. The results of the analysis indicated that 98.50% of 666 wheat samples analyzed were found to be damaged by Sunn pest and Wheat sting bug and as a result 61.26% of the samples had price reduction compared to the Wheat Grain Office (TMO) wheat purchase scale. They reported that the average rate of destruction in wheat samples with Sunn pest and Wheat sting bug damage was 1.43%, and this rate was low as the price reduction rate compared to the TMO wheat purchase scale and had an effect of a 0.5% decrease in the price.

Hüdaverdi and Muştu (2018), reported that there were 42 wheat varieties grown in 38 villages in the central district of Edirne Province and 78 wheat varieties cultivated by the Thrace Agricultural Research Institute in Edirneandthe average of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage in wheat varieties coming from Edirne Commodity Exchange from the villages of the central district of the city determined as 0.80% in the harvest season of 2017. They also reported that the highest average damage ratio was in the village of Budakdoğanca with 1.25% and the ratio of damaged grain in bread wheat varieties taken from Trakya Agricultural Research Institute trials ranged between 0.20-3.04%.

In the current study, Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage determinedto be 1.09%, 0.79%, 0.86%, 0.84% and 1.09%,fortheharvestseasons of 2014 to 2018, respectively. Compared to theother years, the lowest loss rate was in 2015 and the highest loss rate was in 2014 and 2018. Looking at thecumulativedata, the average of Sunn pest and Wheat sting bug damage ratio of all neighborhoods were below 1.00%, protein averages were 12.5% and hectoliter weight averages were 78.0.

In a study conducted by Bilici (2013), as a re-sult of the physical analysis of bread wheats from Karatay District, the rate of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage was determined as 0.93-2.09% in the 2011-2013 harvest seasons. In this study in the 2014-2018 seasons, the rate of Sunn pest and Wheat sting bug damage was observed as 0.71-1.49%. When as a result the two studies were compared that had been determined there was a decrease the rate of Sunn pest and Wheat sting bug in the harvest seasons 2014-2018 according to 2011-2013. In the study conducted by Bilici (2013), in wheats grown in Konya in 2010 and coming to Konya

Commodity Exchange, the average Sunn pestand Wheat sting bug damage rate was 1.43%, in the wheats coming from Karatay District in the 2011-2013 harvest period the average of the damage rate was below 1.50%. In this study the average Sunn pest and Wheat sting bug damage rate was determined to be below 1.00% during the 2014-2018 harvest seasons.

Hüdaverdi and Muştu (2018), in Edirne, it was determined that the average of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage ratio was 0.80% in 2017. Compared this two study, the loss rate of sunn pests in Edirne is lower than the average of sunn pests determined in Karatay (Konya) district in 2014-2018 harvest seasons. While the highest Sunn pest and Wheat sting bug damage ratio was 1.25% in Edirne, the highest damage rate was determined as 1.49% during the five harvest seasons in Karatay District. The degree and shape of the Sunn pest and Wheat sting bug damage can vary depending on the biological periods (nymph and adult) and density of the pest, the variety and phenological period of the grain, climatic conditions (especially temperature and precipitation) (Özkan&Barbaroğlu, 2015). It is thought that the low damage rate in Edirne Province was resulted in by climate conditions, selection of resistant varieties, and better irrigation facilities. In Konya Province, it is thought that low rainfall and high temperatures caused by climate conditions caused high damage rates.

Table 7 showed how the Sunn pest and Wheat sting bug loss affects the price of wheat during the harvest seasons between 2014-2018 only in Karatay (Konya) District..

Table 7

The effect of Sunn pest and Wheat sting bug damage on price in 2014-2018 harvest seasons only in Karatay(Konya) District

Year	Total Amount (Tons)	Sunn pest and Wheat sting bug Loss Rate Average (%)	Unit Price Discount Rate (TL/Ton)	Total Price Discount Rate (TL/Ton)
2014	78.995.794	1.09	- 1.0 TL	- 861.054 TL
2015	127.466.125	0.79	-	-
2016	74.807.871	0.86	-	-
2017	83.852.328	0.84	-	-
2018	78.432.328	1.09	- 1.0 TL	- 857.626 TL

According to the 2018 Wheat Purchase Scale, if Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage ratio is between 3.01-3.50%, the price was cut up to 8.0 TL per tonne, the most important criter effecting discounted price. This damage rate is a reason for a serious price decrease and economic loss. For this reason, over the years, great importance has been given to the fight against Sunn pest and Wheat sting bug and quality and product losses have been tried to be minimized. During the five harvest seasons, the average of Sunn pest and Wheat sting bug damage was determined as 0.94% in all neighborhoods of Karatay District. It has been determined that this value, according to the scale, can be considered to be below the limit of low quality or that the wheat cannot be purchased (3.50%), and it does not cause a price reduction.

In the harvest seasons between 2014 and 2018; the average protein value of bread wheat from all neighborhoods was determined to be 12.9%, and this value was found to be above the limit to be discounted. The average protein value had been the highest at 14.1% in 2016, the lowest at 11.8% in 2015. The average weights of hectoliter were determined as 78.2 kg and it was determined that discount of price was above the limit to be applied. Hectoliter weight average had been determined the highest was 79.4 kg in 2015 and the lowest was 76.8 kg in 2018.

In the current study, Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage was observed in bread wheat from all neighborhoods in the harvest seasons between 2014 and 2018; and the average damage rate was 0.94%. When the neighborhoods of Karatay District are compared; the minimum rate of damage during the five harvest seasons was found to be in the Sub Region VI and ranged from 0.71% to 0.93%. During the five harvesting seasons, the highest rate of Sunn pest and Wheat sting bug damage rate was insub region II. and varies between 0.84% and 1.49%. In these regions, besides the Sunn pest and Wheat sting bug damage; wheat variety cultivated, soil structure, irrigation and cultivation techniques are other factors that affect quality of the production.

In Karatay District, the fact that the rate of Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage was found to be below the economic tolerance level shows that it is a district with high quality production. For many years, Sunn pest and Wheat sting bug are known to cause to significant yield and quality losses almost all wheat producing areas in Turkey As a result of the transfer of the information obtained through research in different regions of Turkey, seems to have helped in the control ofSunn pest and Wheat sting bug Mostly, it is aimed to protect the natural balance and increase the number of the natural enemies of the Sunn pest and Wheat sting bugand to reduce the

losses caused by the pests in the areas where these pests are fought.

As a result, efficiency and quality increase in recent years, with biological control and beneficial insect release, state policies and training studies for farmers have been determined to reduce the Sunn pest (*Eurygaster*spp.) and Wheat sting bug (*Aelia* spp.) damage to a great extent. Biological control is given more importance than chemical control. Nowadays, it is seen that Turkish farmers are also conscious of higher quality production and thus higher quality production is made.

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Seed Yield and Characteristics in a Half-Diallel Pumpkin Population

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ABSTRACT

In addition to fresh and roasted pumpkin seeds used in human nutrition, they are used as an additive to bread, salami, sausage, mayonnaise and many food products because of their high protein content. The most common problem encountered in the cultivation of confectionary pumpkin is the lack of varieties with good seed yield and quality in the market. In this study, it was aimed to reveal the promising hybrids with superior characteristics by determining the yield and seed characteristics of the 13 pumpkin inbred lines (*Cucurita pepo*) and 74 hybrid lines which are obtained by crossing between inbred lines and two local varieties (3-Hatun Tirnagi and 4-Cercevelik) as control. As a result of the study, the highest positive correlation was found between seed thickness and 1000 seed weight and between seed length and seed width. The crosses of 31x34, 23x28, 13x23, 38x40, 29x37, 30x31 and 23x29, especially 40x29, in the positive region of both components showed superior performance compared to their parents in all parameters. These hybrids have emerged as promising crosses to develop the F_1 hybrid confectionary pumpkin varieties.

1. Introduction

The Cucurbitaceae family includes important species such as melon, watermelon and pumpkin, which have economic importance in the world. Species included in the family differ greatly in aspects such as plant characteristics, fruit and seed structure. Pumpkin fruits, one of the important species of the family, are used as fresh consumption and making desserts, as well as mature seeds, are used in human nutrition.

A total of 27.6 million tons of squash is produced on an area of approximately 3 million ha in the world. China (8 million tons) takes the first place in this production, while India (5.5 million tons), Ukraine (1.3 million tons) and Russia (1.1 million tons) are important producer countries. Turkey meets 2.23% of world production and in eighth place with a production of about 0.6 million tons (FAO, 2018). In the confectionary pumpkin, Turkey production is 50.265 tons at 706.894 da area. The provinces with the highest production are Kayseri (16 706 tons), Nevşehir (16 673 tons), Aksaray (4 849 tons), Konya (4 468 tons) and Eskişehir (2 598 tons) (TUİK, 2019).

In addition to fresh and roasted pumpkin seeds used in human nutrition, they are used as an additive to bread, salami, sausage, mayonnaise and many food products because of their high protein content (Rangahau, 2002). On the other hand, it has medical uses in terms of human health. Some researchers reported that it improves the immune system (Chew and Park, 2004), reduces the risk of stomach, breast, lung and colon cancer (Stevenson et al., 2007), plays an important role in lowering cholesterol levels and treating advanced prostate utilizing phytosterols (Hong et al. al., 2009). Pumpkin seeds are among the oilseeds with 28-40% protein (Achu et al., 2005) as well as 35-50% oil content (Seymen et al., 2016; Türkmen et al., 2015). Besides, it is rich in minerals such as potassium, phosphorus, calcium, magnesium and iron, which are important in human nutrition (Seymen et al., 2016), and is known as a good source of vitamins A, C and E (Eleiwa et al., 2014).

Production is increasing day by day because confectionary pumpkin farming can be done mechanically in large areas, yielded in less irrigated semi-arid regions, there is no storage problem and it is more profitable than some agricultural products in some regions. However, the most common problem encountered in cultivation is the lack of varieties with good seed yield and quality in the market. The way to produce high-yield and quality seeds is a variety of breeding studies. In the breeding studies, heterosis has been applied to many species and varieties with high commercial value have been developed (Gergerli et al., 2018). Although heterosis occurs in different plant species, it is seen at

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different rates from species to species. In general, heterosis is higher in open-pollinated plants such as squash compared to self-pollinated plants. Many methods are used to determine heterosis. One of these methods is principal component analysis (PCA) and thus superior hybrids can be revealed (Chahal and Gosal, 2002).

In this context, it is aimed to determine the yield and seed characteristics of 13 confectionary pumpkin inbred lines selfed at S7 stage, 74 hybrid lines obtained by half-dial hybridization between these inbred lines and 2 local varieties (3-Hatun Tirnagi and 4-Cercevelik) and put forward the promising candidates with superior features

2. Materials and Methods

The study was conducted at the research area of Selcuk University, Faculty of Agriculture for two years. 13 ibred lines (6, 9, 13, 17, 23, 29, 30, 31, 34, 37, 38, 40 and 41) which are collected from different parts of Turkey, selfed and their purities are determined by the molecular test used as plant material.

In the first year, the seeds were planted at 1x0.5 m distances on 13 May 2019. Sixty plants were cultivated from each inbred line for crossing and selfing without repetition. In the flowering time, crosses were made according to the half-diallel hybrid program, as well as selfing was realized in inbred lines to the production of the seeds of the parents. As a result of the cross-breeding, 74 confectionary pumpkin hybrid candidates were obtained. During the experimental year, cultural practices such as fertilization, irrigation, and disease and pest management were made regularly and timely. The fruits obtained by selfing and crossing were harvested on September 16, 2019, and seeds were extracted individually and dried.

In the second year, with 74 hybrids, 13 parent lines, 2 local cultivars (3-Hatun Tirnagi and 4-Cercevelik) with the highest cultivation in the market and have different seed characteristics were used as plant material. On May 15, 2020, seed sowing was made with a total of 15 plants from each genotype at a distance of 1x0.5 m without repeating. Cultural processes were carried out regularly in plants until harvest. The seeds of the fruits harvested on September 14, 2020, were extracted individually and dried.

After the seeds dried, the parcel yields were determined and the seed yield was expressed as g / plant according to the number of plants in each plot. The total number of fruits in the parcel was divided by the number of plants in the parcel and the number of fruits per plant was determined. 100 seeds from each plot were counted and weighed on precision scales and the weight of 100 seeds was calculated as g. The length, width and thickness of 10 seeds from each parcel were measured with a digital calliper and the average was taken and the seed length, width and thickness were determined.

PCA analysis was carried out to evaluate the seed yield and quality measurements taken from the parents and crosses of confectionary pumpkins and reveal the superiority of the hybrid lines. As a result of the multiple comparison test performed with the JMP 14 statistics program, the Loading plot and Score plot graphs were drawn and the parent and hybrid lines were interpreted.

3. Results and Discussion

The average seed yield was 73.43 g/plant. Among the hybrids, 40x29 (167.0 g), 9x41 (160.05 g) and 31x34 (149.2 g) gave the highest seed yield (Table 1). Turkmen et al. (2016) reported that the average seed yield of 81 different genotypes in confectionary pumpkins was 114 g / plant and the highest yield was 226 g / plant. In another study conducted in Turkey, seed yield ranged from 98 to 107 kg (Ünlükara and Bakır, 2018). Our results are following previous reports and the yields of hybrid lines were found to be above the country average. When the number of fruits per plant was examined, an average of 1.12 fruits was obtained. The number of fruits in confectionary pumpkin is directly correlated with the yield and generally, 1 fruit is obtained per plant. 9x41 hybrids had the highest number of fruits per plant with 2.5, 6x29 and 40x29 hybrids with 2 fruits (Table 1). Turgut (2015) found an average of 1.96, Seymen et al. (2012) and Yegül (2007) reported 1.2 and 1.45 fruit per plant, respectively. The high number of fruits obtained from hybrids has made significant contributions to the yield.

An average of 1000 seeds weight was 226.58 g. The highest 1000 seed weights were obtained from 31x41 (378 g), 34x41 (368 g) and 40x30 (360 g) hybrids (Table 1). 1000 seed weights were reported 134 g (Warid et al., 1993), 203 g (Joshi et al., 1993) and 178 g (Türkmen et al., 2014). The high 1000 seed weights in our study are thought to be due to heterosis effect and well designed a fertilizing program. The average seed length was measured as 20.25 mm, and the highest seed lengths were obtained from 37x38 (23.68 mm) and 31x37 (23.56 mm) hybrids. The average seed width was 10.84 mm and the highest values were obtained from 13x41 (13.61 mm), 40x23 (13.48 mm), 17x38 (13.36 mm) and 38x40 (13.00 mm) hybrids (Table 1). The average seed thickness was 2.94 mm and the highest value was obtained as 4.13 mm from inbred line 29. In different studies, it has been reported that the seed lengths are 20.05 mm (Türkmen et al., 2016) and 16.91 mm (Joshi et al., 1993). Seed widths were reported to vary between 8.78-10.73 mm (Ermiş, 2010). Seed thickness varied between 3.20-4.32 mm (Paris and Nerson, 2003). Our results following the previous reports, and the seed size changes according to the seed structure in confectionary pumpkin seeds and seed shape directly affects the ease of cracking.

Table 1
Seed yield and characteristics of inbred and crosses pumpkins

Inbred line/Crosses	SY	NF	TSW	SL	SW	ST
3	63.13	1.25	198	21.35	8.33	3.23
4	74.52	1.00	204	20.34	9.87	3.01
6	49.65	1.00	196	20.37	9.56	2.76
9	55.48	1.50	202	18.64	8.92	3.02
13	54.43	1.00	162	18.56	9.77	2.79
17	61.87	1.00	288	20.19	11.63	3.11
23	79.50	1.00	210	20.11	10.34	3.11
29	85.97	1.67	198	16.77	8.99	4.13
30	44.30	1.00	212	21.23	11.09	2.33
31	42.83	1.00	246	22.71	11.66	3.17
34	11.80	1.00	64	18.75	9.84	1.95
37	58.95	1.00	240	21.26	10.66	3.66
38	28.33	1.00	170	19.91	10.69	2.67
40	27.80	1.00	218	19.69	10.68	3.03
41	51.05	1.00	220	18.83	11.32	2.97
6x13	44.70	1.00	156	18.50	9.25	2.45
6x17	74.70	1.33	238	20.62	10.01	2.40
6x23	80.60	1.00	282	22.05	11.08	3.39
6x29	109.9	2.00	190	17.62	9.63	2.40
6x34	54.23	1.00	162	19.38	10.08	2.36
6x37	69.07	1.00	224	18.67	10.57	2.59
6x41	46.10	1.00	208	19.57	10.41	2.55
9x13	88.07	1.33	198	18.55	10.35	3.17
9x17	75.45	1.00	218	19.32	10.79	3.10
9x23	46.17	1.00	158	18.43	9.07	3.36
9x29	88.70	1.33	234	19.18	10.22	2.84
9x30	94.80	1.00	282	18.37	10.32	3.62
9x31	106.00	1.00	260	21.86	10.88	3.37
9x34	55.93	1.00	186	20.61	10.67	3.53
9x41	160.05	2.50	236	18.85	9.71	2.87
13x17	97.05	1.00	286	20.35	10.87	3.34
13x23	125.97	1.33	238	20.97	10.95	3.15
13x29	84.17	1.00	240	22.26	12.39	3.18
13x30	93.00	1.00	220	19.75	10.27	3.08
13x34	118.30	1.00	284	19.53	10.75	2.59
13x38	71.77	1.33	242	20.94	11.49	2.66
13x41	79.73	1.00	220	22.07	13.61	3.12
17x23	45.60	1.00	156	18.98	10.68	2.24
17x29	80.80	1.00	196	18.09	10.26	2.97
17x30	37.75	1.00	204	18.94	11.25	3.29
17x34	38.35	1.00	126	17.88	9.40	2.24
17x38	104.45	1.00	316	23.49	13.36	3.33
17x40	98.25	1.00	278	21.26	11.13	3.25
17x41	37.15	1.00	166	17.59	9.76	2.55
23x29	105.30	1.50	206	21.43	12.11	2.83
23x30	76.03	1.00	268	21.65	11.36	3.01
23x31	70.10	1.00	202	22.61	10.23	2.97
23x34	41.25	1.00	186	20.16	9.95	2.57
23x38	135.10	1.50	254	20.41	11.31	2.84
23x41	61.25	1.00	210	19.59	10.57	2.84
29x30	43.95	1.00	168	17.88	10.76	2.81
29x34	28.55	1.00	120	17.19	9.45	2.48

Table 1
Seed yield and characteristics of inbred and crosses pumpkins

29x37	108.20	1.33	280	21.79	12.37	3.36
29x38	101.10	1.00	324	21.55	12.28	3.38
29x41	58.35	1.00	174	18.62	10.19	2.42
30x6	32.20	1.00	212	20.86	11.66	2.82
30x31	94.90	1.50	220	20.33	11.61	2.91
30x34	44.65	1.00	204	20.58	11.43	2.62
30x37	46.45	1.00	182	19.85	10.45	3.12
30x41	51.60	1.00	208	19.55	10.46	3.18
31x6	68.25	1.00	230	20.37	11.08	2.66
31x13	75.97	1.00	188	19.74	9.71	2.74
31x29	61.17	1.00	188	18.93	10.07	2.92
31x34	149.20	1.50	214	20.58	10.17	3.08
31x37	102.95	1.00	308	23.56	11.24	3.15
31x38	59.50	1.00	226	21.93	12.49	2.94
31x40	66.60	1.50	184	19.83	9.59	2.82
31x41	57.50	1.00	378	18.88	12.55	3.34
34x37	36.80	1.00	192	20.63	11.18	2.81
34x38	25.85	1.00	134	20.15	11.81	2.31
34x40	25.25	1.00	146	18.96	10.03	2.21
34x41	128.40	1.00	368	23.28	13.15	3.32
37x9	85.65	1.00	252	20.37	10.46	2.94
37x13	88.55	1.00	240	15.62	11.12	2.69
37x17	43.40	1.00	174	18.60	9.26	2.86
37x23	77.20	1.00	340	23.44	11.35	3.47
37x38	86.67	1.00	342	23.68	11.88	3.54
37x40	104.50	1.00	306	22.05	11.31	3.32
38x6	108.25	1.00	310	22.05	11.39	2.92
38x9	66.97	1.00	228	22.32	11.65	2.50
38x40	115.63	1.67	256	23.45	13.00	2.46
38x41	50.80	1.00	182	19.52	10.57	2.29
40x6	85.35	1.00	308	21.52	10.78	3.33
40x9	90.53	1.67	188	19.07	10.36	2.58
40x13	56.40	1.00	276	21.28	11.69	2.99
40x23	46.80	1.00	266	22.08	13.48	3.73
40x29	167.00	2.00	220	19.22	11.17	3.15
40x30	97.80	1.00	360	23.23	12.17	3.70
40x41	110.80	1.00	312	21.56	11.54	2.93
Mean	73.43	1.12	226.58	20.25	10.84	2.94

Seed yield-g/plant (SY); Number of fruit-number/plant (NF); 1000 seed weight-g (TSW); Seed length-mm (SL); Seed width-mm (SW); Seed thickness-mm (ST).

PCA was made with yield and seed properties obtained from different inbred lines and hybrids (Table 2). As a result of the PCA. the study was explained in two components. and it had a rate of 72.82%. It has been reported that PCA analyzes were used in different studies and the study was strongly explained by PCA (Kamrani et al., 2018; Mozafari et a, 2019; Seymen et al., 2019; Yavuz et al., 2020; Seymen, 2021). As a result of PCA, the first component (PC1) explained 47.41% of the study, and the SY, TSW, SL, SW and ST were the most positively explained parameters. The second component (PC2) explained 25.41% of the study, and the SY and NF parameters were strong and positive parameters.

Using PC1 and PC2 components, a loading plot chart was created to examine the correlative relationship between seed yield and characteristics (Figure 1). It has been reported that there is a positive relationship if the angle between the vectors in the figure is $<90^o$, there is a negative relation if the angle is$> 90^o$, and if the angle between the vectors is 90^o, there is no significant relationship (Yan and Kang, 2003; Yavuz et al., 2020). When the figure is examined, the highest positive relationship was seen between ST and TSW and SL and SW. Likewise, a score plot graph was created using PC1 and PC2 components to evaluate the seed yield and characteristics of confectionary pumpkin inbred lines and hybrids (Figure 2). In the figure, 34x41, 40x30, 17x38 and 37x38 hybrids located in the positive direction of PC1 have emerged as the best hybrids in terms of the parameters explained in PC1. The 40x29 and 6x29 hybrids were the best hybrids in

terms of the parameters described in PC2 and located in the positive region of PC2. The hybrids 40x29, 31x34, 23x28, 13x23, 38x40, 29x37, 30x31 and 23x29, which are prominent in terms of seed yield and characteristics in the positive region of both components, performed better results than their parents.

Table 2
PCA results regarding of seed yield and characteristics of inbred and crosses pumpkins

Items	PC1	PC2
Eigenvalue	2.84	1.52
Percentage of variance	47.41	25.41
Cumulative variance	47.41	72.82
Eigenvectors		
SY	0.37000	0.56330
NF	0.01542	0.75056
TSW	0.54147	-0.02025
SL	0.46443	-0.23490
SW	0.44385	-0.25087
ST	0.39619	0.02882

Principle component (PC); Seed yield (SY); Number of fruit (NF); 1000 seed weight (TSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

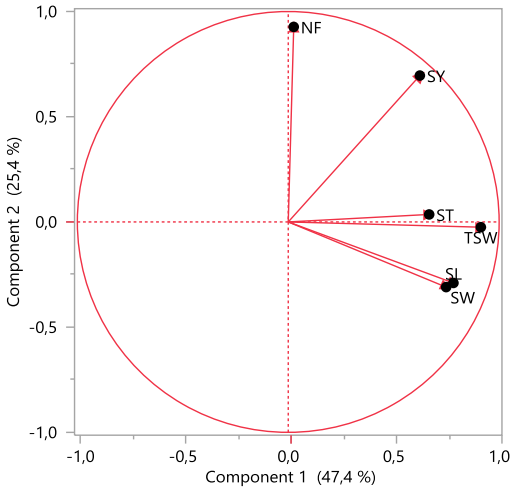

Figure 1
Loading plot based on PC 1 and 2 obtained from PCA using seed yield and characteristics of inbred and crosses pumpkins.

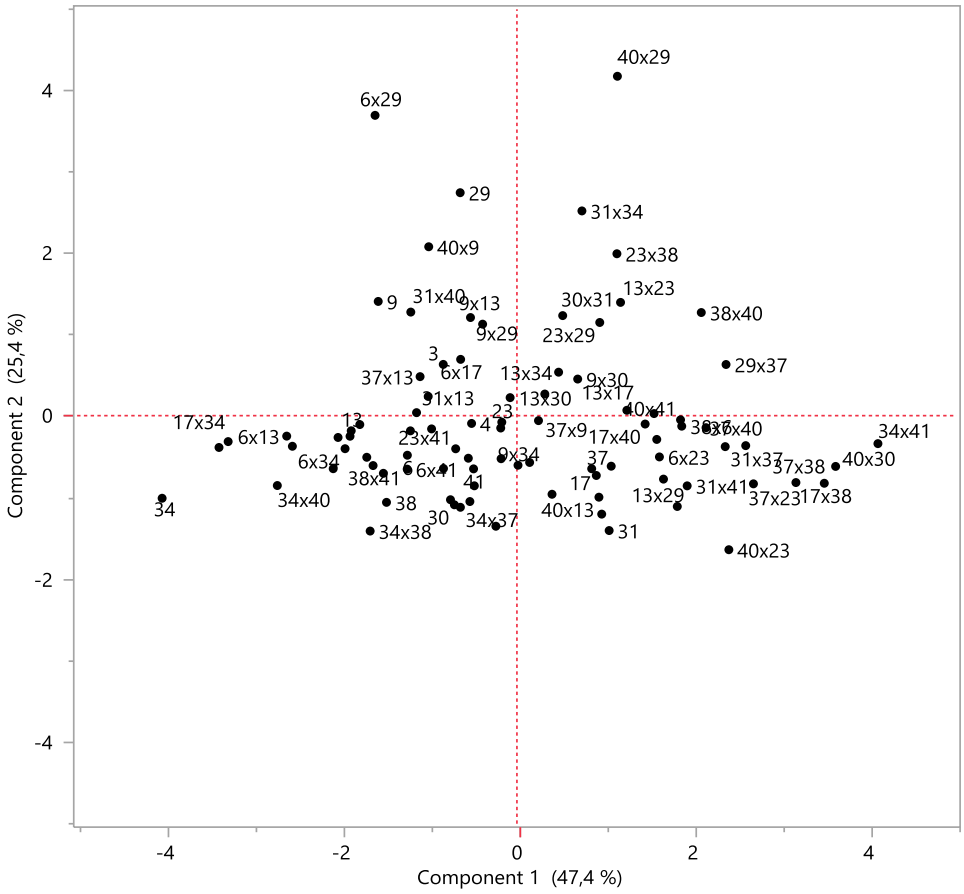

Figure 2
Score plot based on components 1 and 2 obtained from PCA using seed yield and characteristics of inbred and crosses pumpkins.

4. Conclusion

In the study conducted to determine the hybrids that show heterosis effects in terms of seed yield and properties in confectionary pumpkins. it has been revealed that some hybrids show superior traits than rootstocks. As a result of the PCA, all parameters are explained in the study described in two components. The highest positive correlation between the parameters was found between seed thickness (ST) and 1000 seed weight (TSW) and between seed length (SL) and width (SW). The crosses of 31x34, 23x28, 13x23, 38x40, 29x37, 30x31 and 23x29, especially 40x29, in the positive region of both components showed superior performance compared to their parents in all parameters. These hybrids have emerged as promising to develop the F_1 hybrid confectionary pumpkin variety. Determining the performance of these hybrids in larger trial fields by reproducing will give clearer results and give more clear information in the development of F_1 varieties.

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Environmental Mitigation Through Irrigation Management in Sugar Beet Production

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ABSTRACT

This study assesses the greenhouse gases (GHG) emissions of sugar beet production under different irrigation and nitrogen fertilizing strategies. This manuscript is an evaluation of the production inputs used in a research carried previous on sugar beet and its conversion into GHG emissions equivalent. This paper evaluates the potential for environmental mitigation, including the reduction of total GHG emissions from agricultural inputs in sugar beet production by managing irrigation and nitrogen fertilizing. In this context, the nine treatments based on three different irrigations (full irrigation, conventional deficit irrigation, partial root drying irrigation) and three nitrogen fertilization strategies (full nitrogen, partial deficit nitrogen, moderate deficit nitrogen) were assessed. The results of evaluation showed that DI-N_1 strategy can reduce irrigation water and nitrogen use up to 25% compared to control treatment (FI-N). In addition, this strategy saved 25% of electricity consumption use for irrigation. The analyse of pollution in this study led to very important findings: more environment-friendly irrigation and fertilization practices by using less water and nitrogen have a considerable potential for environmental mitigation in sugar beet production.

1. Introduction

Agriculture is both an energy user and energy supplier system. When using solar energy to produce biomass, plants capture atmospheric carbon dioxide (CO_2) as their main source of carbon. Agriculture supplies energy by growing crops that convert solar energy into biomass, which in turn supplies energy to human beings and animals. On the other hand, agriculture uses large quantities of energy inputs such as diesel fuel, electricity, fertilizer, plant protection, chemicals, machinery and human labor. Besides the energy consumption, greenhouse gases (GHG) emission and global warming potential (GWP) issues are also critical in the agricultural production systems in recent twenty years (Khoshnevisan et al. 2013). Because, greenhouse gases produced as a result of agricultural activities, enhance the natural greenhouse effect. However agricultural crops bind CO_2 from the air via the photosynthesis process, but crop production on farmer's field is also a source of the GHG emissions. Also, for each crop the CO_2 fixation is much higher than the CO_2 emissions associated with the production of the crops (Küsters 1999).

Today the agriculture sector is one of main contributors for energy consumption and GHG emissions (Barker et al. 2009; Devi et al. 2009). Each year, agriculture emits 10–12% of the total estimated GHG emissions (Niggli et al. 2009). Studies of the direct energy use of on-farm operations suggest that grounwater pumping for irrigation is one of the highest energy consumption processes (Lal 2004; Mushtaq et al. 2009; Qiu et al. 2018). On a global scale, agricultural irrigation consumes approximately 70% of the world's fresh water supply; 90% of this irrigation takes place in arid and semi-arid areas (Viala 2008). Water resources are usually scarce in these areas and irrigation often requires electric energy to pump or divert water. Therefore, agricultural irrigation consumes both water and energy (Jimenez-Bello et al. 2015). Irrigation is important for achieving high yields in arid and semi-arid regions. Globally, 17% of irrigated cropland leads to 40% of the total production (Postel 1999). Yet, irrigation is a very carbon intensive practice. Irrigated agriculture around the world relies heavily on energy resources to extract freshwater and to convey it to application sites. This is especially the case in arid and semi-arid regions, where large amounts of irrigation water are required to sustain crop production. As a result, the availability and cost of energy are among major factors influencing the economic viability of irrigated agriculture in these regions. In addition, ener-

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gy consumption for irrigation has major environmental consequences, mainly due to the emission of GHG (Khan et al. 2014; Pradeleix et al. 2015; Handa et al. 2019).

Turkey produces about 18 million ton /year sugar beet root from 320 000 ha cultivation land area. Sugar beet is grown throughout Turkey under irrigated conditions. Konya basin produces about 42% of total sugar beet production in Turkey. Sugar beet is a major commercial field crop in this region which is the largest producer of Turkey (TÜİK 2020). The Konya basin, Middle Anatolian region in Turkey, lies within a semi-arid area with annual rainfall ranging from 280 to 500 mm (average 323 mm), and is one of the most important agricultural and agro-industrial regions. Water loss by evapotranspiration is very high during the growing season in the basin. However, available water resources of Konya basin are fairly scant. Thus, water is an essential component and the single most important factor in limiting crop production in the region (Topak et al. 2008). Irrigation water for crops is obtained mainly from ground water resources (Göçmez and İşçioğlu 2004) and there are approximately 100 000 deep wells in the basin (WWF 2014). This study is undertaken for making realistic assessment of GHG emissions from groundwater irrigated sugar beet production in Konya region in Turkey and evaluate the impact of irrigation management strategies for reducing the GHG emissions.

2. Materials and Methods

This study is used the data regarding the production inputs and yields of the field treatment of a project, which carried out on sugar beet by Topak et al (2014) in Konya conditions. In the article, we evaluated the effects of irrigation techniques and nitrogen doses on GHG emissions of sugar beet production, which is not within the scope of the project. In this context, the treatments are defined based on three different irrigation techniques and three nitrogen amounts as follows:

FI-N: Full irrigation + full nitrogen.

FI-N_1: Full irrigation + 75% of full nitrogen.

FI-N_2: Full irrigation + 50% of full nitrogen.

DI-N: 75% of full irrigation + full nitrogen. (DI: Conventional deficit irrigation)

DI-N_1: 75% of full irrigation + 75% of full nitrogen.

DI-N_2: 75% of full irrigation + 50% of full nitrogen.

PRD-N: 50% of full irrigation + full nitrogen. (PRD: Partial root drying irrigation)

PRD-N_1: 50% of full irrigation + 75% of full nitrogen.

PRD-N_2: 50% of full irrigation + 50% of full nitrogen.

In the mentioned project, irrigation water was taken from the deep well adjacent to the trial field with a flow rate of 75 m^3h^{-1}. The field experiment was irrigated by drip irrigation system. Irrigation was applied when 35–40% of the available soil moisture was consumed in the 0.90-m root zone in the FI treatment during the irrigation periods. The FI treatment was designated to receive 100% replenishment of soil water depletion. Depletion was defined as the difference between the depth of water held in the root zone at field capacity and the depth of water actually held in the root zone at the time of an irrigation decision. Fertilizers were applied on the basis of soil analysis. In soil samples, soil nitrogen before sowing was determined as 57.5 kg ha^{-1}. Diammonium phosphate fertilizer (18% N, 46% P_2O_5) was applied to the soil at a rate of 200 kg ha^{-1} prior to seeding. The remaining nitrogen amounts (N: 126.5; N1:85.9; N2: 45.3 kgha^{-1}) for the treatments were applied in the form of a urea fertilizer (46% N) in four equal parts during the first four irrigation cycles using a fertigation system. Total irrigation water, nitrogen amounts and root yields related to the treatments are given in Table 1. Production inputs and quantities related to the treatments examined are given in Table 2 and Table 3. Except for beet harvester, the input data were obtained from mentioned project records. Information associated with beet harvester was taken from farmers.

Since 1990, in Konya basin, over-exploitation from the groundwater resources is present. Long-term groundwater over-exploitation has led to a continuous decline in the groundwater depth in Konya basin and the groundwater table in the plains has decreased as well as notable. This decline of the groundwater table has led to an increase in energy consumption for groundwater exploitation. Therefore, electricity consumption per m^3 of groundwater has been revised according to today's conditions and taken into account as 0.5 kWh per m^3 water.

Table 1
Yield values with the amounts of nitrogen and water applied to treatments

Treatments	Nitrogen (kg ha^{-1})	Irrigation Water (mm)	Crop water use (mm)	FRY (kg ha^{-1})	SRY (kg ha^{-1})	RDMY (kg ha^{-1})	SY (kg ha^{-1})
FI-N	162.5	851.1	961.8	93433	110951	21075	17803
FI-N_1	121.87	851.1	961.8	88715	107678	20450	17245
FI-N_2	81.25	851.1	961.8	88838	108660	20411	17405
DI -N	162.5	643.3	784.2	80818	101628	19344	16253

Table 1
Yield values with the amounts of nitrogen and water applied to treatments

DI-N_1	121.87	643.3	784.2	81653	105077	19915	16820
DI-N_2	81.25	643.3	784.2	79435	100187	18803	16028
PRD-N	162.5	435.6	588.5	66905	88733	16937	14210
PRD-N_1	121.87	435.6	588.5	65102	86870	16688	13913
PRD-N_2	81.25	435.6	588.5	66710	89266	16954	14295

FRY: Fresh root yield; RDMY: Root dry matter yield; SY: Sugar yield; SRY (Standardized Root Yield): root yield calculated according to the standard 16% sugar ratio.

Table 2
The inputs of sugarbeet production

Treatments	İnputs							
	Electricity (kWh ha^{-1})	Diesel fuel (L ha^{-1})	Nitrogen (kg ha^{-1})	Phosphorus (P_2O_5) (kg ha^{-1})	Potassium (K_2O) (kg ha^{-1})	Human Labor (h ha^{-1})	Drip system (Φ110 mm)* (m ha^{-1})	Drip system (Φ 16 mm)** (m ha^{-1})
FI-N	4255.5	105	162.5	92	70	240	120	22220
FI-N_1	4255.5	105	121.87	92	70	240	120	22220
FI-N_2	4255.5	105	81.25	92	70	240	120	22220
DI –N	3216.5	105	162.5	92	70	240	120	22220
DI-N_1	3216.5	105	121.87	92	70	240	120	22220
DI-N_2	3216.5	105	81.25	92	70	240	120	22220
PRD-N	2178	105	162.5	92	70	240	120	22220
PRD-N_1	2178	105	121.87	92	70	240	120	22220
PRD-N_2	2178	105	81.25	92	70	240	120	22220

*: Life 15 years; **: Life 7 years

Table 3
The energy input from agricultural machinery

Agricultural machinery	Machine Weight (kg)	Energy equivalent (MJ kg^{-1})	Useful life (h)	Energy equivelent (MJ h^{-1})	Working time (h ha^{-1})	Machine energy (MJ ha^{-1})
Beet harvester (6 rows)	24000	71.38*	12000	142.8	2	285.5
Tractor	3340	71.38*	16000***	14.9	8	117.7
Plow	350	49.35**	2000***	8.64	2.5	21.6
Cultivator	560	49.35**	2000***	13.8	1	9.87
Rotatil	700	49.35**	1500***	23.03	1.2	27.64
Fertilizer Spreader	100	49.35**	1200***	4.94	0.3	1.0
Sowing machine	530	49.35**	1500***	17.44	2	34.88
Row crop cultivator	430	49.35**	2000***	10.6	1	6.17
Total Machine Energy (MJ ha^{1})						512.79

*Acaroğlu ve Aksoy (2005); ** Hacıseferoğulları ve Acaroğlu (2015); ***ASAE (1999).

The required energy in farm machinery manufacturing was calculated as:

$E_M = (W_M / L_M) \times E \times T$ (MJ ha^{-1})

Where EM is the energy of the mobile and stationary mechanical power per unit area (MJ ha^{-1}); W_M is the weight of mechanical power (kg); L_M is the economic life of the mechanical power (h); E is the energy coefficient (MJ kg^{-1}); and T is the work hours per unit area per year (h ha^{-1}).

2.1. GHG emissions assessment

To determine the impact of irrigation level and nitrogen doses on environmental pollution from sugarbeet production, an assessment of GHG emissions was performed. The total GHG emissions for different treatments was obtained by calculating the emissions separately for input as fuel, electricity, human power, agricultural machinery, fertilizers, and drip system. Taking into account the different units of measurement, the GHG emissions for the total production inputs were calculated in a unified CO_2eq system using the conversion equivalents presented in Table 4.

Total GHG per hectare emissions is computed as:

$GHG_T = E \times EF_1 + D \times EF_2 + F \times EF_3 + M \times EF_4 + DS \times EF_5 + HP \times EF_6$

where:

GHG_T – total GHG emissions for irrigated sugarbeet production (kg CO_2 eq ha^{-1}),

E – electricity consumption for irrigation (kWh ha^{-1}),

EF_1– emission factor for electricity (kg CO_2 eq kWh^{-1}),

D – diesel fuel consumption for field works (L ha^{-1}),

EF_2 – emission factor for diesel fuel (kg CO_2 eq L^{-1}),

F– amount of fertilizer applied (kg ha^{-1}),

EF_3– emission factor for fertilizers (kg CO_2 eq kg^{-1}),

M – input energy for machinery use (MJ ha^{-1}),

EF_4– emission factor for machinery (kg CO_2 eq MJ^{-1}),

DS – drip irrigation system for irrigation (m ha^{-1}),

EF_5– emission factor for drip system (kg CO_2 eq m^{-1}),

HP – human power for hoeing (h ha^{-1}),

EF_6 – emission factor for human labor (kg CO_2 eq h^{-1}).

Table 4
GHG emission equivalent values of agricultural inputs

Inputs of production	Emission factor	References
Electricity	0.55 kg CO_2 eq kWh^{-1}	Dulkadiroğlu (2018)
Diesel fuel	2.76 kg CO_2 eq L^{-1}	Dyer and Desjardins (2003)
Human power	0.7 kg CO_2 eq h^{-1}	Nguyen and Hermansen (2012)
Nitrogen	7.759 kg CO_2 eq kg^{-1}	Chen et al. (2015)
P_2O_5	2.332 kg CO_2 eq kg^{-1}	Chen et al. (2015)
K_2O	0.660 kg CO_2 eq kg^{-1}	Chen et al. (2015)
Machinery	0.071 kg CO_2 eq MJ^{-1}	Dyer and Desjardins (2006)
Polyethylene (PE) production	2.51 kg CO_2 eq kg^{-1}	Bai et al (2006)
PE Φ110 mm tube	3.56 kg CO_2 eq m^{-1}	Calculated
PE Φ 16 mm tube	0.114 kg CO_2 eq m^{-1}	Calculated
Output		
Beet root (Dry matter)	0.45 kg C eq kg^{-1}	Epstein ve Bloom (2005) ;Bolinder et al(2007); Sánchez-Sastre et al (2018)

Due to the GHG emissions is based on carbon dioxide equivalent, to determine the carbon content this amount should be multiplied on ratio of carbon to carbon dioxide that it is 12/44. Moreover, for treatments, carbon (C) yield in root biomass was determined. The carbon yields of treatments per hectare is calculated as follows:

$Y_C = RDMY \times C$

where:

Y_C – carbon yield beet roots (kg ha^{-1}),

C – carbon content beet roots (%).

In order to show the results of GHG emissions, two functional units were chosen: 1 tone of product (root and sugar) and 1 ha of farmland. Therefore, specific GHG emissions (kg CO_2eq t^{-1}) and areal GHG emissions (kg CO_2eq ha^{-1}) were computed.

3. Results and Discussion

Table 5 displays the estimates of GHG emissions for different inputs used in sugarbeet production. They were calculated from the farming inputs detailed in Table 2 and Table 3 and by applying the emissions factors presented in Table 4. The GHG emissions of sugarbeet production varied under different irrigation techniques and nitrogen doses, and both root yield and GHG emissions decreased as the irrigation and nitrogen amount decreased (Table 6). Application of the deficit irrigation and reducing the nitrogen amount had a positive effect on environmental pollution based on decreasing GHG emissions. The comparison of different irrigation and nitrogen strategies in sugarbeet production showed that the highest GHG emissions (4746.6 kg CO_2eq ha^{-1}) was in the control treatment (FI-N). The lowest GHG emissions (2973 kg CO_2eq ha^{-1}) was observed under the PRD technique when %50 nitrogen deficit was used. Compared to control treatment (FI-N), the DI-N_1 treatment decreased the standardized root yield by only 5.0%. On the other hand, the GHG emissions per unit of area from DI-N_1 treatment was decreased by 18.7%, when compared to the FI-N treatment.

The results indicated that the main component of GHG emissions was electricity for irrigation. An analysis of the impact of sugarbeet cultivation on environmental pollution showed that the greatest proportion of GHG emissions was related to electricity for irrigation (from 33.2 % under PRD-N to 57% under FI-N_2) and nitrogen (from 15.3% under FI-N_2 to 42.4% under PRD-N). This results show that the GHG emissions per unit of area increased as the irrigation water and nitrogen amounts increased. Some previous studies have reported that the main components of GHG emissions were electricity for irrigation. For example, it was found this indicate was 49.6–75.4% for irrigated winter wheat production (Wang et el. 2016), 73% for irrigated sugar beet production (Yousefi et al. 2014), and also 63% for soybean production (Mohammadi et al. 2013).

GHG emission was achieved by the control (FI-N) treatment (42.8 kg CO_2eq t^{-1} SRY and 266.6 kg CO_2eq t^{-1} SY), followed by FI-N_1 treatment (41.2 kg CO_2eq t-^{1}

SRY and 257 kg CO_2eq t^{-1} SY) and DI-N treatment (41.1kg CO_2eq t^{-1} SRY and 256.8 kg CO_2eq t^{-1} SY), while the lowest GHG emission was found in PRD-N_2 treatment (33.3 kg CO_2eq t^{-1} SRY and 208 kg CO_2eq t^{-1} SY). As it can be seen in Table 6, the FI group required the highest total carbon inputs, which ranged from 1119.6 kg ha^{-1} (FI-N_2) to 1291 kg ha^{-1} (FI-N), whereas the PRD group required the lowest total carbon inputs, and the difference between the carbon inputs of these two groups were affected by deficit irrigation and nitrogen. Meanwhile, the FI-N, FI-N_1, and FI-N_2 treatments returned the highest carbon outputs 9483.8, 9202.5, and 9185 kg ha^{-1}, respectively, and the PRD group, returning the lowest carbon outputs, which ranged from 7509 kg ha^{-1} (PRD-N_1) to 7629 kg ha^{-1} (PRD-N_2). Compared to control treatment (FI-N), the DI-N_1 treatment decreased the SRY, SY and output carbon by only 5.0%. On the other hand, the GHG emissions per unit of area from DI-N_1 treatment was decreased by 18.7%, when compared to the FI-N treatment.

As can be seen from these results, carbon amount accumulated inside sugarbeet roots is almost 8 times more than the amount of carbon emitted in its production. In brief, sugarbeet is a plant with a high level of carbon fixation capacity.

Table 5
GHG emissions related to inputs of sugar beet production (kg CO_2 eq ha^{-1})

Treatments	İnputs of sugarbeet production								
	Electricity	Diesel fuel	Nitrogen	P_2O_5	K_2O	Agricultural machinery	Drip system (Φ110 mm)	Drip system (Φ 16 mm)	Human Power
FI-N	2340.5	289.8	1260.8	214.5	46.2	35.8	28.5	361.9	168
FI-N_1	2340.5	289.8	945.6	214.5	46.2	35.8	28.5	361.9	168
FI-N_2	2340.5	289.8	630.4	214.5	46.2	35.8	28.5	361.9	168
DI -N	1769	289.8	1260.8	214.5	46.2	35.8	28.5	361.9	168
DI-N_1	1769	289.8	945.6	214.5	46.2	35.8	28.5	361.9	168
DI-N_2	1769	289.8	630.4	214.5	46.2	35.8	28.5	361.9	168
PRD-N	1197.9	289.8	1260.8	214.5	46.2	35.8	28.5	361.9	168
PRD-N_1	1197.9	289.8	945.6	214.5	46.2	35.8	28.5	361.9	168
PRD-N_2	1197.9	289.8	630.4	214.5	46.2	35.8	28.5	361.9	168

Table 6
GHG emission indicators of sugar beet production

Treatments	Areal GHG emissions			Specific GHG emissions	
	Total GHG emissions (kg CO_2 eq ha^{-1})	Input Carbon (kg C ha^{-1})	Output Carbon (kg C ha^{-1})	SRY (kg CO_2eq t^{-1})	SY (kg CO_2eq t^{-1})
FI-N	4746.6	1291	9483.8	42.8	266.6
FI-N_1	4431.4	1205.3	9202.5	41.1	257
FI-N_2	4116.2	1119.6	9185	37.9	236.5
DI -N	4174.5	1135.5	8705	41.1	256.8
DI-N_1	3859.3	1049.7	8961.8	36.7	229.5
DI-N_2	3544.1	964	8461.4	35.4	221.1
PRD-N	3603.4	980.1	7621.7	40.6	253.6
PRD-N_1	3288.2	894.4	7509.6	37.9	236.3
PRD-N_2	2973	808.7	7629.3	33.3	208

SRY: Standardized root yield; SY: Raw sugar yield

4. Conclusions

This paper compares the potential for environmental mitigation, including the reduction of total GHG emissions from agricultural inputs in sugarbeet production by managing irrigation and nitrogen fertilizing. This article shows that sugar beet has a higher performance than many other plants in terms of fixed carbon amount. Although, the control treatment (FI-N) required the highest carbon inputs, produced the highest carbon output value. On the other hand, compared to FI-N, the DI-N_1 treatment decreased the output carbon by only 5.0 % and GHG emissions by 18.7 %. The results of this study indicated that although four treatments FI-N, FI-N_1, FI-N_2 and DI-N_1 showed the best SRY performance, the environmental assessment revealed that only one treatment (DI-N_1) had significantly lower environmental pollution compared with the other treatments (FI-N, FI-N_1 and FI-N_2). Moreover, DI-N_1 treatment saved 25% of irrigation water and nitrogen and 25% of electricity use in irrigation. Therefore, DI-N_1 treatment was recommended for sugarbeet production in the region studied.

5. Acknowledgment

This paper is derived the data regarding the production inputs and yield values of the field trial of a project (TÜBİTAK, project number:111O286), which carried out on sugar beet by Topak et al (2014) in Konya conditions.

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Sesamum indicum and *Trichilia heudelotii* N-hexane and Ethanol Extracts: Effective Remedy Against *Callosobruchus maculates* F. (Coleoptera: Chrysomelidae) Infesting Cowpea Grains

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ABSRACT

The use of synthetic pesticidesusage to prevent cowpea weevils have been reported to be harmful, sometimes leading to the demise of consumersby ingestion of contaminated grains. To save lives, efforts are intensely made to seek after safer alternatives one in particular, is the use of plant based biopesticides. A study was conducted to investigate the effectiveness of N-hexane and ethanol extracts of both sesame (*Sesamum indicum*) leaf and seed and;*Trichilia heudelotii* leaf to control *Callosobruchus maculatus*infesting grains of cowpea. The methods employed involved dressing cowpea seeds (100 g) with the botanical crudeextracts at 0 (control), 0.5, 1.0 and 1.5 ml respectively. Next, six pairs of newly emerged adult *C. maculatus* were introduced into glass vials containing treated seeds in three replicatesand observation was conducted for; adult mortality; emergence of larvae, pupae, and new adults of the insect;weight loss of cowpea grains; and qualitative phytochemical screening. The results presented in this paper revealed that the N-hexane and ethanol extracts of both sesame and *T. heudelotii* were significantly ($p < 0.05$) effective mostly at 1.5 ml in controlling the weevil when compared to the control. The phytochemical analysis indicated the presence of some useful bioactive compounds in the extracts.The observation on weight loss of cowpea revealed that all the various treatment especially at 1.5 ml sustained a significant ($p < 0.05$) weight compared to the control (mean = 40.00) which was lower. A plausible usage of homemade biopesticide using sesame and *T. heudelotii* could be suggested as additives to cowpea grains in the control of *C. maculatus*.

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is cultivated for its nutritious grains, leaves, and green pods, which plays an important part in the protein requirement for both rural and urban settlers and also a source of quality fodder for livestock and provide cash inflow to the buyers and sellers of the crop (Wakili, 2013; Langyintuo&Lowenberg, 2006). The protein content of cowpea has been classed to be about 23% making it a desirable source of plant based protein (FAO, 2005). It is also rich in starch with seeds containing about 63.6% carbohydrate in them as reported by Akyaw et al. (2014). The production practices of growing cowpea has been estimated to support over 850 million people worldwide showing that the importance of the crop should not be underestimated especially in supplying the nutrition requirements of the undernourished in sub-Saharan Africa (FAO, 2005). Up to 70% of the world's cowpea supplies come from the dry Savanna and Sahel Zones of West and Central Africa because the crop is relatively well adapted to the agricultural ecosystem of these areas (Timko et al., 2007; Coulibaly et al., 2009).

Despite the afore mentioned benefits and importance of cowpea, the production yield is still considered to be very low at 100 to 500 kg ha^{-1}in farming conditions compared to the potential yield of 1.5 to 3 tons ha^{-1} (Rachie, 1985; Karungi et al., 2000; Asante et al. 2001; Asiwe et al., 2009; Boukar&Fatokun, 2009; Oyewale&Bamaiyi, 2013;

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Singh, 2014). This low yield has been attributed to a combination of both abiotic and biotic stress factors most especially insect pests' infestations and damage having the most negative influence on the production of cowpea,sometimes depleting the average grain yields to up to 90 to 80% under intense infestations (Togola et al., 2017; Singh, 2014; Jackai&Daoust, 1986; Singh &Jackai, 1985).

One of the known most prominent and important insect pest of this crop is the field-to-store cowpea weevil- *Callosobruchus maculatus* (Fabricius)which belong to the Order Coleoptera and Family Chrysomelidae(Brisibeet al.,2011). When cowpea are allowed to wait in the field for longer periods before harvest, the greater the damage to cowpea grains in storage that will be incurred by the insect pest sometimes reaching a 50% damage level within the space of few months in storage (Dugie et al., 2009). The larvae of the bruchid weevil depends on the grains for its nutrition, feeding and developing exclusively on it. Adult weevils emerge from cowpea grains leaving exit holes. Substantial infestation rates causes loss of quality and the growth of mould invariably reducing market quality and loss of farmers income (Mulatu& Gebremedhin, 2000). Enormous losses of about 50 to 100% have been recorded on stored cowpea due to attack by *C. maculatus* (Udo & Harry, 2013).

The use of chemical insecticides remains the commonest measure used so far to control ofthe insect pest. However, the practice is extremely hazardous to users and consumers (Togola et al., 2017) sometimes leading to death of consumers as in the case of people who died in Nigeria after consuming beans containing high levels of pesticides used in preventing the bruchid weevils from attacking cowpea grains. This event was later known as the killer beans spree (Shiabu, 2008; Gwary et al., 2012) thereby prompting both farmers and consumers with the urgent need to search for safer alternatives to chemical insecticides. Efforts are being made to seek eco-friendly alternative including the use of biopesticides (Togola et al., 2017) hence the reason for conducting this investigation. The current study's objective is to investigate the use of plant materials namely *Sesamum indicum* L (Pedaliaceae: Lamiales) and *Trichilia heudelotii* Planch (Meliaceae: Sapindales) as a biopesticide alternative to the use of chemical insecticidesin the control of *Callosobruchus maculatus* affecting cowpea grains. These plants are easily sourced within sub-Saharan Africa where major cowpea production yield have been reportedand have also been observed to grow as part of natural vegetation in the region, thereby the above mentioned plants as biopesticidewas investigated to study their effectiveness against the bruchid beetle.

2. Materials and Methods

2.1. Culture of Callosobruchus maculatus

Mass culture of *C. maculatus* was maintained using the procedure described by Strong et al. (1968).The variety of cowpea used for this experiment was the variety IT96D-610 provided by the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Seeds were described to be susceptible to attacksand infestation by the insect pest *Callosobruchus maculatus*. The insects were acquired from stock cultures from the Nigerian Stored Products Research Institute (NISPRI), Ilorin, Nigeria. Six pairs of 0-1 day-old adults (male and female) of *C. maculatus* were isolated and introduced into small glass vials (7.5 × 2.5 cm) containing untreated cowpea seeds (Khalequzzaman et al., 2007). The insects were given 7 days to mate and oviposit in the cowpea. Glass vials were covered with muslin cloth and secured firmly with rubber bands to prevent escape of the insect.After one week, when oviposition had been noticed, the parent stocks of *C. maculatus* were removed and vials were left under laboratory conditions (temperature 24-28^{0}C, relative humidity of 70%) till the emergence of new filial progenies which were used for the experiment.

2.2. Sample preparations and extraction

Sample preparation and extraction was done according to the methods described by Uddin II et al (2020). The plant materials, *Sesamum indicum*seeds and leaves and,*Trichilia heudelotii* leaves were air-dried at room temperature, turning regularly to ensure even and thorough drying to avoid moulding of the samples. The dried plant materials were ground using an electric grinder and sieved using a 90-micron mesh sieve to obtain fine powder of the samples.The powdered leaves (650 g for *Trichilia heudelotii* and 700 g for *Sesamum indicum*) and seeds (1.1 kg) were extracted twice by maceration, sequentially with a non-polar and polar organic solvents, hexane (Hex) and ethanol (EtOH) respectively. Yields of 14.26 g (Hex) and 10.24 g (EtOH) of *Trichilia heudelotii*leaves and 12.24 g (Hex) and 13.04 g (EtOH) of *Sesamum indicum*was obtained after evaporation of the organic solvents. For the sesame seeds, 8.6g (Hex) and 12.8 g (EtOH) was obtained. The crude extracts obtained used for the experiment were then prepared using an aqueous solution of 1% v/v of acetic acid in distilled water and concentrated at 0.5 g per ml.

2.3. Phytochemical screening (qualitative)

Chemical tests were carried out on both the ethanolic and N-Hexane extracts for the qualitative determination of phytochemical constituents as described by Harborne (1973), Trease and Evans (1989) and Sofowora (1993).

Test for Tannins:Few drops of 1% lead acetate was added to 0.2 g of the extract and observed for the formation of yellow precipitate

Test for Alkaloids:Exactly 0.2 g of the extract was stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. One ml of the filtrate was taken individually into two separate test tubes. To the first portion, Mayer's reagent was added and appearance of buff-coloured precipitate was an indication for the presence of alkaloids. To the second portion, few drops of Dragendorff's reagent was added to the filtrate and observed for the formation of an orange-red precipitate.

Test for Terpenoids: Exactly2 ml of chloroform was added to 0.2 g of the extract, 3 ml of concentrated sulphuric acid was added carefully to form a layer. Formation of a reddish-brown colouration at the interface indicates the presence of terpenoids.

Test for Phenolic Compounds: The extract (0.5 g) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

Test for Flavonoids:Exactly 4 ml of dilute ammonia solution was added to a portion of the extract followed by addition of concentrated sulphuric acid. A yellow colouration indicates the presence of flavonoids.

Test for Saponins:Exactly 1 g of the extract was boiled with 5 ml of distilled water and filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persists on warming shows the presence of saponins.

Test for Steroids:Acetic anhydride (2ml) was added to a portion of the extract with 2ml H_2SO_4. Colour change from violet to blue or green indicates the presence of steroids.

2.4. Experimental procedure

The experiment was carried out under ambient laboratory conditions. One hundred gram (100 g) of undamaged cowpea were placed in glass vials (7.5 × 2.5 cm). The seeds were properly mixed with the various levels of the botanical treatment extracts at 0.5, 1.0, and 1.5 ml respectively using a wooden spatula to ensure uniform coating of the seeds. The control (0.0 ml) had no treatment applied to it. Six pairs of newly emerged male and female adult *C. maculatus* were introduced after drying coated seeds for five minutes and glass vials were ensured covered with muslin cloth fastened with rubber bands to permit ventilation and prevent escape of the insect. The experiment was a Completely Randomized Design (CRD) with three replications. Dead beetles were removed from vials every 24 hours interval when beetles were noticed to be inactive and unresponsive to probing of the abdomen using an entomological needle (Uddin et al., 2020).

Oviposition rate was recorded at day 7 after treatment (DAT) and ten seeds were randomly selected from each treatment vials to view eggs laid using a × 100 magnifying lens. Larval and pupae emergence was recorded at 15 and 23 DAT respectively by also selecting 10 seeds at random from the various treatments and control and dissecting seeds gently with a sharp razor blade to check for the presence of *C. maculatus* larvae and pupae in cowpea seeds. Emergence of adult progeny was recorded from 33 days after treatment and the adults removed every 24 hours to avoid the next generationThe percentage loss in cowpea grain weight was recorded at day 38 after release of beetles that have completely emerged (Khalequzzaman et al., 2007). Data collected were subjected to a two-way analysis of variance and T-test and significant mean differences were separated using New Duncan Multiple Range Test set at a P-value of 0.05.

3. Results and Discussion

3.1. Effects of the selectedextracts on adult mortality of Callosobruchus maculatus after treatment

The experiment showed significant results between the various botanical extracts and the control. Post hoc analysis revealed that at 1 DAT, both the N-Hexane extract and the ethanol extract of sesame leaf were the most effective treatment, significantly ($p < 0.05$) able to suppress the population of *C. maculatus* by the mean number of 6.00 in both treatment at the concentration rate of 1.5 ml when compared to the other treatment and the control (Table 1). Further observation also indicated that the next effective botanical treatment were the N-Hexane and Ethanol extract of *T. heudelotii* leaf which were also significantly ($p < 0.05$) able to suppress the insect pest adult population to a mean number of 5.33 at the increasing concentration rate of 1.5 ml when compared to the control (0.0 ml) which had a mortality rate of 2.33 as shown in Table 1. The highest mortality was recorded to occur within 24 hour period after treatment (Table 1).

Table1
Comparative effect of the botanical treatment extracts on adult mortality of *Callosobruchus maculatus*

		Adult Mortality Days after treatment (DAT)				
Treatments	Conc.(ml)	1	2	3	4	5
NHTL	0.5	4.00^{abcd} ±1.00	1.67^{abc}±0.58	1.67^{ab}±0.58	0.67^{abc}±0.58	0.00^{a}±0.00
	1.0	5.00^{bcd}±0.00	1.33^{abc}±0.58	1.00^{ab}±0.00	0.67^{abc}±0.58	0.00^{a}±0.00
	1.5	5.33^{cd}±1.53	1.67^{abc}±0.58	1.00^{ab}±0.00	0.00±0.000^{a}	0.00^{a}±0.00
NHSS	0.5	4.00^{abcd}±1.00	1.33^{abc}±0.58	1.33^{ab}±0.58	1.33^{bc}±0.58	0.00^{a}±0.00
	1.0	4.67^{abcd}±0.58	1.33^{abc}±0.58	1.00^{ab}±0.00	0.67^{abc}±0.58	0.33^{a}±0.58
	1.5	5.00^{bcd}±0.00	2.00^{abc}±1.00	0.67^{a}±1.16	0.33^{ab}±0.58	0.00^{a}±0.00
NHSL	0.5	4.33^{abcd}±1.52	1.33^{abc}±1.16	1.00^{ab}±0.00	1.00^{abc}±0.00	0.33^{a}±0.58
	1.0	5.00^{bcd}±0.00	1.67^{abc}±0.58	0.67^{a}±1.16	0.67^{abc}±0.58	0.00^{a}±0.00
	1.5	6.00^{d}±1.00	1.00^{ab}±0.00	1.00^{ab}±0.00	0.00^{a}±0.00	0.00^{a}±0.00
ETHTL	0.5	4.67^{abcd}±1.16	1.67^{abc}±0.58	0.67^{a}±1.16	0.67^{abc}±0.58	0.33^{a}±0.58
	1.0	5.33^{cd}±1.53	1.00^{ab}±1.00	0.67^{a}±1.16	0.33^{ab}±0.58	0.67^{a}±0.58
	1.5	5.33^{cd}±1.53	0.67^{a}±0.578	1.33^{ab}±0.58	0.67^{abc}±0.58	0.00^{a}±0.00
ETHSS	0.5	4.00^{abcd}±1.00	1.00^{ab}±0.00	1.33^{ab}±0.58	1.00^{abc}±0.00	0.67^{a}±0.58
	1.0	3.67^{abcd}±1.53	1.33^{abc}±1.16	2.00^{ab}±1.00	0.67^{abc}±0.58	0.33^{a}±0.58
	1.5	3.33^{abc}±0.58	1.67^{abc}±0.58	2.33^{b}±1.155	0.67^{abc}±0.58	0.00^{a}±0.00
ETHSL	0.5	4.00^{abcd}±1.00	1.67^{abc}±0.58	1.67^{ab}±0.58	0.33^{ab}±0.58	0.33^{a}±0.58
	1.0	4.67^{abcd}±1.53	1.67^{abc}±1.16	1.33^{ab}±0.58	0.33^{ab}±0.58	0.00^{a}±0.00
	1.5	6.00^{d}±1.00	0.67^{a}±0.58	1.33^{ab}±0.58	0.00^{a}±.00	0.00^{a}±0.00
Control	0.0	2.33^{a}±1.53	1.00^{ab}±0.00	1.00^{ab}±0.00	1.67^{c}±0.58	2.00^{b}±1.73

Means followed by the same letter (s) in a column are not significantly different from each other at $P < 0.05$ according to New Duncan's Multiple Range Test

Keys**:** Conc. =Concentration, NHTL = N-Hexane extract of *T. heudelotii* leaf, NHSS = N-Hexane extract of sesame seed, NHSL = N-Hexane extract of sesame leaf, ETHTL = Ethanol extract of *T heudelotii* leaf, ETHSS = Ethanol extract of sesame seed, ETHSL = Ethanol extract of sesame leaf

3.2 Effects of the selectedextracts on the mean number of eggs, larvae emergence and pupaeofC. maculatus after treatment

There were significant ($p < 0.05$) differences between the treatments and the control. Table 2 showed that all the various treatments were effective in restricting the amount of eggs laid by *C. maculatus*although, the N-Hexane extract of sesame leaf was observed to have the least mean number of eggs laid by the insect pest (1.33) at the concentration level of 1.5 mlwhen compared to the others (Table 2). The control did not show any reduction in the egg laying activities of the insect and as such had the highest rate of oviposition (8.33) as shown in Table 2.

Table 2 also showed that all the various treatments were significantly effective in restricting the larvae emergence of *C. maculatus* at different concentration levels, although the concentration rate of the botanical extracts: N-Hexane *T. heudelotii* leaf, N-Hexane sesame seed, N-Hexane sesame leaf, Ethanol *T. heudelotii* leaf and Ethanol sesame seed at 1.5 ml was observed to have the lowest mean number (1.00) of emerged larvaeof the insect pest (Table 2). The control had the highest mean number (6.00) of larvae that emerged and thus was significantly ($p < 0.05$) higher when compared to the botanical treatments which were able to suppress the numbers of emerging larvae of the weevil as seen in Table 2.

On the amount of *C. maculatus* pupae recorded after treatment, the experiment showed significant results between the various treatment extracts and the control. It was observed that the botanical extracts used were effective in suppressing the numbers of *C. maculatus* pupating (Table 2). Close observation of the mean numbers of pupae seen further indicated that the ethanol extract of *T. heudelotii* leaf at the concentration rates of 1.0 and 1.5 ml had the least amount of pupae at the mean number of 0.67(±0.58) each. This was also the same for the ethanol extract of sesame seed at 1.5 ml (0.67±0.58).The control (4.67±0.58) had the most amount of pupae seen in the experiment when compared to the treated ones shown in Table 2.

Table 2
Effect of the different treatments on the mean numbers of eggs, larvae emergence and pupae of *C. maculatus*

		7 DAT	15 DAT	23 DAT
Treatments	Conc.(ml)	Eggs laid	Larvae emergence	Pupae
NHTL	0.5	4.00^{abcd}±1.00	2.00^{abc}±1.00	1.67^{abc}±0.58
	1.0	3.67^{abcd}±1.53	1.33^{ab}±0.58	1.00^{ab}±0.00
	1.5	3.33^{abcd}±1.53	1.00^{a}±0.00	1.00^{ab}±0.00
NHSS	0.5	3.67^{abcd}±1.53	1.67^{abc}±0.58	1.67^{abc}±0.58
	1.0	3.33^{abcd}±1.16	1.67^{abc}±0.58	1.33^{abc}±0.58
	1.5	2.00^{abc}±0.00	1.00^{a}±0.00	1.00^{ab}±0.00
NHSL	0.5	2.67^{abcd}±1.53	1.33^{ab}±0.58	1.33^{abc}±0.58
	1.0	1.67^{ab}±1.16	1.33^{ab}±0.58	1.00^{ab}±0.00
	1.5	1.33^{a}±0.58	1.00^{a}±0.00	1.00^{ab}±0.00
ETHTL	0.5	4.00^{abcd}±1.00	1.67^{abc}±0.58	1.33^{abc}±0.58
	1.0	3.33^{abcd}±1.16	1.33^{ab}±0.58	0.67^{a}±0.58
	1.5	3.00^{abcd}±1.00	1.00^{a}±0.00	0.67^{a}±0.58
ETHSS	0.5	4.33^{bcd}±0.58	1.67^{abc}±0.58	2.00^{abc}±0.00
	1.0	2.67^{abcd}±1.16	1.67^{abc}±0.58	1.67^{abc}±1.16
	1.5	2.67^{abcd}±0.58	1.00^{a}±0.00	0.67^{a}±0.58
ETHSL	0.5	3.33^{abcd}±0.58	1.67^{abc}±1.16	1.33^{abc}±0.58
	1.0	1.67^{ab}±1.16	2.00^{abc}±1.00	1.00^{ab}±0.00
	1.5	1.67^{ab}±0.58	1.33^{ab}±0.58	1.00^{ab}±0.00
Control	0.0	8.33^{e}±1.53	6.00^{d}±1.00	4.67^{d}±0.58

Means followed by the same letter (s) in a column are not significantly different from each other at $P < 0.05$ according to New Duncan's Multiple Range Test,

Keys:DAT= day after treatment, Conc. =Concentration, NHTL = N-Hexane extract of *T. heudelotii* leaf, NHSS = N-Hexane extract of sesame seed, NHSL = N-Hexane extract of sesame leaf, ETHTL = Ethanol extract of *T heudelotii* leaf, ETHSS = Ethanol extract of sesame seed, ETHSL = Ethanol extract of sesame leaf

3.3. Effects of the selectedextracts on the mean number of newly emerged C. maculatus adults

Table 3 showed the effects of the different botanical treatments against the emergences of*C. maculatus*adults. All of the treatment extracts were effective in restricting the emergence of the adults and were thus significantly ($p < 0.05$) potent when compared to the control which had the highest population of newly emerged *C. maculatus* adultsfrom 33 to 37 DAT with the mean numbers of 2.67(±1.16), 3.00(±1.73), 2.67(±1.16), 3.00(±1.00) and 2.33(±0.58) respectively. Consideration of the mean numbers of the treatments indicated that N-Hexane sesame leaf (0.0±0.00) and Ethanol sesame leaf extract (0.0±0.00) at 1.5 ml had no emergence of *C. maculatus* adults recorded at 33 DAT (Table3).

Table 3
Effect of treatments on the number of newly emerged *C. maculatus* adults

		Days after treatment (DAT)				
Treatment	Conc.(ml)	33	34	35	36	37
NHTL	0.5	0.67^{abc}±0.58	1.00^{ab}±1.00	1.67^{abc}±0.58	1.33^{ab}±1.16	1.33^{bcde}±0.58
	1.0	1.33^{abcd}±0.58	1.33^{ab}±0.58	1.00^{ab}±0.00	1.00^{ab}±1.00	0.67^{abc}±0.58
	1.5	1.00^{abc}±1.00	0.67^{ab}±0.58	1.33^{abc}±0.58	0.67^{ab}±0.58	0.33^{ab}±0.58
NHSS	0.5	0.67^{abc}±0.58	1.00^{ab}±1.00	0.67^{ab}±0.58	0.33^{ab}±0.58	1.00^{abcd}±0.00
	1.0	0.67^{abc}±0.58	0.67^{ab}±1.16	1.00^{ab}±0.00	0.33^{ab}±0.58	0.33^{ab}±0.58
	1.5	0.33^{ab}±0.58	1.00^{ab}±1.00	0.33^{a}±0.577	0.67^{ab}±0.58	0.33^{ab}±0.58
NHSL	0.5	0.33^{ab}±0.58	0.67^{ab}±0.58	1.33^{abc}±0.58	1.00^{ab}±1.00	0.33^{ab}±0.58
	1.0	0.67^{abc}±0.58	0.33^{a}±0.58	0.67^{ab}±0.58	0.00^{a}±0.00	0.33^{ab}±0.58
	1.5	0.00^{a}±0.00	0.33^{a}±0.58	0.67^{ab}±0.58	0.33^{ab}±0.58	0.00^{a}±0.00
ETHTL	0.5	0.67^{abc}±0.58	1.00^{ab}±1.00	1.33^{abc}±0.58	1.00^{ab}±1.00	1.00^{abcd}±1.00
	1.0	1.00^{abc}±1.00	1.00^{ab}±1.00	1.00^{ab}±0.00	1.00^{ab}±1.00	0.33^{ab}±0.58
	1.5	0.67^{abc}±1.16	0.67^{ab}±0.58	0.67^{ab}±0.58	0.67^{ab}±1.16	0.33^{ab}±0.58

Table 3
Effect of treatments on the number of newly emerged *C. maculatus* adults

ETHSS	0.5	0.67^{abc}±0.58	1.00^{ab}±1.00	0.67^{ab}±0.58	0.33^{ab}±0.58	0.67^{abc}±1.16
	1.0	0.33^{ab}±0.58	0.67^{ab}±1.16	0.67^{ab}±0.58	0.67^{ab}±0.58	0.33^{ab}±0.58
	1.5	0.33^{ab}±0.58	0.67^{ab}±0.58	0.33^{a}±0.577	0.67^{ab}±0.58	0.33^{ab}±0.58
ETHSL	0.5	0.67^{abc}±1.16	1.67^{abc}±1.53	1.00^{ab}±0.00	1.00^{ab}±1.00	0.67^{abc}±0.58
	1.0	0.33^{ab}±0.58	1.00^{ab}±1.00	0.67^{ab}±1.16	1.00^{ab}±1.00	0.67^{abc}±0.58
	1.5	0.00^{a}±0.00	0.33^{a}±0.58	0.67^{ab}±1.16	0.33^{ab}±0.58	0.33^{ab}±0.58
Control	0.0	2.67^{d}±1.16	3.00^{d}±1.73	2.67^{c}±1.16	3.00^{c}±1.00	2.33^{e}±0.58

Means followed by the same letter (s) in a column are not significantly different from each other at $P < 0.05$ according to New Duncan's Multiple Range Test
Keys:Conc. = Concentration, NHTL = N-Hexane extract of *T. heudelotii* leaf, NHSS = N-Hexane extract of sesame seed, NHSL = N-Hexane extract of sesame leaf, ETHTL = Ethanol extract of *T heudelotii* leaf, ETHSS = Ethanol extract of sesame seed, ETHSL = Ethanol extract of sesame leaf

3.4. Effects of the treatment extracts on the percentage weight of cowpea

The experiment revealed that the various treatment sustained a significant amount of cowpea weight (g) when compared to the control. However, the ethanol extract of sesame leaf retained the highest percentage weight of 93.33%of the cowpea at 1.5 ml concentration followed by the ethanol extract of sesame seed (86.67%), N-Hexane extract of sesame leaf (86.66%) and N-Hexane extract of sesame seed (86.66%) at 1.5 ml concentration when compared to the other botanical treatments and the control in Table 4. The control had a drastic loss of weight (40.00%) significantly ($p < 0.05$) lower than the botanical treatments usedas shown in Table 4.

Table4

Effects of thetreatment extracts on the percentage weight of cowpea

Treatment	Conc.(ml)	% weight (g)
NHTL	0.5	66.67^{bc}±6.67
	1.0	73.33^{cd}±6.67
	1.5	73.33^{cd}±6.67
NHSS	0.5	66.67^{bc}±6.67
	1.0	80.00^{de}±6.67
	1.5	86.66^{ef}±6.67
NHSL	0.5	73.33^{cd}±0.00
	1.0	80.00^{de}±6.67
	1.5	86.66^{ef}±6.67
ETHTL	0.5	73.33^{cd}±6.67
	1.0	73.33^{cd}±6.67
	1.5	73.33^{cd}±6.67
ETHSS	0.5	73.33^{cd}±6.67
	1.0	86.66^{ef}±6.67
	1.5	86.67^{ef}±6.65
ETHSL	0.5	73.33^{cd}±6.67
	1.0	80.00^{de}±6.67
	1.5	93.33^{f}±6.67
Control	0.0	40.00^{a}±6.67

Means followed by the same letter (s) in a column are not significantly different from each other at $P < 0.05$ according to New Duncan's Multiple Range Test

Keys:Conc. = Concentration, NHTL = N-Hexane extract of *T. heudelotii* leaf, NHSS = N-Hexane extract of sesame seed, NHSL = N-Hexane extract of sesame leaf, ETHTL = Ethanol extract of *T heudelotii* leaf, ETHSS = Ethanol extract of sesame seed, ETHSL = Ethanol extract of sesame leaf

3.5. Qualitative Phytochemical screening of the different botanical treatment extracts

The qualitative phytochemical screening (Table 5) indicated that there was relatively moderate amount of alkaloid present in the N-hexane and ethanol sesame seedand in the N-hexane *T. heudelotii* extracts. Flavonoids were detected to be in trace amount in all the treatment extracts. Saponin was indicated to be moderately available in the ethanol sesame seed, and in the N-Hexane and ethanol *T. heudelotii* leaf. Tannin was mostly present in the ethanol sesame leaf extract with the other extracts having tannin in trace amount. Steroid was abundantly present in all the treatment extracts except in the ethanol sesame leaf and seed which were in moderate amount as shown in Table 5.

Table 5
Qualitative phytochemical analysis of the various treatment extracts

Phyto-chemicals	NH SL	ETH SL	NH SS	ETH SS	NH TL	ETH TL
Alkaloids	+	-	++	++	++	+
Flavonoids	+	+	+	+	+	+
Terpenoids	-	+	-	++	++	+
Saponin	+	+	+	++	++	++
Tannin	+	++	+	+	+	+
Steroid	+++	++	+++	++	+++	++

Keys**:** NHTL = N-Hexane extract of *T. heudelotii* leaf, NHSS = N-Hexane extract of sesame seed, NHSL = N-Hexane extract of sesame leaf, ETHTL = Ethanol extract of *T heudelotii* leaf, ETHSS = Ethanol extract of sesame seed, ETHSL = Ethanol extract of sesame leaf, - = Absence of the Secondary metabolites, + = Trace presence of Secondary metabolites, ++ = Presence of Secondary metabolites, +++ = Abundance of Secondary metabolites

Extracts from different plants have been studied to possess insecticidal properties against a wide range of insect pests (Abdullah & Muhammad, 2004). The study conducted showed promising bio-pesticidal potentials of two plant botanical extracts namely sesame and *Trichilia heudelotii* against various life stages of the ruthless insect pest *Callosobruchus maculatus* infesting cowpea grains.

The current study investigated the use of N-hexane and Ethanol extracts of *Trichilia heudelotii* leaf, sesame leaf and seed. The extracts of the treatment used were mostly effective at an increased rate of 1.5 ml invariably resulting to the control of *C. maculatus* in cowpea.Sesame and *T. heudelotii* extracts increased adult mortality, reduced oviposition and larvae emergence rate, constricted the population of the next generation of *C. maculatus* adults fromemerging and also had the lowest cowpea weight loss when compared to untreated ones. Several reports have suggested the bio-insecticidal action of the selected plant extracts. Ahmed et al. (2003) made use of sesame oil and discovered that sesame oil controlled the larvae of *Callosobruchus chinensis* inside the cotyledons of azuki beans. There was no evaluation of the use of sesame leaves in his study hence the present study showed that N-hexane and ethanol leave extracts of sesame could be considered a plausible control against *C. maculatus* when used as additives on cowpea grains. In another study, Wheeler andIsman (2001) and López-Olguín(1998) investigated the use of different plant parts of the genus *Trichilia* anddiscovered that it has insecticidal and antifeeding effects against some field insects. This, too, was without consideration of the *Trichilia* species-*heudelotii* as indicated in this research that the leave extracts of the plant could possibly be used to restrict the life cycle of the bean weevil from further development in grains.

The insecticidal properties of the extracts of *Trichilia heudelotii* leaf, Sesame leaf and Sesame seed might be connected to the phytochemical constituents such as alkaloids, flavonoids, steroids, terpenoids and tannin. Anilakumaret al (2010) stated that sesame plants contain many phytochemically important compounds like flavonoids, phenolic acids, alkaloids, tannins, saponins, steroids and terpenoids that could restrict the activities of various insect pests. Similarly, it was reported that the genus *Trichilia* is a good source of different classes of phytochemical compounds with bio-insecticidal potentials in the control of insect pests(Garcia-Gomez et al., 2019; Sengottayan, 2013; Curcino-Vieira et al., 2014).These components may be having the role of biochemical defenses or protectant against *C. maculatus* in cowpea grains.

The antagonistic action of the treatment extracts may be partially attributed to interference in the normal respiration of the insect pest, leading to suffocation (Schoonhoven, 1978). Other factors other than shortages of oxygen supply may also play its unique role in their mode of action (Shaaya & Ikan, 1978). Egg mortality has been previously connected to toxic components of plant materials and also to their physical properties which could cause changes in surface tension and also oxygen tension within the insect eggs (Singh et al., 1978).

Al-lawati et al (2002a,b) stated that materials gotten from plants have been traditionally used and accepted by farmers due to their relatively safe usage in protecting and preserving grains from pest attacks. The study relatively showed that there was better maintenance and very low loss of the grain weight of cowpea after having been treated with the botanicals

is advisable for short grain storage because of high degradability and volatility of the plant material (Salako et al., 2008) this is comparatively safer in preserving cowpea grains for a short period as it is intended to be eaten soonthereby leaving off no toxic chemical residues. Plant materials with insecticidal and preservative potentialscould be easily sourced and acquired from local environmentand also suggested as a cheaper, quick and eco-friendly option in preserving food grains such as cowpea for home usage due to the

developing fears of using chemical pesticides (Mukanga et al., 2010).

4. Conclusion

The use of plant materials as insecticides is increasingly gaining prominence as a sustainable means of pest management in cowpea grains.Previous studies have revealed the negative impacts of chemical insecticides, even the reports of deaths of consumers of cowpea who ingested grains containing lethal dosage of the substance after 24 hours. Biopesticides,on the other hand, poses no deleterious risk to human health, the environment and non-target organisms. The use of sesame (seed and leaf) and *Trichilia heudelotii* (leaf) N-hexane and ethanol extracts indicated active insecticidal potentials against the notorious insect pest-*Callosobruchus maculatus* whose reputation is known to be extremely destructive, drastically reducing cowpea yield and its market values. The plant extracts added to cowpea grains as alternatives to synthetic insecticides effectively reduced the population of the weevil further disorienting the various life cycle of the insect pest from development in cowpea. The solution presented here could be easily adopted by household users in preventing their grain stock from degradation by the insect pest therefore a recommendation of subsequent research to improve the longevity and persistence of the bioactive compounds of these plant extracts on cowpea is suggested.

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Identification and Characterization of White Mold Disease (*Sclerotinia sclerotiorum*) in Globe Artichoke

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ABSTRACT

Globe artichoke (*Cynara scolymus* L.) is an economically important plant that is cultivated over 30 countries in the world. In 2017, wilting and lodging symptoms were observed on globe artichoke plants in three locations (Serik-Gebiz, Serik-Karadayı and Gazipaşa-Bakılar) in the Western Mediterranean Region of Turkey. To determine the cause of the symptoms, samples from necrotic tissues were cultured in vitro. Based on the morphological and ITS sequences, the causal agent was identified as *Sclerotinia sclerotiorum* (Lib.) de Bary. Pathogenicity test was conducted using cv. Bayrampaşa in the greenhouse. Three isolates (Ser3, Ser4 and Gzp3) of *S. sclerotiorum* were characterized on the basis of mycelial compatibility, colony radial growth, sclerotia formation, sclerotia size, sclerotia weight and sclerotia number. Virulence of the isolates was also determined using detached leaf technique. No mycelial compatibility was detected among the isolates. Significant ($P<0.01$) differences were found in the examined morphological features and virulence of the isolates. Gzp3 was the most virulent isolate forming average 8.60 cm lesion length. However, lesion lengths formed by the other isolates, Ser3 and Ser4, were average 4.16 and 1.93 cm, respectively. This is the first detailed characterization of *S. sclerotiorum* causing crown and stalk rot in globe artichoke.

1. Introduction

Globe artichoke (*Cynara scolymus* L.) is a herbaceous plant from Asteraceae family. It is mostly grown in the Mediterranean Basin and has various bioactive phenolic compounds, inulin, fibre, minerals and cynarin. Therefore, it is a functional food source and constitutes significant part of the Mediterranean cuisine (Lattanzio et al 2009). With 39477 t annual globe artichoke production, Turkey is the 11th producer in the world (FAO 2020).

Globe artichoke production is negatively affected by fungal pathogens. One example of which is *Sclerotinia sclerotiorum* (Lib.) de Bary. Usually called as white mold, it is a necrotrophic fungus infecting over 400 plant species (Allan et al 2019). Infection of the fungus appears as whitish, cottony and dense mycelial mats on host tissue. The fungus can infect miscellaneous parts of plants depending on germination of sclerotia. Thus, the disease caused by *S. sclerotiorum* might be designated differently. In addition to white mold, it is also called as stem rot, watery soft rot, cottony rot, white blight, root rot, stem break, stalk rot or white canker (Kapatia et al 2016).

Sclerotia (aggregates of hyphae containing thick melanin) are essential inoculum sources and long-term survival structures of the fungus. They can survive up to 8 years in soil (Bolton et al 2006). Depending on the environmental conditions, sclerotia could germinate carpogenically or myceliogenically (Smolińska & Kowalska 2018). If myceliogenic germination occurs, sclerotia form hyphae that infect root, crown and stalks of host plants, which is called basal infection of the fungus (Lane et al 2018). In this infection phenomenon, the fungus could form appressoria on host surface or directly penetrate cuticle through its infective hyphae at basal stem of host. Ensuing penetration, hyphae develop inter and intracellularly in cells by excreting cell wall degrading enzymes, results in tissue collapse of host. The fungus maintains its colonization on dead tissue of host, which is seen as white fluffy mycelial mats (Davar et al 2012).

In addition to basal infection, if the carpogenic germination of sclerotia occurs, the fungus induces rots on different parts of host (e.g. pod rot on bush bean, fruit rot on garden pea, head rot on cauliflower and blossom rot on salvia) (Rahman et al 2020). All of

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these indicate destructiveness of the fungus on many agricultural crops. As regards this, Grabowski & Malvick (2017) reported that *S. sclerotiorum* caused stem and crown rot and consequently wilt and death of a wide range of annual flowering plants.

Due to difficulty in controlling of *S. sclerotiorum*, there is a steady increase in losses stem from the fungus in horticultural crops in the world (Smolińska & Kowalska 2018). To give some examples, it was reported that *S. sclerotiorum* led to substantial yield and quality losses in bean (*Phaseolus vulgaris*), oilseed rape (*Brassica napus* and *B. juncea*), lettuce (*Lactuca sativa*), sunflower (*Helianthus annuus*), perilla (*Perilla frutescens*) and cabbage (*Brassica oleracea*) production in Brazil, Australia, UK, China, Korea and Sri Lanka, respectively (Meinhardt et al 2002; Li et al 2006; Clarkson et al 2014; Liu et al 2018; Afroz et al 2019; Mahalingam et al 2020). With regard to globe artichoke, too little is known about *S. sclerotiorum* on globe artichoke.

The aims of the study were to i) identify causal agent causing crown and stalk rot on globe artichoke in the Western Mediterranean Region of Turkey, ii) characterize isolates of white mold based on morphologic and genetic features, and iii) determine virulence of isolates of *S. sclerotiorum* on globe artichoke.

2. Materials and Methods

2.1. Isolation

Disease symptoms were observed in globe artichoke growing fields of Serik-Karadayı, Serik-Gebiz and Gazipaşa-Bakılar locations of the Western Mediterranean Region of Turkey in 2017. Samples from crown and stalk parts of lodging plants were taken and tagged in paper bags. They were taken to the Mycology Laboratory of Batı Akdeniz Agricultural Research Institute. Initially, the samples were washed under tap water and then necrotic tissues were cut into small pieces. They were exposed to NaOCl (2%) for two min and rinsed with sterile distilled water. Afterwards, they were put on sterile filter papers for drying for nearly 45 min and then transferred on potato dextrose agar (PDA). Petri dishes were kept at 25 °C at 6 days. Fungal colonies developing on PDA were subcultured by taking tips of hyphae of each colony.

2.2. Identification

Three isolates of *S. sclerotiorum* were designated as Ser3, Ser4 and Gzp3.

2.2.1. Morphological identification

Morphological features (structure of mycelium, colony color and pattern) were examined using an Olympus BX43 microscope with SC100 digital color camera. Sclerotia numbers of the isolates were calculated by counting sclerotia in each petri. Size and weight of sclerotia were determined using a caliper and precision scales (Bolton et al 2006).

2.2.2. Molecular identification

DNA of each isolate was extracted using purification protocol of Promega. Following DNA extraction, rDNA fragments were amplified using primer pairs ITS-1 (5' TCC GTA GGT GAA CCT GCGG 3') and ITS-4 (5' TCC TCC GCT TAT TGA TATGC 3') (White et al 1990). Amplifications were performed in a SimpliAmp Thermocycler (Applied Biosystems, USA) and consisted of 1 cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, annealing temperature at 58.5ºC for 1 min, 72°C for 1 min, with a final extension at 72°C for 7 min. PCR products were separated in 2% agarose gels, stained with safe DNA dye and visualized under UV light. Sequence analysis was done by GENOKS (Çankaya-Ankara). The ITS sequences of the isolates, Ser3, Ser4 and Gzp3, were deposited at GenBank (http://www.ncbi.nlm.nih.gov) with the accession numbers of MH593866, MH593867 and MH593868, respectively.

In addition, a phylogenetic tree was constructed using neighbour-joining method in MEGA version 7.0 program to compare relatedness of our isolates with the other isolates of *S. sclerotiorum* in the genbank.

2.3. Pathogenicity test

Two month-old seedling of globe artichoke (cv. Bayrampaşa) was planted per pot containing autoclaved soil and vermiculates (1:1). Mycelial plugs (0.5 cm) were taken from the edges of 7 day-old colonies of each isolate. These mycelial plugs were attached to just below the crown of seedlings. A total of 4 seedlings were inoculated for each isolate. In the controls, only agar plugs were used. Fifteen days after inoculation, wilting symptoms were observed on inoculated seedlings, while no symptom was seen on the control seedlings. Samples from the necrotic tissues of the inoculated seedlings were taken and each isolate of *S. sclerotiorum* was re-isolated separately.

2.4. Colony radial growth and sclerotia formation

Mycelia plugs (0.5 cm) of each isolate were put on the center of petri dishes (9 cm) containing PDA. One plug was used per petri and incubated at 25 °C for 5 days. Colony radial growth of each isolate was measured daily. The experiment was carried out according to completely randomized design with four replicates. Experimental unit consisted of one petri dish. Sclerotia formation, sclerotia size, sclerotia weight and sclerotia number per petri were recorded (Mert-Türk et al 2007).

2.5. Mycelial compatibility groupings (MCGs)

One mycelial plug (0.5 cm) of each isolate was put nearly 2 cm away from the edge of Petri dish containing PDA with red food coloring (six drops/L). Each pairing was performed with four replications. In addition, self-pairings for each isolate were done as aforementioned (Kohn et al 1990; Schafer & Kohn 2006).

2.6. Virulence of the isolates of S. sclerotiorum

Virulence of each isolate was determined using detached leaf technique. One mycelial plug (0.5 cm) of each isolate was put on the center of two-month-old leaf of globe artichoke seedling (cv. Bayrampaşa) placed on moist sterile filter paper per Petri dish. The experiment was conducted using completely randomized design with three replications. In the controls, only agar plugs were put on the leaves. Four days after inoculation, lesion lengths on the detached leaves were measured. Mean lesion length of each isolate was compared using variance analysis (Zanatta et al 2019; Rahman et al 2020).

2.7. Statistical analysis

For variance analysis, SAS 9.1 software program (SAS Institute Inc., Cary, NC, USA) was used. Means of colony radial growth, sclerotia size, sclerotia weight, sclerotia number and lesion lengths lesions were calculated using $LSD_{0.01}$.

3. Results and Discussion

3.1. Symptoms of white mold on globe artichoke in the field

Plants infected by white mold (*S. sclerotiorum*) initially showed wilting symptoms. Then, rotted areas were observed on the stalks. The fungus progressed from crown to upper parts of the stalks. As a result of entire rotting of the stalks, plants lodged and fell over the ground. When the infected stalks were cut longitudinally, sclerotia were observed inside of those stalks (Figure 1).

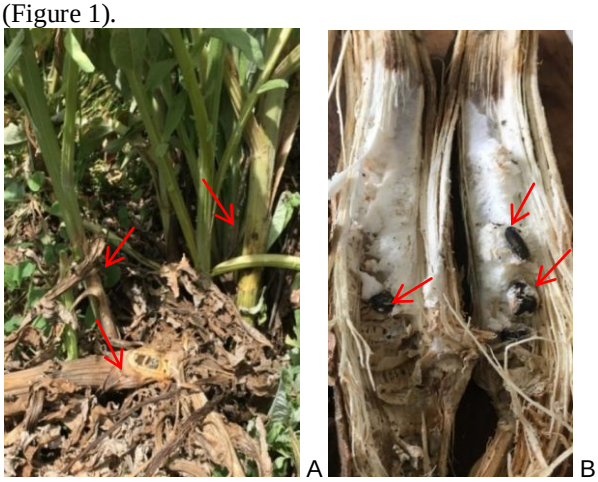

Figure 1

Wilting, crown and stalk rot symptoms caused by *S. sclerotiorum* on globe artichoke plants (A) and sclerotia of *S. sclerotiorum* in stalks of globe artichoke (B) (Gazipaşa, Antalya).

3.2. Pathogenicity of the isolates of S. sclerotiorum

Fifteen days after inoculation, all the isolates (Ser3, Ser4 and Gzp3) initially led to wilting with necrotic tissues and then rot on crown and stems of the globe artichoke seedlings (Figure 2). The isolates were re-isolated from those necrotic tissues and their pathogenicity to globe artichoke plants was confirmed. In the pathogenicity test, globe artichoke seedlings were severely affected by infections by the isolates of *S. sclerotiorum* (Figure 2).

Figure 2

Wilting symptoms (death of lower shoots with red arrows) of seedling of cv. Bayrampaşa inoculated with Gzp3 isolate of *S. sclerotiorum* (left) and non-inoculated (control) seedling (right).

3.3. Morphological features of isolates of S. sclerotiorum

Hyphae of all isolates of *S. sclerotiorum* were septate, branched and transparent in color (Figure 3). These are typical features of hyphae of *S. sclerotiorum* described by Bolton et al (2006).

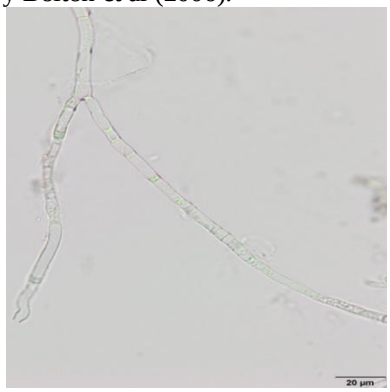

Figure 3

Hyphae of Ser3 isolate of *S. sclerotiorum*

Colonies of the isolates were white to off-white. Colony pattern of Ser3 and Ser4 isolates were similar in forming loose mycelial growth. However, Gzp3 isolate displayed dense velvety aerial mycelia in the center of the petri. Five-six days later, initially mycelium mats appeared as drop like and then became rigid and darkish-black in color. Shapes of the sclerotia of the isolates were irregular to globe (Figure 4).

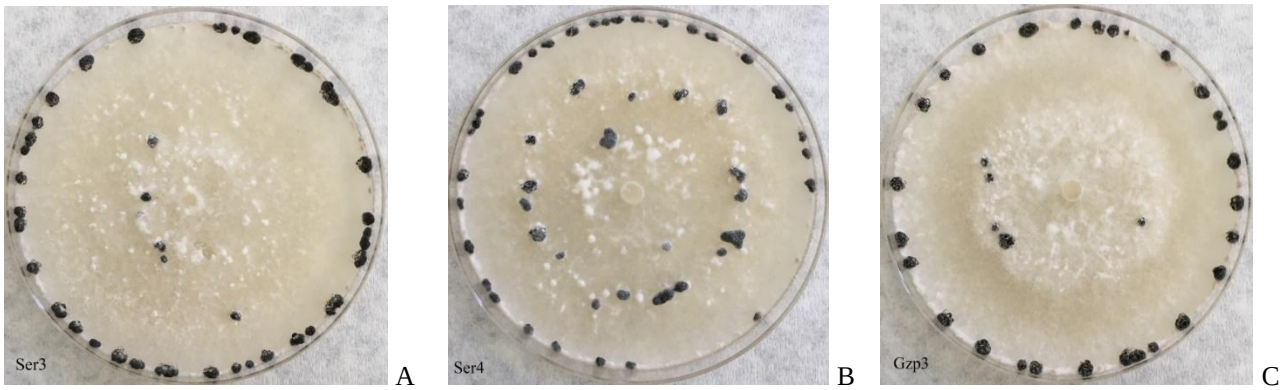

Figure 4
Colony development and sclerotia formation of isolate Ser3 (A), Ser4 (B) and Gzp3 (C) on PDA

Significant differences were found in colony radial growth, sclerotia size, sclerotia weight and sclerotia number per petri (Table 1).

Colony radial growths of Gzp3 and Ser3 isolates were not different statistically (P<0.01). However, the least colony radial growth, 37.42 mm/48 h, was detected in Ser4 isolate. Mean of sclerotia size of Gzp3 isolate was the largest (2.62 mm), while mean of sclerotia sizes of the other isolates were not different statistically (P<0.01). Similarly, mean of sclerotia weight of Gzp3 isolate was 18.63 mg, whereas mean of sclerotia weights of the other isolates were not different statistically (P<0.01). As for sclerotia number, differences in sclerotia numbers of all the isolates were significant (P<0.01). Mean of sclerotia numbers of Gzp3, Ser4 and Ser3 isolates were 23.25, 40.25, 50.25, respectively (Table 2).

Table 1
Variance analysis of colony radial growth, sclerotia size, sclerotia weight and sclerotia number per petri

Source	Degree of freedom	Colony radial growth (mean of squares)	Sclerotia size (mean of squares)	Sclerotia weight (mean of squares)	Sclerotia number per petri (mean of squares)
Isolate	2	100.053**	1.618**	104.513**	745.333**
Error	9	0.293	0.049	2.896	18.694
Total	11	11	11	11	11
CV(%)		1.25	11.69	13.29	11.40

**Significant at P<0.01

Table 2
Colony radial growth, sclerotia size, sclerotia weight and sclerotia number of *S. sclerotiorum* isolates

Isolates	Colony radial growth (mm/48 h)	Sclerotia size (mm)	Sclerotia weight (mg)	Sclerotia number per petri
Gzp3	46.62 a	2.62 a	18.63 a	23.25 c
Ser3	45.42 a	1.43 b	9.11 b	50.25 a
Ser4	37.42 b	1.64 b	10.65 b	40.25 b
LSD	1.24	0.51	3.91	9.93

Values within each column above are means of four replications and the same letters in each column did not differ statistically (1%)

3.4. Mycelial compatibility groupings (MCGs)

In mycelial compatibility, there is no formation of either a distinctive barrier or aerial mycelial development in the interaction zone of colonies. In the assessment of mycelial compatibility, PDA containing red food coloring was used to distinguish compatibility. As a result of the pairings, distinct barriers composed of aerial mycelial developments occurred in the interaction zones of the isolates. Thus, there was no mycelial compatibility between each isolate, which was also confirmed with emergence of red distinct line in the interaction zones (Figure 5).

3.5. Phylogenetic analysis

ITS sequence sizes of the isolates [(MH593866; Ser3), (MH593867; Ser4) and (MH593868; Gzp3] were 478, 460, 470 bp, respectively. The phylogenetic tree was constructed using other *S. sclerotiorum* isolates displaying 100% homology in the genbank with our isolates. In the phylogenetic tree, all of our isolates (MH593866, MH593867 and MH593868) of *S. sclerotinia* were on the different clades (Figure 6), which indicated genetic variability of the isolates obtained from globe artichoke growing locations of the Western Mediterranean Region of Turkey.

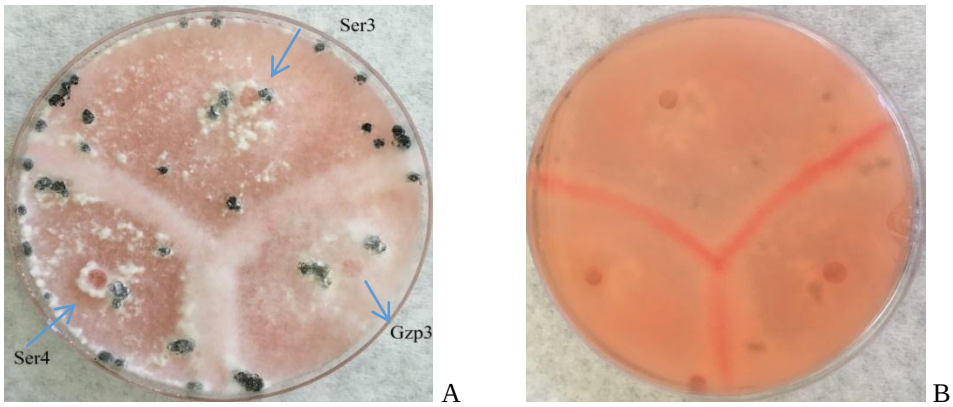

Figure 5
Mycelial compatibility grouping of the isolates of *S. sclerotiorum*, surface side (A) and reverse side (B)

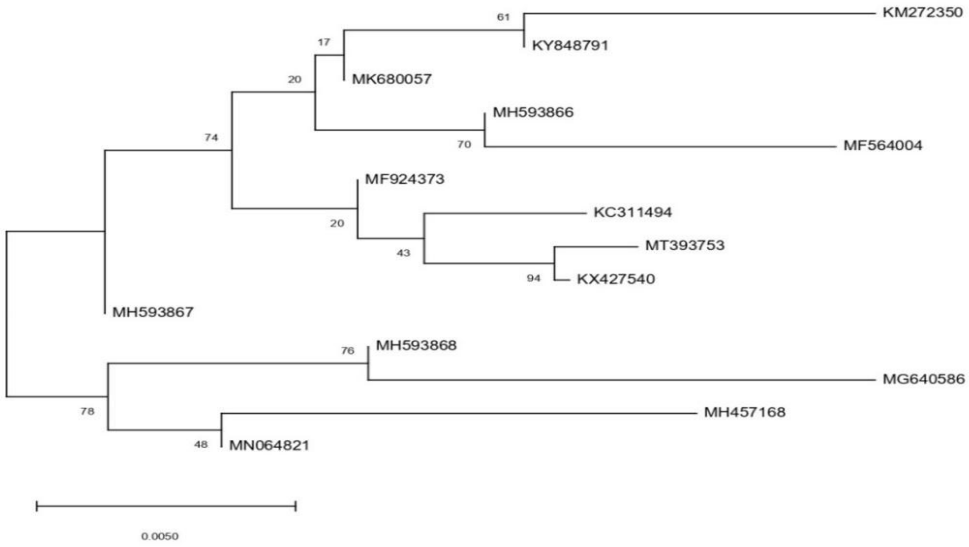

Figure 6
Phylogenetic tree constructed with isolates of *S. sclerotiorum* showing 100% homology in the genbank with our isolates (MH593866, MH593867 and MH593868)

3.6. Virulence of the isolates of S. sclerotiorum

From the point of contact of mycelial plugs of each isolate, lesions (necrotic tissues) occurred on the detached leaves in petri plates. Lesion lengths varied depending on each isolate (Figure 7).

Significant ($P<0.01$) differences occurred in lesion lengths of the isolates on the detached leaves of globe artichoke (Table 3).

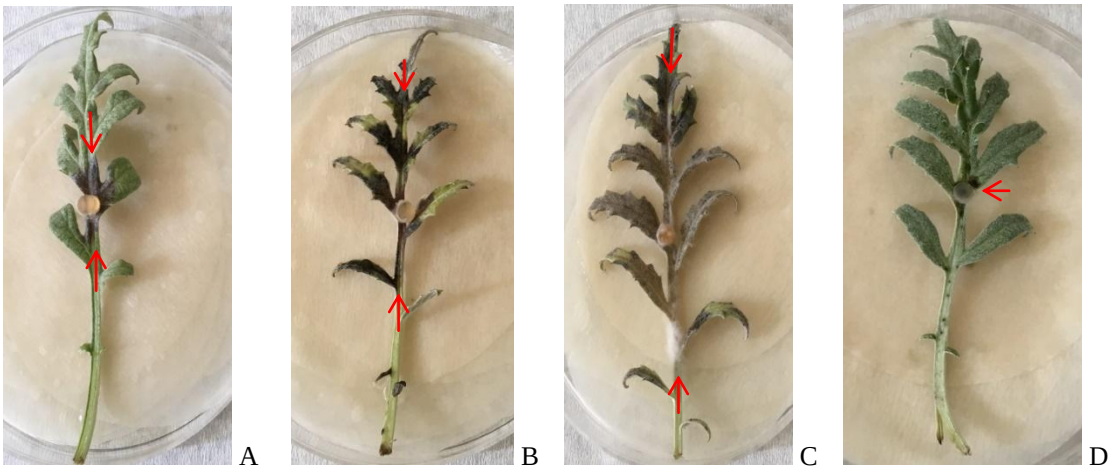

Figure 7
Lesion lengths caused by the isolates [Ser4 (A), Ser3 (B) and Gzp3 (C)] of *S. sclerotiorum* on leaves of globe artichoke (cv. Bayrampaşa) and no lesion development on the control (D)

Table 3
Variance analysis of lesion lengths caused by the isolates of *S. sclerotiorum* on detached leaves in petri

Source	DF	Mean of squares	F value	P>F
Isolate	2	34.543**	661.47	<0.01
Error	6	0.052		
Total	8			
CV(%)	4.6			

**Significant at P<0.01

Mean lesion length caused by each isolate on the detached leaves was significantly (P<0.01) different from each other, indicating difference in virulence of each isolate. Based on this comparison, it was concluded that the most virulent isolate was Gzp3 forming 8.60 cm mean lesion length. However, the other isolates, Ser3 and Ser4, caused 4.16 and 1.93 cm mean lesion lengths, respectively (Table 4).

Table 4
Comparison of mean lesion lengths caused by each isolate of *S. sclerotiorum* on detached leaves of cv. Bayrampaşa

Isolates	Mean lesion lengths on detached leaves in petri
Gzp3	8.60 a
Ser3	4.16 b
Ser4	1.93 c

$LSD_{0.01}$: 0.69

If sclerotia of *S. sclerotiorum* germinate myceliogenically, they form hyphae that infect root, crown and stalks of host plants, which is called basal infection (Lane et al 2018). In our study, this type of infection of *S. sclerotiorum* was detected on globe artichoke plants in the field. Similarly, Brosten & Sands (1986) found that *S. sclerotiorum* was pathogenic to thistle (*Cirsium arvense* L.; Asteraceae), causing crown and root rot and death of shoots of thistle in Canada. These findings might indicate that *S. sclerotiorum* may be prone to induce basal infection on hosts from asteraceae. However, it may not be associated with the host. Because, when sclerotia of *S. sclerotiorum* germinate carpogenically, they produce ascospores that act as an airborne manner by infecting above ground parts of host (Foley et al 2016). For example, in this way, the fungus causes pod rot on bush bean, fruit rot on garden pea, head rot on cauliflower, blossom rot on Salvia (Rahman et al 2020). In this context, environmental conditions in particular moisture and temperature are primary factors affecting carpogenic or myceliogenic germination of sclerotia (Smolińska & Kowalska 2018).

Mycelial compatibility is the capability of two fungi to constitute one continuous colony with anastomoses. If mycelia of two fungi don not anastomose, a distinct reaction line occurs in the interaction zone of the two fungi, which implies incompatible reaction (Schafer & Kohn 2006). Likewise, in our study, there were distinct reaction lines among three isolates of *S. sclerotiorum*. This indicates mycelial incompatibility of all the isolates. This is also means that there is a genetic variability in our isolates. Because, mycelial compatibility/incompatibility is a way of establishment of genetic variability of *S. sclerotiorum* isolates (Kohn et al 1990). Moreover, in our study, in the phylogenetic tree, all the isolates are on different clades, indicating genetic variability of the *S. sclerotiorum* isolates in globe artichoke. Similarly, Mert-Türk et al (2007) found a high genetic diversity among *S. sclerotiorum* isolates obtained from oilseed rape fields in the Çanakkale Province of Turkey. Yanar & Onaran (2011) also detected a wide range of mycelial incom-

patibility groups among *S. sclerotiorum* isolates from cucumber in Kumluca, Finike and Demre locations of Antalya Province of Turkey. These are supported our findings. In addition, genetic diversity of *S. sclerotiorum* was reported on various crops and regions around world (e.g. Barari et al 2012; Liu et al 2018; Mahalingam et al 2020). Outcrossing and evolutionary potential of the fungus might play a significant role in its genetic diversity (Mert-Türk et al 2007). Genetic diversity of *S. sclerotiorum* isolates might be also associated with coinfection of the fungus. In this regard, Sexton et al (2006) reported that even a single canola stem could be infected by multiple isolates of *S. sclerotiorum*, inducing outcrossing/recombination and consequently emergence of new genotypes.

In our study, based on mean lesion length caused by each isolate, virulence levels of the isolates of *S. sclerotiorum* were determined. Difference in virulence of each isolate was significant ($P<0.01$). Oxalic acid, cell wall-degrading enzymes and other secreted proteins have primary role in virulence of *S. sclerotiorum* (McCaghey et al 2019). There was a significant positive correlation between the lesion length caused by *S. sclerotiorum* on sunflower leaves and oxalic acid production of the fungus (Liu et al 2018). These justified the method we used in determining virulence of the isolates. In the detached leaf technique, measurement of lesion length can be done easily. Moreover, comparison on the basis of this measurement gives accurate results in determining virulence of *S. sclerotiorum* isolates and evaluation of host reactions as well. Apart from this, there are numerious studies reporting virulence differences of *S. sclerotiorum* isolates in various crops (soybean, cucumber, canola, drybran, cabbage, pear, rapeseed, sunflower, dry bean, pinto bean and tomato) (Kull et al 2004; Davar et al 2011; Otto-Hanson et al 2011; Yanar & Onaran 2011; Barari et al 2012; Karimi et al 2012). *S. sclerotiorum* has a broad-range of pathogenicity mechanisms enabling infection of a great number of host (Sexton et al 2006). In addition to soilborne and airborne nature, that *S. sclerotiorum* could be spread to long distance through sunflower seeds, indicating seedborne nature of the fungus as well (Zancan et al 2015). Therefore, seeds might also harbor mycelium of *S. sclerotiorum* and constitute a risk factor in spreading of the fungus.

4. Conclusions

Limited information is present about *S. sclerotiorum* on globe artichoke. With our study, isolates of the fungus were characterized in detail for the first time.

Sclerotinia sclerotiorum is a fungus which infects numerous agricultural crops. However, management of the fungus through either cultural or chemical way is hard in any agricultural crop. Because, the fungus has multiple pathogenesis mechanisms to overcome its host and long term survival structures (sclerotia). Therefore, using host resistance is the only plausible approach in the management of the fungus. Selecting virulent isolates of *S. sclerotiorum* is important for evaluation of globe artichoke genotypes for resistance to white mold disease. In this regard, virulent Gzp3 isolate can be used as inoculum source for screening globe artichoke genotypes against *S. sclerotiorum* in further studies..

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