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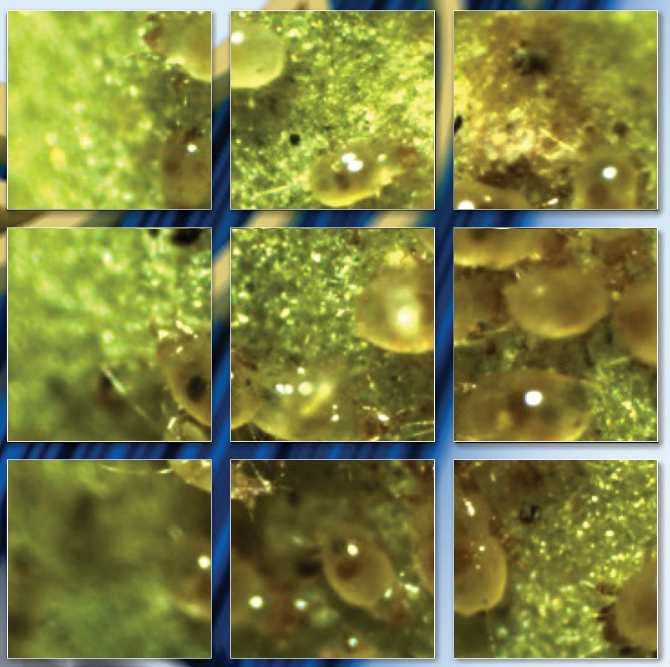

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Determination of Genotype x Environment Interactions of Some Chickpea (*Cicer arietinum* L.) Genotypes by Using Different Stability Methods

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ABSTRACT

This study was carried out to determine the productive responses of 10 chickpeas (*Cicer arietinum* L.) genotypes to different places and years. Hasanbey, Aksu, Seckin, Damla 89, Gulumser, Cagatay, Sezenbey, Inci, Gokce and Uzunlu 99 chickpea genotypes were used as plant material. This research was conducted in Yozgat, Kirikkale and Kirsehir Provinces of Turkey in 2014, 2015 and 2016. The experimental design was a randomized block with 4 replicates. Environmental variance, variation coefficient, ecovalance, stability variance, superiority measure, regression coefficient, deviation from regression and coefficient of determination methods were used for stability calculations. Aksu genotype had the highest stability level, whereas Seckin, Damla 89 and Uzunlu 99 chickpea genotypes also successfully grown with respect to stability parameters. Cagatay chickpea genotype showed the highest yield potential, if grown in ideal environmental conditions. To conclude, the ideal yield would be obtained in the event that the requirements of if the genotypes are fulfilled by desired environmental conditions.

Keywords: Chickpea; Genotype; Environment; Location; Stability; Yield

1. Introduction

Chickpea has been an important legume plant for Turkey, especially in Central Anatolia Region including Kirikkale, Yozgat and Kirsehir, consisting of 12.4% of the cultivation area of Turkey. It has been an important source of dietary protein for human nutrition. As well known, chickpea is self-pollinated, diploid annual grain legume crop (Babagil 2013). Legumes restore soil structure and fertility through biological nitrogen fixation as well as conserving, and improving physical properties of soil via their deep root system and leaving a quite amount of biomass (i.e., nitrogen) to the soil from their leaves due to falling from pulse crops, which will reach to 40 kg N ha^{-1} (Singh 2016). Local farmers have used local populations and they have been reluctant to switch to other populations for many years. Their local populations can be only sowing in summer season and are highly susceptible to anthracnose (*Ascochyta rabiei* (pass.) Labr.). Anthracnose emerges especially in heavy spring

rains and causes huge production losses. Although the studies have shown that sowing chickpeas during fall season yield successful results, it takes time to adapt sowing season. Chickpeas do not resist cold, which is the biggest challenge for fall sowing (Acikgoz et al 2009). Changes in the environment have been important determinants in genotypic performance, identifying the genotypes that can tolerate the changes in the environment is important (Singh & Bejiga 1990).

The main production goal in legume production has been seed yield and, thus, it is desired to get the sustainable high yield production results with related agronomic properties. The components of genotype x environment interaction have been recommended for commercial cultivation to get higher yields (Singh et al 2010).

The quantitative properties, such as grain yield, in different plant genotypes grown in a wide environment vary from one environment to another (Altinbas & Sepetoglu 1994). This phenomenon leads to get different production results from the genotype x environment interactions in different cultivation conditions (Kilic 2014). The effects of genotype x environment interaction at significant levels reduce the relationship between genotypic values, preventing the genetic progression expected in breeding, which aim to breed high-quality genotypes (Comstock & Moll 1963).

Yadav et al (2014) determined that genotype x environment interaction was statistically significant with respect to the studied parameters. High productivity and adaptability to environment depend on the physiological responses of cultivars used in certain environmental conditions (Costa et al 2004). Atta & Shah (2009) found significant differences in grain yields among genotypes, attributed to these differences to the magnitude of genotypes responses to the environments. According to Farshadfar et al (2011) found out that the environmental effect on yield was 86.44%, whereas the effects of genotype and genotype x environment interaction were only 2.48% and 11.08%, respectively. Moreover, breeding genotypes that tolerate environmental conditions has been the cheapest way to control possible negative outcomes and minimizes yield losses (Tsenov et al 2015).

This study was aimed to determine the productive responses of 10 different chickpea genotypes in 3 different environments during 3 years by using different stability parameters.

2. Material and Methods

For the current study, 10 registered chickpea genotypes (*Cicer arietinum* L.) developed by Turkish Research Institutes were used. These were Hasanbey, Seckin, Inci (Eastern Mediterranean Agricultural Research Institute); Cagatay, Sezenbey, Damla 89, Gulumser (Black Sea Agricultural Research Institute); Gokce, Uzunlu 99 (Field Crops Central Research Institute) and Aksu (East Mediterranean Transitional Zone Agricultural Research of Institute) chickpea genotypes. This study was conducted in the locations of Sarikaya/ Yozgat, Keskin/Kirikkale and Center of Kirsehir in Turkey during the period of 2014 and 2016. Altitudes of locations were between 800 and 1300 m. Climate data (Table 1) showed that these three years were similar with respect to the mean monthly temperature and relative humidity. Total amount of precipitation in April and March in all three locations and years was lower than that of rainfall seasons of all three locations and three years. In July, it was excessive.

The trials in all three locations were carried out in a randomized block experimental design with 4 replicates. Seedings were manually performed on rows determined with markers. The trial parcels were made up of 4 rows with 45-cm inter-row spacing and 8-cm intra-row spacing and total parcel area was 5 m x 1.8 m= 9 m^{-2}. Harvest area was determined to be 4 m x 0.9 m= 3.6 m^2. The sowing processes were modified according to climate conditions. All planting processes took place in March. Sowings were done in March on 17-19 days, on 20-22 days and on 18-20 days, respective to Yozgat, Kirikkale and Kirsehir locations. Harvesting times were in July on 13-15 days, in March on 10-12 days and in

Table 1- Climate data for Keskin, Sarikaya and Kirsehir*

	Months	*Average temperature (^{0}C)*				*Total rainfall (mm)*				*Average relative humidity (%)*			
		2014	*2015*	*2016*	*Long term*	*2014*	*2015*	*2016*	*Long term*	*2014*	*2015*	*2016*	*Long term*
Keskin/ Kirikkale	March	6.4	5.4	7.3	6.9	67.0	52.0	61.6	35.9	64.2	74.2	61.9	66.3
	April	11.8	8.0	13.7	12.2	7.2	18.0	22.2	44.8	49.3	58.9	45.0	50.7
	May	14.1	15.0	14.0	16.9	61.6	27.9	58.0	51.0	63.2	51.1	65.4	58.4
	June	17.6	17.6	20.3	21.2	35.8	75.4	18.8	36.8	55.2	68.0	50.5	63.5
	July	23.5	22.1	23.0	24.6	1.4	0.0	1.2	10.9	38.1	45.2	41.7	42.4
Sarikaya/ Yozgat	March	7.0	5.7	6.7	2.8	86.4	74.9	38.4	64.7	62.0	73.2	60.1	63.5
	April	12.4	7.7	13.4	8.3	14.2	29.6	20.2	59.4	52.3	64.0	44.8	55.6
	May	14.9	14.9	13.9	13.1	50.2	54.4	57.9	66.8	61.5	60.3	64.6	60.7
	June	17.9	16.9	19.1	16.7	46.4	43.5	8.9	43.2	58.3	73.0	58.6	64.9
	July	22.9	20.4	21.2	19.5	0.5	2.1	0.0	12.0	45.0	55.8	50.8	52.3
Center/ Kirsehir	March	7.4	7.0	7.1	5.2	56.0	87.8	44.8	39.0	64.4	76.2	60.7	67.5
	April	13.2	8.8	13.8	10.7	23.2	26.4	24.0	42.2	54.8	66.2	47.4	59.7
	May	16.3	16.0	14.9	15.5	46.6	27.4	98.2	44.8	61.3	58.1	63.7	56.2
	June	19.9	18.4	21.0	19.7	36.0	141.1	18.5	33.9	54.1	66.9	53.0	50.9
	July	25.5	23.0	24.2	23.1	13.4	20.3	5.8	6.6	39.2	47.0	42.5	38.4

*, Turkish State Meteorological Service

March on 7-9 days, respective to Yozgat, Kirikkale and Kirsehir locations.

In sowing time at all locations, 25 kg ha^{-1} pure nitrogen and 50 kg ha^{-1} pure phosphorus fertilizers were used. During the trial, among the stability parameters, environmental variance (Lin et al 1996), variation coefficient (Francis & Kannenberg 1978), ecovalance (Wricke 1962), stability variance (Shukla 1972), superiority measure (Lin & Binns 1988), regression coefficient (Eberhart & Russel 1966), deviation from regression (Becker & Leon 1988) and coefficient of determination (Pinthus 1973) methods were used in stability calculations. From these methods, coefficients and their deviations from regressions were considered to be stable. In addition, environmental and genotype indices were calculated. The results were evaluated by applying variance analysis in accordance with the different years and repeated randomized block experimental design used in the SPSS 17 package program.

3. Results and Discussion

The variance analyses of the experiment are shown in Table 2, revealing that the differences among the years, locations and genotypes and their interactions were statistically significant (P<0.01).

Table 2- Analysis of variance results of chickpea yield for different location and years

Source	*Degree of freedom*	*Mean square*
Year	2	37,915.84**
Location	2	356,415.58**
Year x Location	4	319,348.96**
Genotype	9	31,677.61**
Year x Genotype	18	10,112.34**
Location x Genotype	18	9,980.14**
Year x Location x Genotype	36	8,017.56**
Error	244	1,106.11**
Total	360	
Coefficient of variation	10.32%	

**, P≤0.01

According to Table 2, Year x Location x Genotype interaction was statistically important. Also, it is observed that the observed differences between years seriously affected the properties of the genotypes by combining with the locational characteristics. The efficacies of the Year x Genotype and Location x Genotype interactions had quite importance, showing that these effects were very strong. Both the location and the year had significant impacts on the formation of genotypes, resulting different productive outputs between the present cultivated genotypes. The significant location x genotype interactions with respect to yield were shown on the efficacy of environment on the genotypes by affecting the productive yields of the experimental plants.

This result supports the report of Farshadfar et al (2011) in which they determined that the contribution of environment on yield change was 86.44%. This result is also in line with that of Altınbas & Sepetoglu (1994) in which they stated that the responses of the characters vary depending on the environment. The differences among the cultivars were of great importance because of the fact that all investigated properties of the cultivars showed similar behaviors (Sabanduzen & Akcura 2017). The significance of Genotype x Environment interaction was also determined in the studies of Arshad et al (2003), Bakhsh et al (2006), Abbas et al (2008), Ali & Sarwar (2008) and Karasu et al (2009), on chickpeas, white beans, green peas and soy beans, respectively.

Table 3 shows the yields of the genotypes with respect to the locations in which they were grown for 3 years. Table 3 reveals that, in the Sarikaya/Yozgat location, the highest yield was obtained in Cagatay genotype (1,832.2 kg ha^{-1}), whereas the lowest yield was obtained in Gokce genotype (1,544.6 kg ha^{-1}). In Keskin/Kirikkale location, Cagatay genotype (1,904.3 kg ha^{-1}) was the most prominent genotype and showed the highest yield, while the lowest-yielding genotype was Gokce genotype (1,696.5 kg ha^{-1}).

In Kirsehir location, Cagatay genotype did not reach the performance as reached in the other two locations. The highest yield was obtained in Aksu genotype (1,678.2 kg ha^{-1}). Gokce genotype was more successful in this location and was among the highest-ranking genotypes. The lowest yield was observed in Hasanbey genotype (1,406.8 kg ha^{-1}).

In general, it can be argued that the genotypes demonstrated significant differences among each other and these differences varied depending on the sowed locations. The comparison between the average yields of the locations showed that Sarikaya/Yozgat and Keskin/Kirikkale locations were in the same group, while Center/Kirsehir location was different from the other two locations and had the

Table 3- Yield situations of genotypes according to locations (kg ha^{-1})

Genotypes	*Sarikaya/Yozgat*	*Keskin/Kirikkale*	*Center/Kirsehir*	*Mean*	*Genotype index*
Cagatay	1,832.2	1,904.3	1,532.4	1,756.3	82.9
Aksu	1,755.3	1,761.4	1,678.2	1,731.6	58.4
Seckin	1,675.9	1,774.3	1,621.4	1,690.5	17.1
Uzunlu 99	1,719.3	1,709.6	1,613.2	1,680.7	7.3
Damla 89	1,700.6	1,713.8	1,621.7	1,678.7	5.3
Gulumser	1,778.6	1,708.2	1,496.3	1,661.0	-12.4
Hasanbey	1,825.9	1,705.3	1,406.8	1,646.0	27.4
Sezenbey	1,764.1	1,697.0	1,467.1	1,642.7	-30.7
Inci	1,623.7	1,714.9	1,564.2	1,634.3	-39.1
Gokce	1,544.6	1,696.5	1,594.8	1,612.0	-61.4
Mean	1,722.0 A	1,738.5 A	1559.6 B	1,673.4	
Environment index	48.6	65.1	-113.8		

lowest yield. This difference is attributable to the ecological factors.

Plant physiology is highly susceptible as a result of their sensitive mechanism of action and it gains further importance when their yield capacities are in question. The degree of their reactions is not only dependent on their genotypic structure but also is affected by factors interacting with the environment and environment (Sehirali & Ozgen 1988; Kabak & Akcura 2017). Therefore, different genotypes in different environments may show different performances (Acikgoz & Acikgoz 1994; Altinbas et al 1999). It was reported that the yield and certain properties of plants showed significant variations depending upon the environment, most likely, affecting the yield at significant levels (Silim & Saxena 1993; Yucel & Mart 2014).

Chickpea can show different phenological reactions or responses to climate conditions. This consequently will affect plant growth and productivity in different way. Additionally, location effect contributed this efficacy. Climate changes will affect early growth and flowering by changing dry matter content, the numbers of fertile and dropped flowers (Garcia Del Moral et al 2003). Rainy conditions in different locations affected the environmental responses of plants. The difference in adaptation abilities of genotypes plus rainy conditions both increased the intense of their environmental responses. However, plants would have eliminated the negative consequents of climate changes when they grew up sufficiently (Saidi et al 2008). In our present study, it can be said that the genotypes affected from environmental factors in lesser extent, showed the better growth performance than the others.

Figure 1 shows the mean yield values of genotypes for experimental years and locations, revealing that Hasanbey, Gulumser and Sezenbey chickpea genotypes showed poor performances in all environments; Cagatay genotype was different from the other genotypes and yielded high in good environments, although its yield decreased in environments where its physiological requirements were not met. Aksu, Seckin, Damla 99 and Uzunlu 99 genotypes maintained their yields under all

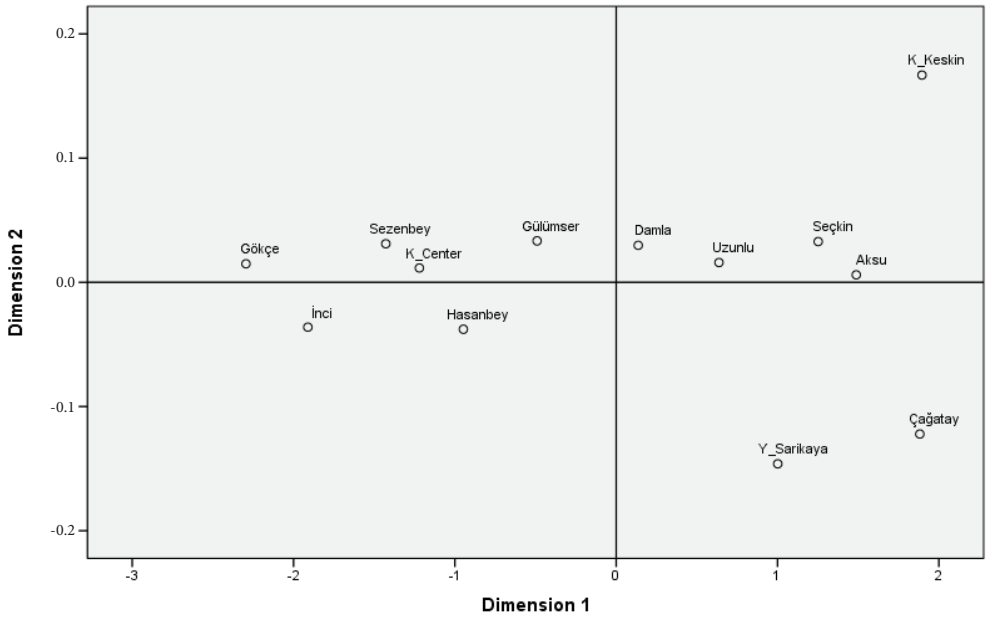

Figure 1- Multi dimentional scaling of location, year and genotypes performance

conditions while Inci and Gokce genotypes maintained their yields at acceptable levels, even under unfavorable conditions.

Table 4 shows the results of the different stability parameters applied to the chickpea genotypes used in the present study. Table 4 reveals that, in the view of the investigated parameters, Aksu, Seckin, Uzunlu 99 and Damla 89 genotypes were more stable than the other genotypes in terms of all years and locations, whereas Hasanbey, Gulumser and Sezenbey genotypes were not stable in any environment or location and showed significant changes, depending on their sowed environments and years.

Regression coefficient and deviation from regression indicated the stability of a cultivar: the closer the regression coefficient was to 1 and the smaller the deviation from regression, the more stable the cultivar. Furthermore, coefficient of determination (R^2) shows how much of the variation in a dependent variable can be explained with the regression equation and therefore, cultivars with higher R^2 values can be accepted as more stable cultivars (Unay et al 1990; Aleksoska et al 2015).

Eberhart & Russel (1966) evaluated the genotypes as stable if their regression coefficients (b_i) are '1.0' and their deviations from regression can be statistically accepted as "(S^2_{di}=0)" and stated that genotypes with higher performances in all environments were desired. Therefore, it can be concluded that the regression coefficient used by Eberhart & Russel (1966) was the same as that of Finlay & Wilkinson (1963).

Table 4- Stability parameters of genotypes for different location and years

Genotypes	$\bar{X}$	S^2_{xi}	b_i	S^2_{di}	R^2	W^2_i	σ^2_i	CV_i	P_i
Hasanbey	164.5	4.26	1.65	3.58	0.86	6.38	0.61	32.1	2.58
Aksu	173.1	1.68	1.08	1.14	0.95	2.18	1.10	24.3	1.14
Seckin	169.0	1.88	0.96	1.06	0.94	2.07	1.06	23.8	1.16
Damla 89	167.8	2.61	0.95	0.94	0.94	2.36	1.21	24.4	1.18
Gulumser	166.0	8.36	0.68	4.26	0.76	7.11	3.16	32.6	3.54
Cagatay	177.0	3.22	0.92	1.20	0.89	2.84	1.28	25.1	2.20
Sezenbey	164.2	7.69	0.69	3.91	0.79	6.74	3.37	35.6	3.94
Inci	163.4	3.56	1.10	1.36	0.88	2.66	2.47	27.1	2.21
Gokce	161.1	3.54	1.09	1.42	0.87	2.71	2.64	27.6	1.96
Uzunlu 99	168.0	2.05	0.94	1.14	0.94	2.11	1.18	23.8	1.29

4. Conclusions

The results of the present study showed that, according to the parametric stability tests, the Aksu cultivar had the highest stability level. Seckin, Damla 89 and Uzunlu 99 chickpea genotypes grew up successfully. Cagatay chickpea genotype has the highest yield potential, if it grew up under proper breeding conditions; however, such conditions cannot be continuously provided and, therefore, successful results cannot be expected from this genotype. Inci and Gokce chickpea genotypes should be considered as the potential successful genotypes. The tendency of the higher yields were observed in the Keskin/Kirikkale location without showing any statistical difference between locations.

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Crown Gall Disease Susceptibility of Some Stone Fruit Rootstocks in Turkey

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ABSTRACT

Rhizobium radiobacter formerly known as *Agrobacterium tumefaciens,* is the most important bacterial pathogen causing crown gall disease on over 750 different plant species including ornamentals, vegetables and fruit trees. This pathogen causes crown galls on root and stems of plants and rarely on, above-soil parts of plants. Biological control is successfully used in disease management; however, such strategies are quite ineffective in disease contaminated soils or seedlings. Thus, growers and scientists are mostly focused on disease resistant cultivars or rootstocks.

In this study, crown gall disease reactions of three widely used rootstocks Garnem, Myrobolan and GF-677 for stone fruit trees were evaluated. A drop of bacterial suspension of 10^9 cfu mL^{-1} were inoculated onto three individual wounds over 90 shoots per rootstock. The weights and sizes of the tumor formed were measured five months after rootstock inoculations. The size of produced galls on rootstocks Garnem, Myrobolan and GF-677 were 0.16-5.28 mm, 0.09-4.42 mm and 0.09-0.36 mm, respectively. However, the weights of the galls varied between 0.02-2.85 g in Garnem, 0.01-1.58 g in Myrobolan and 0.02-0.11 g in GF-677 rootstocks. According to statistical analyses of gall formations, rootstocks were placed in different groups, additionally, none of them were found resistant to crown gall disease. Among the three rootstock tested, the study revealed that Garnem was highly susceptible, Myrobolan was moderately susceptible and GF-677 rootstock was less susceptible to crown gall disease. When an orchard will be intended to be newly planted with stone fruit trees, hence, GF-677 type rootstock would be a good choice to be preferred for planting in new orchards.

Keywords: *Rhizobium radiobacter*; Rootstock; Gall; Reaction

1. Introduction

Crown gall disease agent, *Rhizobium radiobacter* (synonym *Agrobacterium tumefaciens)* causes economically deleterious damages in over 750 plant species including woody plants like pome and stone fruit and nuts and ornamental woody crops like roses, as well as on grapevines and raspberries (Kado 2002). This disease is of great significance for nurseries and results in scrapping of diseased trees reaching 80% or more (Moore 1990). Galls are formed on woody roots and at the base or crown of the infected plants. Gram-negative bacterium *Rhizobium radiobacter* is a rhizoplane pathogen, strictly aerobic and non-fastidious. Fresh crown

galls are spheroidal, white to cream and smooth. As these galls age in years, the enlarged galls are crumbled and become brown and black. Aged galls are easily detached and diseased plant tissues are dead. Best sources of this pathogen are fresh galls, when there aren't any alive bacterium in plant tissues (Gelvin 2000). Crown gall disease has threatened the nurseries of stone (Aysan et al 2003), pome (Soylu et al 2011) fruits and rose (Aysan et al 2003) growers in Turkey since plants with tumors are unsalable and have to be discarded. Soil fumigation is a way to control, but it does not prevent entirely and use of chemicals is limited with new regulations, (Bliss et al 1999; Moriya et al 2008). Biological control using *Agrobacterium radiobacter* strain K84 or K1026 can provide a potential protection from the disease (Cooksey & Moore 1982; Farrand 1990; Rhouma et al 2004). Recently, plantations of stone fruit trees that are fruiting early in the season increased in Turkey. Since the growers prefer seedlings in polythene bags or pots for plantations, it is impossible to use biological control agents for disease management. Use of resistant rootstocks in cultivation is the most effective strategy for disease control. In a field experiment, crown gall disease decreased with the usage of crown-gall resistant grapevine rootstocks (Sule & Burr 1998). Many studies on crown gall disease resistance have been reported in woody plants, including fruit trees, grapevine and aspen (Szegedi et al 1984; Bliss et al 1999; Mahmoodzadeh et al 2004). A crown gall resistant wild apple was identified by Moriya et al (2008). This study suggested that a possible major gene could control the resistance in that wild apple, but probably, an additional minor genes were also responsible for the resistance. The knowledge about the crown gall resistance mechanisms is limited. A study reported that the crown gall reactions of aspen (*Populus* sp.) cultivars were related to differences in sensitivity to cytokinin (Beneddra et al 1996). The concentration of the *Rhizobium radiobacter* suspension can influence the gall occurrence in tested plant shoots. Moriya et al (2008) demonstrated that when the suspensions of strain CG8331 at a concentration of 10^7 cfu mL^{-1} used in inoculation tests, the concentration was inadequate for gall occurrence in the tested genotypes. Thus, they have adapted to 10^9 cfu mL^{-1} concentration of suspensions for evaluating crown gall disease resistance. Galls developed on genotypes with a frequency from 0.31 to 6.44. Crown gall production often occurs on wounded plants. Wounds are the main openings for the pathogen to infect the plants. However, when the bacteria enter into the host plant, disease reaction may vary among several susceptible plants. Recently, stone fruit plantations including plum, apricot, nectarine and peaches have increased in the Eastern Mediterranean Region of Turkey. In these plantations, due to some advantages of rootstocks such as resistance to nematodes, calcareous soil and groundwater, growers mostly use hybrids of *Prunus persica* x *Prunus amygdalus* GF-677 and *Prunus dulcis x Prunus persica* Garnem in nectarine and peach and Myrobolan for plum and apricot trees as rootstocks. In Turkey, the resistance of these rootstocks to *Rhizobium radiobacter* is unknown. The objective of this study was to screen three individual rootstocks used in stone fruit plantations for reaction to *Rhizobium radiobacter*. Evaluations were made according to emerged tumor weights and sizes at inoculated sections of 90 shoots.

2. Material and Methods

2.1. Plant materials

The peach rootstock of GF-677, almond x peach hybrid rootstock of Garnem and plum and apricot rootstock of Myrobolan were supplied from a commercial nursery (Vitroplant, 01120, Adana, Turkey) in December 2014 (Table 1). Since these three rootstocks were used for stone fruit trees of the region, they were tested for crown gall resistance. They were grown in pots containing sterilized soil. All seedlings were kept in a glasshouse at Plant Protection Department Research and Development Station of Cukurova University. The temperature of the glasshouse was 15-20 °C. Other cultural practices such as irrigation, fertilization, pest management were routinely performed.

Table 1- Geographic and genetic origin of rootstocks

Rootstock name	*Originated from*	*Genetic origin*
GF 677	France	*Prunus persica x Prunus amygdalus*
Garnem	Spain	*Prunus dulcis x Prunus persica*
Myrobolan	USA	*Prunus cerasifera*

2.2. Preparation of bacterial inoculum

Rhizobium radiobacter strain ECG-1 isolated from an infected apple tree was used in resistance studies (Soylu et al 2011). Bacterial pathogen was grown on King's Medium B (King B) (10 g L^{-1} proteose pepton, 10 g L^{-1} tryptone, 1.5 g L^{-1} $MgSO_4.7H_2O$, 1.5 g L^{-1} K_2HPO_4, 10 mL L^{-1} glycerol, 15 g L^{-1} agar, pH 7.2) for 48 h at 25 ºC. The bacterium were transferred in a glass tube containing 12 mL of the growth medium and preserved at +4 ºC for short use. Additionally, the bacterial culture was stored at -20 ºC in 20% glycerol for longer storage. Before inoculation to shoots, the prepared bacterial suspension was measured using a spectrophotometer with dilution plate technique (Klement et al 1990) and adjusted to 10^9 colony forming unit (cfu) mL^{-1} for inoculation.

2.3. Pathogenicity of Rhizobium radiobacter strain ECG-1

The pathogenicity of *Rhizobium radiobacter* strain ECG-1 was tested on kalanchoe (*Kalanchoe daigramontiana*) seedlings and fresh carrot (*Daucus carota*) slices. Kalanchoe plants were grown in pots in a chamber room under the following conditions: 16 h light/8 h dark. The average height of plants used for inoculations was 10 cm. *Rhizobium radiobacter* strain ECG-1 yielded onto KB at 25 ºC for 48 h. The bacterium was inoculated by wounding ten individual kalanchoe leaves and stems using sterilized toothpicks. Inoculated kalanchoe seedlings were kept at 25 ºC under 16 h light/8 h dark photoperiod for 45 days to monitor gall formations. Carrot tubers about 20 cm height were disinfected with 70% ethanol for 5 minutes and right after rinsed in distilled water. Three carrot tubers were peeled with a sterile knife and 5.0 mm slices were placed onto sterilized blotters in 90 mm petri dishes. Bacterial culture was inoculated onto the slices by wounding. All petri dishes including carrot slices were moisturized with 1.5 mL sterile distilled water and kept in an incubator at 25 ºC for 10-14 days. Sterile distilled water was inoculated to kalanchoe seedlings and carrot slices as control treatment.

2.4. Rootstock inoculations

The methods used by Bliss et al (1999), Moriya et al (2008) and Moriya et al (2010) were modified for rootstock inoculations. The inoculation was conducted in December, 2014 while all the new shoots were 25-30 cm long. The shoots were inoculated from three sites of each potted plant at the third and fourth internodes by wounding tissues (0.5x0.5x0.5 cm) with a scalpel. One drop of $6x10^9$ cfu mL^{-1} (OD_{600}= 0.2) bacterial suspension (20 µL) was injected into each wound. 30 potted plants of each rootstock were included in the study. Since three sites of each potted plant was used for inoculations, in total, 90 sites of each rootstock was inoculated. Each wound was covered with parafilm to avoid drying of the bacterial suspension. Distilled water was injected into control plant wounds. Inoculated plants were kept at 15-20 ºC in a glasshouse of Cukurova University.

2.5. Evaluation of crown gall resistance of rootstocks

Five months after inoculation, each inoculated site was visually monitored and the diameter of formed galls were measured for each wound on plants and galls were picked to be weighed. Statistical analyses were conducted using analysis of variance (ANOVA) with CoStat Statistics Software (CoHort Software, Pacific Grove, CA, U.S.A. Version 6.4). Treatment means were compared with the Duncan's

multiple range test (P≤0.05). Resistance levels of each rootstock were classified into three groups as described by Sule et al (1994) and Moriya et al (2008) according to statistical analyses of gall sizes and weights.

3. Results and Discussion

The *Rhizobium radiobacter* strain ECG-1 formed galls on wounded kalanchoe leaves and stems 45 days after inoculations. Gall formations were observed on carrot slices 10-14 days after inoculations. No gall formations were produced on control seedlings and carrot slices.

Five months after inoculations, *Rhizobium radiobacter* strain ECG-1 formed typical crown galls on wounded tissues of three individual rootstocks. Table 2 shows the results of mean gall sizes and weights after shoot inoculations with *Rhizobium radiobacter* isolated from apple. Crown gall disease resistance levels of three individual rootstocks was defined by statistical analysis and marked with different letters (Table 2). The mean gall sizes and weights ranged between 0.20-2.35 mm and between 0.05-1.04 g in all tested rootstocks. *Rhizobium radiobacter* strain ECG-1 induced large tumors as between 0.16-5.28 mm on Garnem rootstocks and the weights of tumors were between 0.02-2.85 g. Gall formations on Garnem genotype were significantly different from the other rootstocks. Moderate tumors were induced by *R.radiobacter* on inoculated shoots of Myrobolan rootstock up to 4.42 mm in size and 1.58 g in weight. According to statistical evaluation, gall formations on Myrobolan were significantly different from the other rootstocks. GF-677 rootstocks had small galls with 0.09-0.36 mm in size and 0.02-0.11 g in weight. In statistical analysis on gall sizes and weights, GF-677 genotypes could be placed as a separate group different from Garnem and Myrobolan rootstocks. According to statistical analysis, the results demonstrated that Garnem rootstock was highly susceptible rootstock, however, Myrobolan was moderately susceptible and GF-677 was the least susceptible rootstocks to crown gall disease caused by *Rhizobium radiobacter* (Table 2). Pierronnet & Salesses (1996) tested 87 *Prunus* genotypes and reported that *Prunus cerasifera* including Myrobolan rootstock were susceptible to crown gall. Zoina & Raio (1999) tested six peach rootstocks (Barrier 1, GF677, Mariana GF8-1, Mr.S.2/5 and Peach seedling) for crown gall disease susceptibility levels by using several strains of *Agrobacterium tumefaciens*. Plants were wounded and inoculated in the roots and shoots, two inoculation methods were highly correlated. Except Mr.S.2/5, all the tested rootstocks showed high sensitivity to crown gall disease. Thomidis et al (2005) evaluated the susceptibility of five *Prunus* rootstocks (GF677, Antafuel, St. Julien 655/2, Peach seedling and Gisela 5) to *Agrobacterium tumefaciens* and the virulence of three individual *Agrobacterium tumefaciens* isolates from *Prunus* species. The researchers indicated that none of the tested rootstocks was resistant to crown gall disease and among all tested rootstocks, St. Julien 655/2 was the most resistant.

Among the different strains used, the level of susceptibility did not vary with the tested rootstocks. Rhouma et al (2005) also reported the same results indicating that almond and peach rootstocks were highly susceptible to *A. tumefaciens*. In the present study, according to produced mean gall sizes and weights, three tested rootstocks Garnem, Myrobolan

Table 2- Crown gall sizes and weights of Garnem, Myrobolan and GF-677 rootstocks after shoot inoculations with *Rhizobium radiobacter* strain ECG-1

Rootstocks	*Range of gall size*	*Mean gall size (mm)*	*Range of gall weight*	*Mean gall weight (g)*
Garnem	0.16-5.28	2.35[a]	0.02-2.85	1.04[a]
Myrobolan	0.09-4.42	1.68[b]	0.01-1.58	0.59[b]
GF-677	0.09-0.36	0.20[c]	0.02-0.11	0.05[c]

and GF677 were susceptible to *Rhizobium radiobacter*, like indicated in previous works. Various authors (Bush & Pueppke 1991; Sule & Burr 1998; Zoina & Raio 1999) established that less susceptible plant genotypes permit the transfer of T-DNA, but these are failed in T-DNA integration. Integration of T-DNA is critical for tumor inducing. This limited tumor induction may be caused by a low rate of T-DNA integration in host plant.

The susceptibility level of crown gall disease can vary among different strains and concentration of the suspensions. Moriya et al (2008) indicated that concentration 10^7 cfu mL^{-1} of *A. tumefaciens* suspensions was inadequate for evaluating disease sensitivity of apple rootstocks. Researchers suggested 10^9 cfu mL^{-1} suspension for the evaluation of crown gall resistance. This high concentration (10^9 cfu mL^{-1}) was also adapted in this study for better results of an inoculation test to evaluate *Rhizobium radiobacter* sensitivity of rootstocks widely used in Turkey. Galls were produced at 90 individual inoculation sites of all tested rootstocks.

Garnem, Myrobolan and GF677 are widely used rootstocks in Turkey, however they are susceptible to crown gall like the other stone fruit rootstocks. Several authors were tested rootstocks of GF-677 and Myrobolan for crown gall disease (Pierronnet & Salesses 1996; Bliss et al 1999; Zoina & Raio 1999; Rhouma et al 2005; Thomidis et al 2005). None of studies tested both three rootstocks in the same work. In this study, susceptibility of rootstock Garnem to crown gall disease was also investigated. Current outcomes are probably the first report indicating that the rootstock Garnem was highly susceptible to crown gall infections.

4. Conclusions

Crown gall is one of the most destructive diseases of pome and stone fruits worldwide. Pathogenic bacterium, *Rhizobium radiobacter,* is soil-borne and can be encountered in most soils. Since the pathogen can infect the plants via wounds, use of disease resistant rootstocks enhance the disease control in newly planted orchards. Sources of disease resistance have been reported in some plant species, but the knowledge about the susceptibility level of pome and stone fruit rootstocks is very poor. In this study, crown gall disease reactions of most commonly used rootstocks Garnem, Myrobolan and GF677 in Turkey were tested. Statistically significant differences were observed among three rootstocks with regard to their resistance to the strain of *Rhizobium radiobacter*. None of tested rootstocks was resistant to the disease. Garnem was the most susceptible, Myrobolan was moderately susceptible and GF 677 was the least susceptible to crown gall disease infection.

The virulence of tested *Rhizobium radiobacter* strain suggests that the pathogen was a devastating threat to stone and pome fruit trees grafted onto GF677, Myrobolan and Garnem rootstocks. Although none of the tested rootstocks were resistant to *Rhizobium radiobacter*, the rootstock GF677 would be a good choice to be preferred for growing in Turkey.

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Effects of Alginate Based Coatings with Pomegranate Peel Extract on the Microbial Quality of Mackerel Fillets

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ABSTRACT

In this study, antibacterial effects of the pomegranate peel extract against food pathogens were investigated. Different concentrations of pomegranate peel extract (2.5, 5, 7.5 and 10%), which showed the highest antibacterial effect *in vitro* experiments were added to the alginate film. Mackerel fillets coated with the film solution prepared in this way were stored at 4±1 °C for 13 days. *L. monocytogenes*, total aerobic mesophilic bacteria, total aerobic psychrophilic bacteria, total yeast-mould and enterobacteriaceae counts were determined on 0, 3rd, 8th, and 13th day of storage period.

At the end of the study; it was determined that the pomegranate extract was effective and the inhibition effect was also increased based on the increase in extract concentration. Different concentrations of pomegranate peel extract showed antibacterial effect against *L. monocytogenes, E. coli* O157:H7, and *S. aureus* ($P<0.05$). As a result of 13 days storage of mackerel fillets coated with alginate enriched with 2.5, 5, 7.5 and 10% PPE at 4 °C, the number of *L. monocytogenes* significantly decreased ($P<0.05$). During the storage period total aerobic mesophilic bacteria and enterobacteriaceae number were found to be significantly lower in the experimental groups used pomegranate peel extract in comparison with the control group. As a result, it was determined that pomegranate peel extract has a potential to be used as a natural preservative in seafood products.

Keywords: Pomegranate peel extract; Antimicrobial film; *L. monocytogenes*

1. Introduction

The *Listeria monocytogenes* is a gram-positive, asporous, facultative anaerobic bacterium that causes listeriosis from foodborne diseases. It has been reported that most of the listeriosis outbreaks occur through ready-to-eat foods such as dairy products, salads, seafood, processed meats and poultry (Chan & Wiedmann 2009). Synthetic antimicrobial agents are widely used to prevent the development of pathogenic bacteria such as *L. monocytogenes* in foods, but concerns about the safety of these chemicals are increasing day by day (Owen & Palombo 2007). For this reason, the fruits, vegetables, spices, plants or their extracts that are enrich in terms of phenolic compounds are used to avoid the development of pathogenic or spoilage bacteria and to prolong the shelf life of food (Rawdkuen et al 2012).

Today, the use of agricultural food industry's by-products or wastes has become one of the

environmental and economic priorities. It is known that the roots, peels and leaves of many fruits and vegetables have therapeutic properties (Balasundram et al 2006). It was revealed that processing wastes rich in bioactive compounds can be an alternative of synthetic chemical additives because of their antioxidant and antimicrobial features in many studies (Negi & Jayaprakasha 2003; Kanatt et al 2010).

Pomegranate (*Punica granatum* L.) is a fruit commonly produced in our country, and consumed after being processed into by-products such as both fresh and pomegranate syrup or pomegranate vinegar. PP accounts for almost 50% of the total fruit weight, concurrently it contains elagic acid and its derivatives and bioactive compounds such as alegrate and hexahydroxydiphenic acid (Gullon et al 2016). Pomegranate extract is very rich in terms of phytocompounds such as phenol, tannin and flavonoids (Jurenka 2008). Because of the compounds contained in this fruit, it was stated to be able to use as antioxidants and antimicrobials both in the food industry and in the medicine (Al-Zoreky 2009).

Edible films are thin-bedded, eatable with food, and non-synthetic materials, derived from natural sources that are used to protect food and extend shelf-life (Dursun & Erkan 2009). Antioxidants, antimicrobials, colorants and spices can be added to the edible films to able to achieve functional properties to the products (Emiroğlu et al 2010).

The purpose of this study is to determine the antibacterial effect of the alginate based coatings with pomegranate peel exctracts on the mackerel fillets in refrigerated storage.

2. Material and Methods

2.1. Material

The mackerel fish (*Scomber scombrus*) used in the study was procured from Izmir fish market. Pomegranate whose antibacterial effects were determined were procured from local markets.

The *L. monocytogenes*, *Escherichia coli*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Salmonella enteritis* and *Staphylococcus aureus* were procured from İzmir Katip Celebi University Aquaculture Faculty culture collection.

2.2. Method

2.2.1. Preparation of the extract

The pomegranate peel were dried in drying oven at 50 °C. These dried samples were shredded to small pieces by a wiring blender. 20 g of dried samples were extracted with 100 mL solvent (80% ethanol). The mixture was kept in a shaking water bath for 6 hours at 50 °C and was filtered. This process was repeated 4 times. This latter was evaporated in a rotary evaporator, finally freezed and lyophilised (Feresin et al 2000; Keyrouz et al 2011).

2.2.2. Preparation of bacterial inoculums

The stock cultures of *L. monocytogenes*, *E. coli, E. coli* O157:H7*, B. cereus, S. enteritis* and *S. aureus* that are in liquid form were stored at -20±2 °C on glycerol containing (20%; v v^{-1}). For the experiments, 100 µL of stock cultures were transferred to 10 mL MHB medium, and the bacteria were activated by incubation at 30 °C for 24 hours.

2.2.3. Disk diffusion method (Kirby-Bauer method)

The disk diffusion method was used to determine the antibacterial activities of pomegranate peel extracts (Melendez & Capriles 2006). 25 µL (0.25, 0.50, 1, 1.5, 2.5, 5, 7.5 and 10% concentrations) of pomegranate peel extracts were soaked into sterile empty discs placed in petri dishes in which planting was made. The petri dishes in which planting were made were left to the incubation process at 30 °C for 24 hours. The activation process was repeated twice.

2.2.4. Preparation of film solution and application to mackerel fillets

Sodium alginate (Kimbiotek) and $CaCl_2$ (Merck) were used to prepare the film solution. 30 g of sodium alginate was mixed with 1000 mL of distilled water and adjusted to 80 °C. The mixture kept in

a magnetic stirrer. After having been cooled until 50 °C, the pomegranate extracts at concentrations of 2.5, 5, 7.5 and 10% were added into it. 50 g of mackerel fillets were inoculated by spreading *L. monocytogenes* to a final concentration of 10^4 CFU g^{-1}. Then the fillets were immersed in 3% alginate solution containing 2.5, 5, 7.5, 10% PPE and left for 1 minute. The samples coated with alginate film without PPE were used as control. This process has been repeated twice. Fillets coated with alginate were immersed in the 2% $CaCl_2$ solution and kept (3 minute) for solidification (Song et al 2011). The fillets prepared like this were packaged and stored in the refrigerator (4±1 °C) for 13 days.

2.2.5. Determination of the number of L. monocytogenes in mackerel fillets coated with alginate film

The number of *L. monocytogenes* were determined on 0, 3rd, 8th, and 13th day of storage period. For this, the mackerel filet was transferred to the Maximum Recovery Diluent (MRD) solution and homogenized. It was diluted up to the dilutions of 10^6 from homogenate taking into account the 10^{-1} dilution rate, and planting according to the Most Probable Number (MPN) method. For this purpose, 1 mL of the appropriate dilutions was transferred to a 10 mL Fraser broth medium, and incubated at 37 °C for 24 hours. The number of *L. monocytogenes* was determined by being evaluated the tubes in which the growth was occurred after the incubation (Halkman 2005).

2.2.6. The microbiological analysis on mackerel fillets coated with alginate film

The number of total aerobic mesophilic bacteria, total aerobic psychrophilic bacteria, total yeast-mould and enterobacteriaceae were performed on 0, 3rd, 8th, and 13th day of storage period.

The mackerel filet was transferred to the Maximum Recovery Diluent (MRD) solution and homogenized. 0.1 mL was taken from the appropriate dilutions and planted in petri dishes containing nutrient with spreading method. PCA was used for TAMB and incubated for 24-48 hours at 30 °C; PCA was used for TAPB and incubated for 10 days at 6.5 °C; PDA was used for TYM counts and incubated for 4-5 days at 30 °C; and VRBA was used for enterobacteriaceae count and incubated for 24 hours at 37 °C. Bacterial counts were determined by counting the colonies growing on the medium at the end of incubation (Harrigan 1998).

2.2.7. Statistical analysis

The obtained data were analyzed using ANOVA (analysis of variance).The results were evaluated by Duncan Multiple Comparison Test. The SPSS statistical package program was used to test whether there is any difference between the application groups (IBM SPSS 2012).

3. Results and Discussion

3.1. Antibacterial activities of extracts

Antibacterial properties of extracts obtained from pomegranate peel were investigated in this study. The inhibition zone diameters of the extracts against the tested microorganisms were given in Figure 1. Different concentrations of PPE have antibacterial effect against *L. monocytogenes*, *E. coli* O157:H7 and *S. aureus* ($P<0.05$). Similar results have been identified by different investigators. Al-Zoreky (2009) reported that PPE could be used for the inactivation of *L. monocytogenes, S. aureus, E. coli* and *Yersinia enterocolitica.* Prashanth et al (2001) the highest inhibitory effect was obtained in *P. vulgaris* and *B. subtilis*. Ahmad & Beg (2001) determined that extracts of pomegranate displayed antibacterial effect against *S. aureus, E. coli* and *Shigella dysenteriae*.

It was determined that the inhibition effect also increased based on increasing in PPE concentration. The highest inhibition zone was obtained from 10% PPE and the zone diameters for *S. enteritis, B. cereus, E. coli* O157:H7, *E. coli, S. aureus* and *L. monocytogenes* were determined as 18, 22, 24, 17, 16 and 23 mm, respectively. Al-Zoreky (2009) found these zone diameters as 17, 16, 13 and 20 mm for *Bacillus, E. coli, S. aureus* and *L. monocytogenes*, respectively. In the study in which Dahham et al

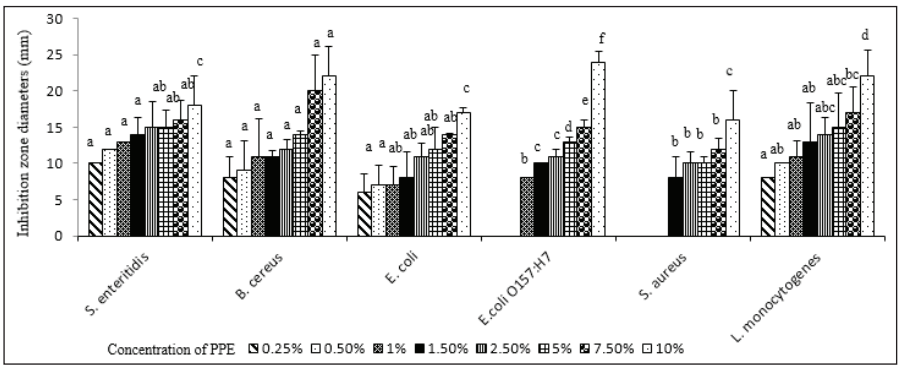

Figure 1- Antibacterial activity of PPE against bacterial species tested using disc diffusion assay

(2010) determined that the inhibition zone diameters obtained from pomegranate peel were in the length of 25 mm for *B. cereus*, 20 mm for *E. coli* and 25 mm for *S. aureus*. Whereas Melendez & Capriles (2006) found these as 12 mm for *E. coli* and 22 mm for *S. aureus*. Mathabe et al (2006) reported that the extracts obtained from pomegranate are effective on *S. aureus, E. coli, Salmonella typhi, Vibrio cholera, S. dysenteriae, S. sonnei, S. flexneri* and *S. boydii*, and that they had the inhibition zones whose diameters were between 12 and 31 mm.

According to the results obtained by Hayrapetyan et al (2012), *L. monocytogenes, Bacillus subtilis, B. cereus, E. coli* and *S. aureus* showed susceptible to PPE, respectively. According to the result that were obtained by them, *S. aureus, B. cereus, E. coli* and *B. subtilis* bacteria were found to be more sensitive to PE compared to *L. monocytogenes*. *S. aureus* were reported to show the highest sensitivity among the tested microorganisms. The results that we obtained are similar to those of other researchers, and it was determined that PPE can be used effectively against many food pathogens.

3.2. Changes in L. monocytogenes number of mackerel fillets during refrigerated storage

The number of *L. monocytogenes* detected during storage (0, 3, 8 and 13. days) of mackerel fillets coated with alginate film containing PPE at 4 °C is given in Figure 2.

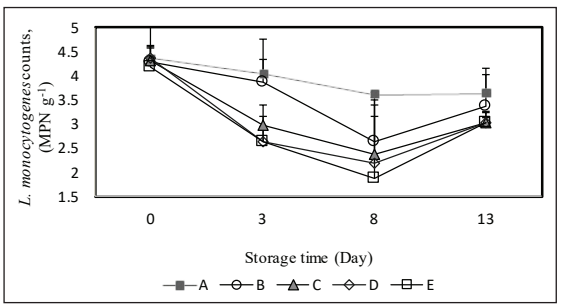

Figure 2- Changes in *L. monocytogenes* number of mackerel fillets during refrigerated storage (A, control group-without PPE; B, samples treated with 2.5% PPE; C, samples treated with 5% PPE; D, samples treated with 7.5% PPE; E, samples treated with 10% PPE)

The number of *L. monocytogenes* on mackerel fillets was significantly higher ($P<0.05$) for untreated samples than for treated samples. But the difference between PPE treated groups was not statistically significant ($P>0.05$). At the beginning of storage, the number of bacteria that had been 10^4 MPN g^{-1}, was determined after 8 day storage as $4.3x10^3$ MPN g^{-1} in the control group and $7.4x10^1$ MPN g^{-1} in the samples treated with 10% PPE. At the end of 8 days of storage, the number of *L. monocytogenes* decreased significantly due to the increase in the extract concentration. The antibacterial effect of PPE was determined on many bacterial species in both

in vitro and *in vivo* conditions. It was determined that the PPE prepared by Al-Zoreky (2009) using 80% methanol has an inhibitory effect against *L. monocytogenes, S. aureus, Escherichia coli* and *Yersinia enterocolitica*. The methanolic of PPE caused decreasing more than 1 log in the number of *L. monocytogenes* of the fish stored at 4 °C. At the last of the 46-day storage period and at 4 °C, Hayrapetyan et al (2012) found that the number of *L. monocytogenes* of meat patties that of the controls and samples treated with 7.5% of were 4.1 log CFU g^{-1}, 9.2 log CFU g^{-1}, respectively. Wu et al (2016), in an experiment conducted on the survival level of *L. monocytogenes* and *V. parahaemolyticus* in the cooked shrimp and raw tuna fish which PPE was applied, they found that the count of *L. monocytogenes* in the samples was 2 log less than the control group.

3.3. Changes in the number of TAMB, TAPB and enterobacteriaceae in mackerel fillets during refrigerated storage

The change in the number of TAMB during 13 days of storage (0, 3, 8 and 13. days) is given in Figure 3a. The count of TAMB was determined less in all groups with PPE than the control group. As the PPE concentration increased, the number of TAMB decreased significantly. At the end of 13 days of storage at 4 °C the lowest number of TAMB was detected in the experimental group to which was applied 10% PPE ($P<0.05$).

The number of TAMB (means±standard errors) of the controls and of samples treated with 2.5, 5, 7.5 and 10% PPE were 6.96±0.03, 6.53±0.20, 6.46±0.11, 6.41±0.04 and 6.18±0.02 log CFU g^{-1}, respectively, at the last of the storage period.

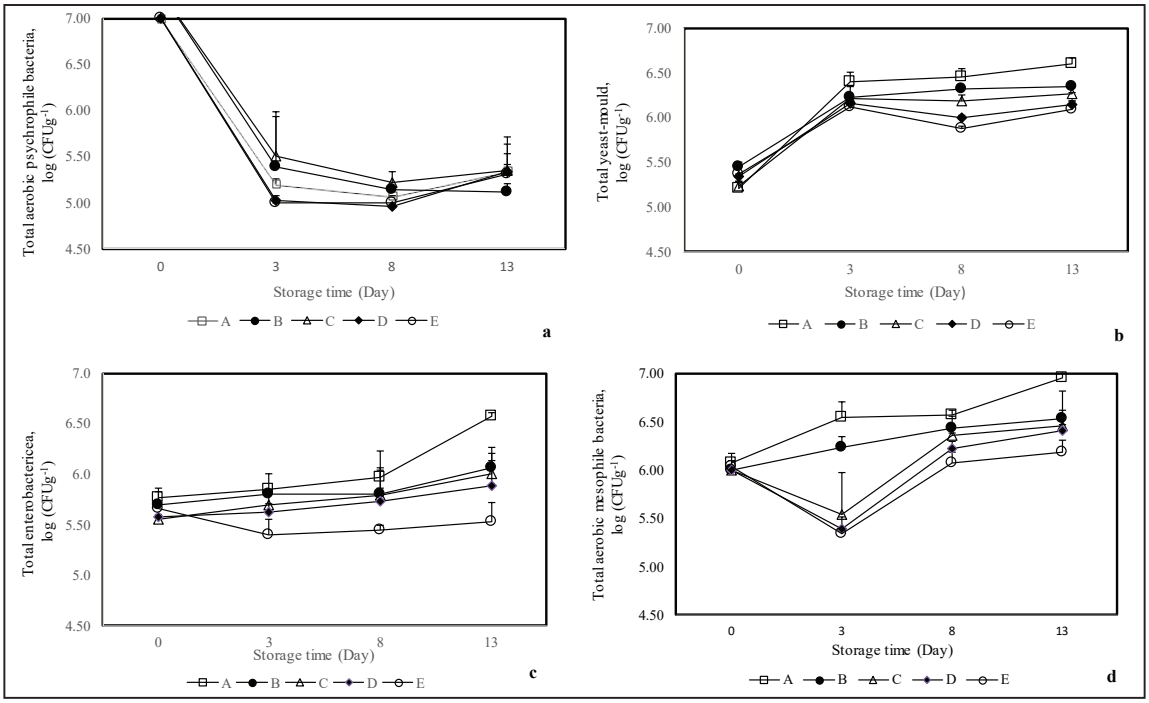

Figure 3- Changes in the number of TAMB (a), TAPB (b), enterobacteriaceae (c) and TYM (d) in mackerel fillets during refrigerated storage. (A, control group-without PPE; B, samples treated with 2.5% PPE; C, samples treated with 5% PPE; D, samples treated with 7.5% PPE; E, samples treated with 10% PPE)

The change in the total number of TAPB is given in Figure 3b. At the end of storage (day 13), the number of TAPB of the controls and of samples treated with 2.5, 5, 7.5 and 10% PPE were determined as 5.35±0.05, 5.11±0.07, 5.35±0.12, 5.34±0.26 and 5.31±0.23 log CFU g^{-1}, respectively. The results indicated that there was no significant differences among the groups in terms of TAPB during the storage period ($P>0.05$).

The change in the TYM counts is given in Figure 3c. On the 13th day of storage, TYM counts of the controls and of samples treated with 2.5, 5, 7.5 and 10% PPE were determined as 6.60±0.05, 6.34±0.02, 6.27±0.01, 6.15±0.05 and 6.09±0.07 log CFU g^{-1}, respectively. Significant differences between TYM levels of control and other groups have not been observed ($P>0.05$).

The change in the number of enterobacteriaceae in the mackerel fillets stored at +4 ^{o}C is given in Figure 3d. When compared with the control group, the number of enterobacteriaceae was determined to be lower in the groups to which samples treated with 10% PPE ($P<0.05$). At the end of storage, the number of enterobacteriaceae controls and of samples treated with 2.5, 5, 7.5 and 10% PPE were determined as 6.57±0.31, 6.07±0.14, 6.01±0.14, 5.88±0.18 and 5.53±0.13 log CFU g^{-1}, respectively.

Although the lowest number of the enterobacteriaceae were observed in the group to which 10% PE was applied, the number of enterobacteriaceae and TAMB showed a significant decrease as the extract concentration increased ($P<0.05$). Berizi et al (2016) investigated the effect of 1, 2, 4% PPE on the quality of trout for 18 months at -18 ^{o}C. At the end of storage, the lowest TAPB counts were detected in trout to which 4% PPE was applied. At the end of storage, number of TAPB controls and of samples treated with 1, 2 and 4% PPE were determined as 5.14, 4.87, 4.86 and 4.27 CFU g^{-1}, respectively. In addition, the count of enterobacteriaceae was determined to be lower in the trout samples to which 1, 2 and 4% PPE was applied in comparison with the control group. It was reported that the development of TAMB significantly was suppressed in the groups to which 0.1, 0.2 and 0.3% PPE was added compared to the control group in the study carried out by Özdemir et al (2014). Furthermore, as the concentration of the extract increased, the number of enterobacteriaceae also decreased significantly. The results that we obtained are similar to these studies, and it was seen that the antibacterial quality increases as the extract concentration increases. Contrary to these studies, it was reported that the increase in extract concentration did not increase the antibacterial effect in the study conducted by Basiri et al (2014). These investigators investigated the impact of vacuum packaging and PPE on the quality of pacific white shrimp stored at +4 ^{o}C for 10 days. TAPB, LAB and enterobacteriaceae numbers were found to be lower than the control group in the PPE supplemented groups.

4. Conclusions

The antibacterial effect of PPE on the food pathogens were investigated in this study. It was determined that the PPE have antibacterial effect and that the antibacterial effect significantly increased as the extract concentration increased. It was determined that PE which has antibacterial effects on many food pathogens, primarily on *L. monocytogenes*, can be used as a natural preservative in seafood products.

Abbreviations and Symbols	
PP	Pomegranate peel
PPE	Pomegranate peel extract
CFU	Colony forming unit
TAPB	Total aerobic psychrophile bacteria
TAMB	Total aerobic mesophile bacteria
MPN	Most probable number
TYM	Total yeast-mould
LAB	Lactic acid bacteria
TAMB	Toplam aerobik mezofilik bakteri
TAPB	Toplam aerobik psikrofil bakteri
TMK	Toplam maya küf

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Rootstocks Affected Postharvest Performance of Grafted 'Crisby' and 'Crimson Tide' Watermelon Cultivars

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ABSTRACT

Watermelon fruit from 'Crisby' (CR) and 'Crimson Tide' (CT) grafted onto Ferro, RS841, Argentario and Macis rootstocks and non-grafted CR and CT were compared for their postharvest quality at 7 °C for 21 days. Changes in rind thickness, weight loss, fruit flesh firmness, taste, total soluble solids, juice pH, titratable acidity, chilling injury and fungal decay, flesh color values, hallow heart, ripening, citric and malic acid, glucose, fructose, sucrose, total sugar, β-carotene and lycopene were determined during storage at a weekly interval. Watermelon fruit cv. CT grafted on Ferro, RS841 and Argentario rootstocks had thicker rind, lower ripening score, higher flesh firmness and lycopene content, more intense red color during storage, compared to non-grafted fruit. In comparison to non-grafted fruit, CR fruit grafted on Ferro, RS841 and Argentario rootstocks had thicker rind and higher flesh firmness, but higher lycopene content and C* values with lower ripening scores were observed only in the fruit grafted on Ferro and RS841 rootstocks. Macis and Argentario may lead an over-ripening, softening and less intense flesh color with lower lycopene content for CR and/or CT fruit during storage. Watermelons could successfully be kept for 21 days at 7 °C. Watermelons grafted on Ferro and RS841 rootstocks retained better postharvest quality, compared to the non-grafted fruit for both cultivars.

Keywords: Watermelon; Grafting; Rootstock; Storage; Quality

1. Introduction

Watermelon is widely grown crop in almost all regions of Turkey and many areas of the world. Soil borne diseases cause a decrease in yield and quality. Use of soil-borne disease resistant rootstocks such as *Cucurbita* and *Lagenaria* was suggested as an environmentally safe alternative to methyl bromide (Miguel et al 2004).

Bottle gourd, interspecific hybrids between C. *maxima* and C. *moschata*, and wild watermelon (*Citrullus lanatus* var. Citroides) are the most common rootstocks for watermelon (Davis et al 2008). Effects of grafting on watermelon quality were reported previously at harvest (Yetişir & Sarı 2003; Yetişir et al 2003; Davis & Perkins-Veazie 2005; Çandır et al 2013). These effects varied

depending upon production environments, type of rootstock, interactions between specific rootstocks and scions, and harvest date (Davis et al 2008). The recommendation conditions for short-term storage or transport to distant markets (>7 days) are 7.2 °C (45 °F) temperature and 85-90% relative humidity (RH) conditions to maintain postharvest quality of watermelons (Suslow 1997). However, watermelons in most of the world are shipped and stored for short-term under non-refrigerated conditions in practice (Chisholm & Picha 1986; Perkins-Veazie & Collins 2006; Radulovic´ et al 2007). This practice leads quality loss in watermelon. There are few reports on the effects of grafting on storage and shelf life of watermelons. In our previous studies, the postharvest quality of 'Crisby' and 'Crimson Tide' watermelon cultivars grafted on Ferro, RS841, Argentario and Macis rootstocks were determined at 21 °C and 70±5% relative humidity for 7 days after 21 days of cold storage (Özdemir et al 2016) and in common marketing condition at 27 °C (Chisholm & Picha 1986) and 50±5% relative humidity for 7 days and at 21 °C and 70±5% relative humidity for 7 days after 21 days of cold storage (Aras et al 2015). It was reported that firmer fruit at harvest are more likely to maintain a desirable consistency during postharvest period and are expected to have a better shelf life than are softer fruit (Roberts et al 2007; King et al 2010). Therefore, effects of grafting on storage performance of watermelon fruit have gained importance. Our objective in this experiment was to determine effects of the commercial rootstock(s) on the postharvest performance of grafted 'Crisby' and 'Crimson Tide' watermelon cultivars.

2. Material and Methods

The rootstocks tested in this study are Ferro, RS841 (*Cucurbita maxima* x *C. moschata*) and Argentario and Macis (*Lagenaria siceraria*). 'Crisby' (CR) and 'Crimson Tide' (CT) watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] cultivars were used as scion. Grafted and non-grafted (control) seedlings were obtained by a commercial seedling company (Grow Fide, Antalya/Turkey). Plants were grafted by one cotyledon grafting technique. The experiment was carried out in the Alata Horticultural Research Institute, Erdemli, Mersin, Turkey. The grafted plants were planted under low tunnel in early spring and regular cultural practices for watermelon were applied.

Fruit were harvested at full maturity when the 75% of tendril and stipule on the same node with peduncle were dried (Özdemir et al 2014). After harvest, fruit were stored at 7±0.5 ºC and 90±5% relative humidity for 21 days. The analyses were done during storage at a 7-day interval. Fruit was cut longitudinally and rind thickness (mm) was measured using an electronic caliper at two points on cross-section of each fruit. The thirty fruits were numbered and the weight loss (%) was calculated by subtracting the final weigh from the initial weigh in percent. The fruit showing chilling injury symptoms (CI) and decay (1= none, 2= <10% of surface area, 3= 11% to 25%, 4= 26% to 50%, and 5= > 50%) were determined according to Risse et al (1990). Fruit flesh firmness was measured using a penetrometer with 12 mm of conic probe (Now FHR-5 Nippon Optical Works Co. Ltd. Tokyo, Japan) at the three points on the mesocarp tissue of the watermelon fruit heart portion and expressed as Newton (N). Fruit was sliced and its rind and seeds were removed and then total soluble solids (TSS) content (%) was assessed in the juice obtained from 5 fruit per replicate with a digital refractometer (Atago Model ATC-1E Atago Co. Ltd., Tokyo, Japan) at 20 °C. Juice pH was measured by digital pH-meter (Orion 5-Star model Thermo Fisher Scientific Inc., MA, ABD). Titratable acidity (TA) was determined by titration of 5 mL of juice with 0.1 N NaOH to a pH of 8.1, and it was expressed as percent of malic acid equivalents. Taste (1-9) of fruit were rated on hedonic scale of 1= disliked (the lowest value) to 9= liked (the best) by a trained panel consisting of 10 people (non-smoker 7 male and 3 female, ages 20 to 45). The taste scores >5 were considered as acceptable quality. Hallow heart (1-5) of fruit were rated on a scale of 1= none to 5= very severe (50% "more than hallow heart) and ripening (1-7) of fruit were rated on a scale of 1= raw fruit and 3= mature to 7= over-ripe extremely by trained ten panelists. Fruit flesh color (L* C* h°) was measured using the CIELAB (L* a* b*) color

space by a CR-300 Minolta Chroma Meter (Konica Minolta, Osaka, Japan) on the two points of the mesocarp tissue of the watermelon fruit heart portion. Sugars (sucrose, glucose and fructose) and organic acids (malic and citric acid) were extracted according to the method described by Çandır et al (2013). The results were expressed as g 100 g^{-1} fresh weight. Carotenoids were extracted by the method described by Perkins-Veazie & Collins (2006). Detection was carried out at 503 nm for lycopene and 452 nm for β-carotene using the PDA detector in the HPLC. The lycopene and β-carotene contents were expressed as μg g^{-1} fresh weight. The study was performed over a 2-year period. Data are represented as the mean of two experimental years. The data were analyzed a completely randomized block design by ANOVA using SAS software of SAS Institute, Cary, N.C. (SAS 2017). The data were obtained from three replications per scion/rootstock combination. Each replicates contained 5 fruit. Mean separation was performed by Fisher's Least Significance Test at $P<0.05$ level.

3. Results and Discussion

At harvest, CT fruit from grafted plants had thicker rind than those from non-grafted plants while grafting did not affect rind thickness for CR cultivar. Rind thickness decreased in non-grafted and at a lesser extent in grafted fruit during storage period in both cultivars, except for RS-841 rootstock. RS-841 rootstock for both cultivars showed similar rind thickness after 21 days storage to initial values. Fruit grafted on RS841, Argentario and Ferro rootstocks had thicker rind compared to non-grafted fruit during storage for both cultivars (Figure 1). Thinning of the rind may indicate an overripe fruit or fruit subjected to prolonged storage (Kyriacou & Soteriou 2015). Rind thickness of CR cultivar grafted on TZ-148, RS-841 and 64-18 of *Lagenaria* rootstock was similar to non-grafted controls (Alan et al 2007). Davis et al (2008) also found rind thickness increased for both seedless and seeded watermelon fruit when grafted to gourd rootstock '451'. In the study with 'Crimson Tide' cultivar, all of the grafted plants on bottle gourd rootstocks produced fruit with a thicker rind than the control plants (Karaca et al 2012).

Weight loss in grafted and non-grafted fruit were very low (<1%) during storage for both cultivars. Effect of rootstocks on weight loss was not significant during storage period in both cultivars (Figure 2). Consistent with our results, Perkins-Veazie & Collins (2006) reported the <1% of weight loss in watermelon fruit at all temperatures (5 °C, 13 °C and 21 °C) after 14 days of storage. However, Neto et al (2000) determined higher weight loss (3.8%) than our results. Suárez-Hernández et al (2016) reported that the some rootstocks caused to reduce in weight loss during a storage period of 14 days at 15 to 17 °C and 80% RH conditions.

Non-grafted or grafted watermelons onto different rootstocks did not exhibit CI symptoms

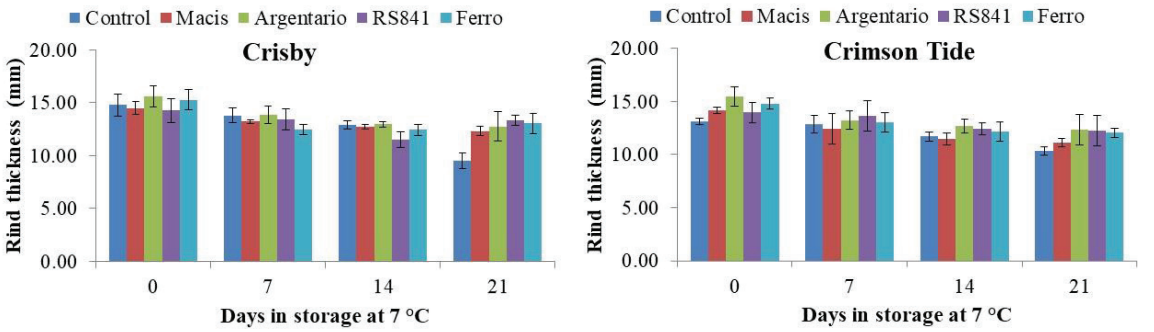

Figure 1- Effects of rootstocks on rind thickness of 'Crisby' and 'Crimson Tide' watermelon fruit during storage at 7 °C

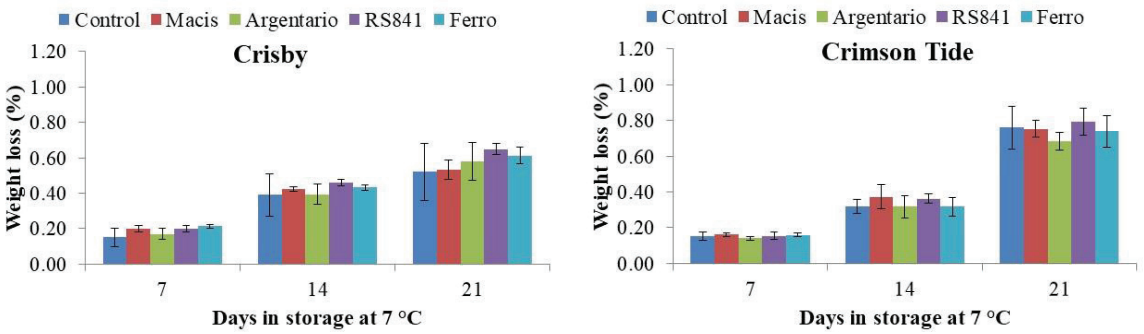

Figure 2- Effects of rootstocks on weight loss of 'Crisby' and 'Crimson Tide' watermelon fruit during storage at 7 °C

during storage period. Fungal decay was not observed on non-grafted and grafted fruit of both cultivars during storage at 7 °C.

Flesh firmness decreased during storage for both cultivars (Data not presented). Watermelons grafted on Ferro (6.98-7.19 N) and RS841 (6.78-7.19 N) rootstocks had the higher flesh firmness for both cultivars (Table 1 and 2), compared to other rootstocks (6.29-6.52 N for Argentario, 5.89-6.23 N for Macis) and control (5.44-5.59 N) after 21 days of storage. Consistent with our study, flesh firmness of watermelon fruit cv. 'Sugar Baby', 'Baby Fun' and 'Minilee' decreased during storage during 4 weeks of storage at 5°, 10°, 15° or 20 °C (Risse et al 1990). At harvest, an increase in flesh firmness due to grafting has been reported (Yetişir et al 2003; Davis & Perkins-Veazie 2005; Roberts et al 2007; Cushman & Huan 2008; Bruton et al 2009; Soteriou & Kyriacou 2015) while grafting on some rootstocks seems not affect watermelon firmness (Karaca et al 2012). The findings of Kyriacou & Soteriou (2015) indicated that *C. maxima* × *C. moschata* hybrids maintained firmness better, compared with non-grafted controls. Authors also reported a greater effect of rootstocks on firmness, in comparison to effects of cultivar and storage period. Suárez-Hernández et al (2016) reported that the some rootstocks retained firmness better than control during storage.

TSS content remained above 10% in fruit of both cultivars throughout storage period (Table 1 and 2), rendering fruit acceptable for perceived sweetness as reported by Kyriacou & Soteriou (2015). In CR cultivar, fruit grafted on Ferro and RS-841 rootstock had higher TSS content after storage period for 21 days at 7 °C, compared to other graft combinations and control (Table 1). In case of CT cultivar, control had higher TSS content than some of the graft combinations after 14 days, but control and grafted fruit had similar TSS content after 7 or 21 days (Table 2). Although some previous studies showed that grafting had no effect on TSS (Miguel et al 2004; Proietti et al 2008; Bruton et al 2009; Bekhradi et al 2011; Soteriou & Kyriacou 2015), grafting on the bottle gourd rootstocks increased TSS contents of watermelons compared to the control fruit in some reports (Karaca et al 2012; Çandır et al 2013; Suárez-Hernández et al 2016). In other studies, grafted watermelons had lower TSS content compared to non-grafted controls (Davis & Perkins-Veazie 2005; Roberts et al 2007; Kyriacou & Soteriou 2015). Our reports are consistent with the previous studies, indicating effects of rootstocks on TSS content, cultivar depending.

Juice pH value slightly decreased during storage (Data not presented). Similarly, lower pH values were reported in 'Charleston Gray' watermelons fruit after a storage period of 14-19 days at 7 °C (Chisholm & Picha 1986). In CR cultivar, non-grafted fruit had higher pH comparing to grafted

fruit after 21 days of storage. In CT cultivar, fruit on RS841 rootstocks resulted in lower pH than those on other rootstocks and non-grafted fruit after 21 days of storage (Table 1 and 2). In agreement with our findings, grafted CT watermelons on some local bottle gourd rootstocks had lower juice pH, compared to control (Çandır et al 2013).

TA content slightly increased in parallel with changes in juice pH during storage for both cultivars (Data not presented). In CR cultivar, there was no significant difference in TA between non-grafted and grafted fruit during storage (Table 1). In CT cultivar, fruit on RS841 rootstock resulted in higher TA than those on other rootstocks and non-grafted fruit after 21 days of storage (Table 2). Higher TA due to grafting was reported in watermelon fruit (Proietti et al 2008; Çandır et al 2013). The malic acid content varied from 0.19% to 0.42% for CR cultivar and 0.19% to %0.65 for CT cultivar (Table 1 and 2) and the citric acid content varied from 0.08% to 0.09% for CR cultivar and 0.10% to 0.14% for CT cultivar during storage (Data not presented). Malic acid was the predominant organic acid for both cultivars. CR fruit on RS841 rootstock had higher malic acid content than other graft combinations and control after 21 days of storage. Although CT fruit non-grafted or grafted on Macis had lower malic acid content at harvest, non-grafted or grafted CT fruit had similar malic acid content during storage. The citric acid content was not affected by grafting during storage for both cultivars (Data not presented).

Flesh color lightness (L* value) decreased during storage for both cultivars (Data not shown). Similarly Perkins-Veazie & Collins (2006) determined lower L* values in the fruit after 14 days of storage at 21 °C, compared freshly harvested watermelons. Grafting did not affect flesh color lightness during storage for both cultivars. In contrast to our findings, Kyriacou & Soteriou (2015) reported that flesh color lightness (L*) of watermelon fruit was affected by rootstock and storage and all hybrid rootstocks invariably maintained darker flesh color during storage. In CT cultivar, C* value peaked after 7 days and then decreased during storage (Data not shown). In CR cultivar, C* value showed gradual decrease toward the end of storage (Data not presented). In CR fruit, grafting did not affected the C* values, but CT fruit grafted on RS841, Argentario and Ferro rootstocks had more intense (higher C*) color than those on Macis and control fruit during storage. The h° values showed a progressive increase in non-grafted fruit with a lesser extent in grafted fruit during storage in both cultivars (Table 1 and 2). This indicated a change of flesh color from red to orange-yellow. These changes in h° value indicate over-ripening and senescence of watermelons which are subjected to prolonged storage (Kyriacou & Soteriou 2015). In CR cultivar, non-grafted fruit had higher h° values than grafted fruits after 14 days of storage, but non-grafted and grafted fruit had similar h° values after 21 days of storage (Table 1). In CT cultivar, non-grafted fruit had higher h° values than grafted fruit after 7 and 14 days, but non-grafted fruit and fruit on Macis had higher h° values than others after 21 days of storage (Table 2). Lycopene content in both cultivars showed similar trend with C* values (Table 1 and 2). Lycopene content significantly decreased at the end of storage for CR cultivar, but peaked after 7 days and then decreased during storage for CT cultivar (Data not presented). In CR cultivar, all grafted fruit had higher lycopene content at harvest, compared to non-grafted fruit, but similar lycopene content was determined among non-grafted and grafted fruit after 21 days of storage (Table 1). In CT cultivar, fruit grafted on RS841, Argentario and Ferro rootstocks had higher lycopene content than those on Macis and non-grafted fruit after 21 days of storage (Table 2). Postharvest color changes and lycopene biosynthesis in watermelons can be affected by storage temperature and cultivar. Perkins-Veazie & Collins (2006) reported that watermelons stored at 21 °C had higher C* value and lycopene content, compared to initial value at harvest whereas no or little change was observed in C* value and lycopene content of fruit held at 5 °C or 13 °C depending on cultivars. Consistent with our results, previous studies have typically shown higher lycopene content in watermelon fruit from grafted plants at harvest (Davis & Perkins-Veazie 2005; Davis et al 2008; Proietti et al 2008;

Table 1- Effects of rootstocks on some quality attributes of 'Crisby' watermelon fruit during storage at 7 °C

Quality parameters	*Scion/rootstock*	*Days in storage at 7 °C*			
		0	*7*	*14*	*21*
Firmness (N)	CR (Control)	7.84 b[x]	6.93 c	5.77 d	5.59 b
	CR/Macis	7.57 c	7.37 bc	6.65 c	5.89 b
	CR/Argentario	8.13 a	7.43 b	7.21 bc	6.29 b
	CR/RS841	8.26 a	8.50 a	8.20 a	7.19 a
	CR/Ferro	8.24 a	8.47 a	7.38 b	7.19 a
TSS (%)	CR (Control)	10.32 a	10.93 a	10.77 a	10.37 b
	CR/Macis	10.20 a	10.57 a	10.63 a	10.53 b
	CR/Argentario	10.57 a	10.77 a	10.90 a	10.46 b
	CR/RS841	10.30 a	10.57 a	10.97 a	11.03 a
	CR/Ferro	10.97 a	10.90 a	11.12 a	11.17 a
Juice pH	CR (Control)	5.65 a	5.46 a	5.55 a	5.51 a
	CR/Macis	5.65 a	5.48 a	5.44 b	5.41 b
	CR/Argentario	5.67 a	5.54 a	5.43 b	5.33 b
	CR/RS841	5.58 a	5.50 a	5.47 b	5.34 b
	CR/Ferro	5.54 a	5.50 a	5.35 c	5.39 b
Malic acid (%)	CR (Control)	0.25 a	0.28 a	0.22 b	0.31b
	CR/Macis	0.24 ab	0.27 a	0.26 ab	0.32b
	CR/Argentario	0.19 c	0.29 a	0.22 b	0.29b
	CR/RS841	0.23 b	0.31 a	0.29 a	0.42a
	CR/Ferro	0.24 ab	0.31 a	0.26 ab	0.32b
h°	CR (Control)	38.17 a	46.55 a	47.73 a	48.88 a
	CR/Macis	35.86 b	45.81 a	43.98 b	46.09 a
	CR/Argentario	36.76 b	44.85 b	44.72 b	47.56 a
	CR/RS841	35.47 b	43.10 c	45.19 b	48.80 a
	CR/Ferro	35.92 b	44.68 b	45.98 b	49.03 a
Lycopene (μg g^{-1})	CR (Control)	38.30 c	31.94 b	25.18 b	26.17 a
	CR/Macis	46.25 a	35.75 b	29.17 ab	28.18 a
	CR/Argentario	41.71 bc	38.90 ab	29.92 ab	24.51 a
	CR/RS841	44.16 ab	45.56 a	31.14 a	27.43 a
	CR/Ferro	44.22 ab	40.38 ab	27.52 ab	26.14 a
Ripening (1-7)	CR (Control)	3.67 a	3.86 a	3.50 a	3.43 b
	CR/Macis	3.17 b	3.52 a	3.40 a	3.53 b
	CR/Argentario	3.59 a	3.72 a	3.70 a	3.93 a
	CR/RS841	3.08 b	3.20 a	3.33 a	3.23 c
	CR/Ferro	3.19 b	3.20 a	3.33 a	3.53 b
Taste (1-9)	CR (Control)	8.29 a	8.11 a	7.19 b	6.34 c
	CR/Macis	8.41 a	8.24 a	7.55 ab	6.81 b
	CR/Argentario	8.03 a	8.19 a	8.02 a	6.67 b
	CR/RS841	8.19 a	8.30 a	7.89 a	7.20 a
	CR/Ferro	8.25 a	8.39 a	7.84 a	6.94 ab

[x], mean separation was performed by Fisher's LSD test. Means (n= 3) followed by same letters within a column are not significantly different at $P<0.05$

Table 2- Effects of rootstocks on some quality attributes of 'Crimson Tide' watermelon fruit during storage at 7 °C

Quality parameters	*Scion/rootstock*	*Days in storage at 7 °C*			
		0	*7*	*14*	*21*
Firmness (N)	CT (Control)	7.37 c[x]	6.02 c	6.10 c	5.44 c
	CT/Macis	7.75 bc	6.06 c	6.44 c	6.23 b
	CT/Argentario	7.96 b	6.88 b	6.75 bc	6.52 b
	CT/RS841	8.31 ab	7.81 a	7.29 ab	6.78 a
	CT/Ferro	8.55 a	8.17 a	7.65 a	6.98 a
TSS (%)	CT (Control)	11.13 a	11.13 a	11.30 a	11.03 a
	CT/Macis	10.63 a	10.83 a	10.67 b	10.63 a
	CT/Argentario	10.80 a	10.83 a	10.57 b	11.03 a
	CT/RS841	10.87 a	11.07 a	10.97 ab	11.00 a
	CT/Ferro	10.57 a	10.93 a	11.13 a	10.50 a
Juice pH	CT (Control)	5.67 a	5.52 a	5.50 a	5.43 a
	CT/Macis	5.66 a	5.50 a	5.37 b	5.41 a
	CT/Argentario	5.69 a	5.47 a	5.37 b	5.36 a
	CT/RS841	5.66 a	5.43 a	5.30 c	5.27 b
	CT/Ferro	5.56 a	5.48 a	5.37 b	5.40 a
Malic acid (%)	CT (Control)	0.23 c	0.22 ab	0.25 b	0.48 a
	CT/Macis	0.21 c	0.19 b	0.29 a	0.47 a
	CT/Argentario	0.27 b	0.23 ab	0.26 b	0.58 a
	CT/RS841	0.30 a	0.26 a	0.27 ab	0.65 a
	CT/Ferro	0.32 a	0.27 a	0.26 b	0.52 a
h°	CT (Control)	40.82 a	44.84 a	44.19 a	48.75 a
	CT/Macis	40.84 a	43.17 b	43.34 b	48.29 a
	CT/Argentario	39.21 b	42.53 bc	43.13 bc	44.89 b
	CT/RS841	39.63 b	41.10 d	41.52 d	44.65 b
	CT/Ferro	39.42 b	41.37 cd	42.60 c	44.50 b
Lycopene (μg g^{-1})	CT (Control)	34.53 c	38.92 c	24.26 b	33.11 bc
	CT/Macis	33.08 c	42.17 bc	38.73 a	29.71 c
	CT/Argentario	55.10 a	46.20 b	35.23 a	42.46 a
	CT/RS841	45.05 b	47.14 ab	34.67 a	42.84 a
	CT/Ferro	46.89 b	52.52 a	41.11 a	43.57 a
Ripening (1-7)	CT (Control)	3.32 a	4.29 a	4.33 a	4.50 a
	CT/Macis	3.31 a	3.63 b	3.67 b	4.13 b
	CT/Argentario	3.18 a	3.42 bc	3.54 b	3.92 b
	CT/RS841	3.13 a	3.19 c	3.63 b	4.07 b
	CT/Ferro	3.13 a	3.13 c	3.38 b	3.96 b
Taste (1-9)	CT (Control)	8.16 ab	8.44 b	7.63 a	7.06 c
	CT/Macis	7.87 b	8.34 b	7.90 a	7.41 c
	CT/Argentario	8.46 a	8.48 a	8.03 a	7.86 ab
	CT/RS841	8.41 a	8.32 b	7.82 a	7.75 ab
	CT/Ferro	8.44 a	8.85 a	8.08 a	8.05 a

[x], mean separation was performed by Fisher's LSD test. Means (n= 3) followed by same letters within a column are not significantly different at P<0.05

Çandır et al 2013) and during storage (Kyriacou & Soteriou 2015). The increase in C* value of watermelon fruit was probably as a result of the increase in lycopene content (Perkins-Veazie & Collins (2006). Degradation in lycopene during senescence of non-grafted watermelon fruit of both cultivar and grafted CR fruit after prolonged storage and consequent shelf life period led to decrease in C* value and increase in h° value. Flesh color changes was observed in non-grafted fruit, suggesting that fruit ripening occurs faster in non-grafted than in grafted fruit during storage.

β-carotene content was not significantly changed and was not affected by grafting during storage (data not presented). In our study, lower storage temperature may suppress increase in β-carotene content. In agreement with our results, a similar β-carotene content was reported between fruit grafted on some local bottle gourd rootstocks and non-grafted fruit (Çandır et al 2013).

We found a slight increase in ripening ratings during storage for both cultivars (Data not presented), indicating fruit became overripe toward the end of storage. Similar findings were reported by Risse et al (1990) for several watermelon cultivars during 4 weeks of storage at 5, 10, 15 or 20 °C. In CR cultivar, fruit grafted on RS841 rootstock had lower ripening scores than those from other rootstocks and control fruit after 21 days of storage (Table 1). In CT cultivars all grafted fruit had lower ripening scores, compared to non-grafted fruit after 21 days of storage (Table 2). Ripening could be retarded by grafting in watermelon fruit at harvest. Soteriou et al (2014) found that as grafting retarded the ripening process, optimum harvest maturity in non-grafted plant was reached at 35-40 days post-anthesis (dpa) compared with 40-45 dpa in grafted plants.

Effects of grafting on hallow heart was not significant during storage for both cultivars (data not shown). Cushman & Huan (2008) found higher incidence of hollow heart in non-grafted watermelon plants than in those grafted. However, the environmental and cultural conditions affect incidence of hollow heart beside to rootstocks.

In CR cultivar, effect of grafting on total and individual sugar contents was not significant during storage (data not shown). In CT cultivar, although sucrose and total sugar contents were not affected by grafting, fructose and glucose content were higher in fruit grafted on RS841, Ferro and Argentario rootstocks than those on Macis and non-grafted fruit after 7 days of storage at 7 °C (data not shown). The differences in fructose and glucose contents between grafted and non-grafted fruit disappeared afterwards. Lower sugar content was reported in grafted watermelon fruit than non-grafted fruit in some studies (Yetişir et al 2003; Davis & Perkins-Veazie 2005; Roberts et al 2007). In contrast, other studies showed similar sugar contents in grafted and non-grafted watermelon fruit (Miguel et al 2004; Proietti et al 2008; Bruton et al 2009; Bekhradi et al 2011). In agreement with our results, Kyriacou & Soteriou (2015) found no significant effect of the hybrid rootstocks on sucrose concentration of watermelon. Previous study have shown that increasing fructose, glucose, and sucrose contents of CT watermelon fruit due to grafting on the local bottle gourd rootstocks in comparison to the non-grafted and grafted CT fruit on commercial rootstocks (Çandır et al 2013). The most abundant sugar was sucrose at harvest and after storage period in both cultivars as reported previously (Chisholm & Picha 1986; Kyriacou & Soteriou 2015). In general, total soluble solid, total and individual sugar contents did not changed significantly during storage. Similarly, soluble solids content, sucrose, glucose, and fructose concentrations of watermelons mostly did not change during storage for 14 days at 0 °C plus 5 days at 23 °C, but all generally were reduced at higher storage temperatures (Chisholm & Picha 1986). In our study, preservation of sugars at lower storage temperature may be attributed to a presumably lower rate of respiration.

Taste scores (1-9) declined to the lowest level for 21 days of storage at 7 °C for both cultivars (Table 1 and 2). In CR cultivar, fruit on RS-841received higher taste scores than those from other rootstocks and control fruit after 21 days of storage (Table 1). In CT cultivar, fruit grafted on Ferro, Argentario

and RS841 received higher taste scores than those on Macis and control fruit after 21 days of storage (Table 2). Lower taste score may be related to becoming of overripe of control fruit and grafted fruit on Macis rootstock. Furthermore, panelists did not detected off-flavors in fruit from grafted plants. Bruton et al (2009) reported similar findings with the fruit from grafted watermelons.

4. Conclusions

Watermelons grafted on Ferro and RS841 rootstocks maintained higher firmness, lycopene content, C* value and taste scores with lower ripening scores, compared to the control for both cultivars. Macis and Argentario may lead an over-ripening, softening and less intense flesh color with lower lycopene content for CR and/or CT fruit during storage. Ferro and RS841 rootstocks provided a 21-day of storage life at 7 °C and 85-90% relative humidity.

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Influence of Pollination on Smut Disease and Yield in Maize

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ABSTRACT

Ustilago maydis, causal agent of smut disease in maize, induces significant yield losses by forming colossal galls (tumours) on cobs. Since infection process of *U. maydis* is parallel with natural pollination of maize, an interaction between maize pollination and the smut fungus is probable. To reveal this interaction perceptibly, a 2-year field study was carried out in Antalya province of Turkey. As host plants, 8-maize-cultivars belonging to different maize variety groups [dent (Ada-523, Pioneer-3394 and Side), flint (Karaçay and Karadeniz Yıldızı), sweet (Merit and Vega) and popcorn (Antcin-98)] were used in the experiment. Inoculations were performed by injecting inoculum into cob silks in pre- and post-pollination periods in plots. In addition, control plots were set up for each treatment. In conclusion, average disease severity, incidence and yield losses of all the maize cultivars in pre-pollination inoculations (PrePI) were 3.8, 20.7 and 45.5%, whereas in post-pollination (PostPI) inoculations, they were 0.9, 15.7 and 35.9%, respectively. It was found that in both years, disease values of the PrePI were higher than those of the PostPI. This study suggested that pollination period of maize is an important factor affecting *U. maydis* infection in cobs and accordingly yield losses.

Keywords: Maize pollination; *Ustilago maydis*; Yield loss

1. Introduction

Maize is a staple crop for human and animal nutrition as well as fodder industry (Kırtok 1998). In 2013, total maize production in the world exceeded one billion tons (FAO 2015). However, *Ustilago maydis* (DC) Corda, called also as corn smut, is one of the primary constraint to maize yield. The disease occurs wherever maize is grown. Unlike other cereal smuts, *U. maydis* gives rise to local infections and severe damages to cobs through formation of huge galls on them (Tunçdemir & Iren 1980). In addition, it particularly causes great economic damage to sweet corn (du Toit & Pataky 1999).

When maize seedlings are infected by the fungus, small galls appear on the leaves and stems, and the seedling may remain stunted or may be killed. On older plants, infections occur on the young, actively growing tissues of axillary buds, individual flowers of the cob and tassel, leaves and stalks. Infected areas are permeated by the fungus mycelium, which stimulates the host cells to divide and enlarge, thus forming galls. Galls are first covered with a greenish white membrane. Later, as the galls mature, their interior darkens and turns into a mass of powdery, dark olive-brown spores (Agrios 2005). *U. maydis* infects stigmas in maize cobs via silks at the tip

of the cob. Sporidia of the fungus accumulate on silks (Shurtleff 1980). Infection process of stigma by the fungus is as follows: when fungus sporidia contact with stigma, sporidia mate in pairs and each pair forms a dikaryotic infection hypha and then enter the stigma. Similarly, fertilization of stigma by pollens occurs as follows: pollens moving from tassels pass through the silk channel and reach to stigma and fertilize ovaries. Following pollination, an abscission zone consisting of layer of dead cells in pollinated silks occurs. *U. maydis* is not able to grow across this abscission zone (Snetselaar & Mims 1993; Snetselaar et al 2001). This phenomenon suggests that when the pollens reach to the ovary earlier than the infective hypha of the fungus, *U. maydis* infection in the cobs become formidable. Correspondingly, plant may escape from the infection.

The main objectives of the study were to perceptibly examine this phenomenon by injecting inoculum into cob silks of different maize varieties in pre and post pollination periods and determine yield losses arising from the fungus infection during pollination period.

2. Material and Methods

Galls on cobs were obtained from naturally infected plants in maize-producing areas of Batı Akdeniz Agricultural Research Institute in 2010. Flint corn varieties [Karaçay (Batı Akdeniz Agricultural Research Institute) (BAARI) and Karadeniz Yıldızı (Black Sea Agricultural Research Institute)], dent corn cultivars [Ada-523 (Maize Research Institute), Pioneer-3394 (Pioneer Firm) and Side (BAARI)], sweet corn cultivars [Merit (May Firm) and Vega (May Firm)], and popcorn variety [Antcin-98 (BAARI)] were used as host plants.

2.1. Isolation

Teliospores of *U. maydis*, also named as chlamydospores, were obtained by crumbling and filtering out the galls. Teliospores were exposed to 1% copper sulfate solution for 20 to 60 h. Afterwards, they were dried up using blotting paper, and transmitted on PDA medium and incubated at 25 °C. Within a week, basidiospores (sporidia) of the fungus appeared. Later, they were transmitted to a 20% carrot solution in 500 mL flasks and incubated at 25 °C for one week. In this way, necessary inoculum was obtained.

2.2. Inoculum

With gently shaking the flasks, sporidia were moved to ensure homogeneity of the spore solution. By means of a hemocytometer, sporidia and teliospore suspensions were arranged to 3×10^6 basidiospores mL^{-1} and 1×10^6 teliospores mL^{-1}, respectively. In this manner, inoculum was arranged according to Tunçdemir (1985).

2.3. Experiments

Experiments were conducted in completely randomized block factorial design with three replicates. Plots, 5 m long, were set up as four rows including controls. Seeds of the each cultivar were sown on 7 June in 2010 and on 3 June in 2011. Mean number of the plants in every plot were one hundred. Furrow irrigation was used and irrigation, a total of 9 times, was done at 15 to 18 days intervals depending on the moisture in the soil. To manage with weeds, the herbicide, foramsulfuron 22.5 g L^{-1} (active substance), was used at 2 to 6 leaf stage of maize. However, deltamethrin 25 g L^{-1} (active substance), was applied at silking stage against earworms. Picking ears by hand from the each plot, harvest was done. Harvest times were on 26 to 29 October in 2010, while they were on 10 to 12 October in 2011.

2.4. Ecological properties of the research area

General soil texture of the research area was clay loam. In sowing-time, the area was fertilized with compound fertiliser (NPK 18:8:8) at the rates of 180 nitrogen, 80 phosphorus and 80 potassium kg ha^{-1}, respectively. Field studies were established in Aksu location of Antalya. The total monthly rainfall when the inoculations of the maize ears were performed in August of 2010 was 4.2 mm, whereas no measurable rainfall was recorded in the same

period in 2011. However, the average temperature and relative humidity in August 2010 and 2011 were 30.5 ºC, 59.1% and 29.6 ºC and 50.0%, respectively (Anonymous 2013).

2.5. Inoculation

The cob inoculation method of Pataky et al (1995) was modified as follows: In total, 3 mL mixed inoculum, consisted of 3×10^6 basidiospores mL^{-1} and 1×10^6 teliospores mL^{-1}, was syringed in primary cob in pre-pollination period (3 days before natural pollination of maize) and in the post-pollination period (3 days after silk browning) per plant. In 2010, inoculations of PrePI and PostPI were performed on August 10th and 20th, while in 2011, those inoculations were applied on August 15th and 25th, respectively. In the control plots, cobs were injected with sterile water.

2.6. Disease assessments

Using 0 to 5 scale of Johnson & Christensen (1935), severity of the disease was determined. Rating of the scale was as follows: 5 = big galls (>10 cm diam); 2.5 = medium galls (5 to 10 cm diam); 1 = small galls (2.5 to 5 cm diam); and 0 = very small galls (<2.5 cm diam). Comparing the number of infected and non-infected cobs, incidence of the disease was detected. Twenty cobs were assessed in each plot for each treatment. In 2010, disease incidence and severity ratings of PrePI and PostPI were done on September 2nd and 10th, whereas in 2011, those ratings were assessed on September 6th and 14th, respectively. To determine yield losses of the hosts, cobs collected from the plots were peeled from the cob leaves and dried. Leaving kernels at 72 °C for 72 h, moisture contents were found. Adjustment of yield was done in compliance with 15% moisture ratio and using Equation (1) of Poehlman (1987) below.

Adjusted weight= Plot weight × (100 – moisture%) / 85 × (kernel/cob) / 100 (1)

Ratio of kernel/cob and plot yield were detected using Equation (2) of Yanıkoğlu et al (1999).

Plot yield (kg ha^{-1})= Adjusted weight × 10000 / Plot area (m^2) (2)

In conclusion, yield losses were designated by comparing yields of inoculated plots with non-inoculated plots.

2.7. Statistical analysis

Analysis of variance was done using *JMP program* (SAS Institute Inc., Cary, North Carolina, USA) and average values established as different were categorized considering $LSD_{0.01}$.

3. Results and Discussion

Average disease incidence (DI) of all the cultivars (hosts) in PrePI was 20.7%, whereas in PostPI, that was 15.7%. However, the highest DI in the PrePI and PostPI were found in cv. Karadeniz Yıldızı and cv. Side, whereas the lowest DI was in cv. Antcin-98 (Table 1). Likewise, average disease severity (DS) of all the hosts in the PrePI was found at the rate of 3.8, while in the PostPI, it was 0.9 (Table 2). In both years, average DS and DI of the hosts in the PrePI were higher than that of the PostPI. In 2010, average yields of the cultivars in the control, PrePI and PostPI plots were 8180, 5240 and 5950 kg ha^{-1}, respectively. However, in 2011, they were 7510, 3060 and 3730 kg ha^{-1}, respectively. In addition, as an average of both years, mean yields of the hosts in the control, PrePI and PostPI were 7850, 4150 and 4850 kg ha^{-1}, respectively. Interactions of the year, cultivar, and year × cultivar × disease in the PrePI and PostPI were significant ($P<0.01$) in both years (Table 3).

In 2010, average yield losses of the hosts in the PrePI and PostPI were 36.5% and 28.0%, while in 2011, they were 55.5% and 44.7%, respectively. Mean yield loss of the hosts over the two years in the PrePI was determined at the rate of 45.5%, whereas in the PostPI, it was 35.9%. Compared all the hosts, the highest yield losses in the PrePI (52.4%) and PostPI (43.8%) were established from Ada-523 (dent corn variety). However, the lowest yield losses in the PrePI (38.4%) and in the PostPI (26.2%) were found in the popcorn variety, Antcin-98 (Table 4).

Table 1- Disease incidence of the cultivars in pre and post pollination inoculations (%)

Cultivar (host)	*Disease incidence in PrePI*[1]			*Disease incidence in PostPI*[2]		
	2010	*2011*	*Mean*	*2010*	*2011*	*Mean*
Ada-523	13.3*	26.6	19.9	8.3	16.6	12.4
Pioneer-3394	15.0	36.6	25.8	10.0	30.0	20.0
Side	13.3	50.0	31.6	8.3	36.6	22.4
Karaçay	3.3	33.3	18.3	3.3	28.3	15.8
Karadeniz Yıldızı	20.0	38.3	29.1	18.3	31.6	24.9
Merit	16.6	20.0	18.3	10.0	15.0	12.5
Vega	13.3	15.0	14.1	10.0	15.0	12.5
Antcin-98	6.6	11.6	9.1	3.3	8.3	5.8
Mean	12.6	28.9	20.7	8.9	22.6	15.7
	Year LSD (0.01)= 2.5 Cultivar LSD (0.01)= 5.1 Year x cultivar LSD (0.01)= 7.3			Year LSD (0.01)= 1.9 Cultivar LSD (0.01)= 3.8 Year x cultivar LSD (0.01)= 5.4		

*, data are means of three replications; PrePI[1], pre-pollination inoculations; PostPI[2], post-pollination inoculations

Table 2- Disease severity of the cultivars in pre and post pollination inoculations

Cultivar (host)	*Disease severity in PrePI***			*Disease severity in PostPI***		
	2010	*2011*	*Mean*	*2010*	*2011*	*Mean*
Ada-523	1.5*	6.8	4.1	0.5	2.0	1.2
Pioneer-3394	1.7	4.2	2.9	0.3	0.8	0.5
Side	2.9	10.5	6.7	0.6	2.6	1.6
Karaçay	0.8	7.4	4.1	0.3	2.2	1.2
Karadeniz Yıldızı	3.2	11.4	7.3	0.8	3.7	2.2
Merit	2.4	2.3	2.3	0.6	0.5	0.5
Vega	2.5	1.7	2.1	0.3	0.6	0.4
Antcin-98	0.5	1.4	0.9	0.1	0.3	0.2
Mean	1.9	5.7	3.8	0.4	1.5	0.9
	Year LSD (0.01)= 0.2 Cultivar LSD (0.01)= 0.4 Year x cultivar LSD (0.01)= 0.6			Year LSD (0.01)= 0.1 Cultivar LSD (0.01)= 0.2 Year x cultivar LSD (0.01)= 0.3		

**, the highest disease severity value was accepted as 10.0; *, data are means of three replications

In both years, average DS, DI and yield losses of all the maize cultivars in the PrePI were higher than those in the PostPI (Table 1 and 2). In the study, inoculations performed in the pre-pollination period induced a higher DS, DI and yield losses on the cultivars than those in the PostPI treatments. These findings revealed that irrespective of the host, injection of *U. maydis* during pre-pollination of maize caused more severe smut infection in cobs than those in the post-pollination period. The results of the present study corroborated the earlier findings of Snetselaar et al (2001). These authors reported that an abscission layer appeared at the bottom of pollinated cob silks, which may preclude ovaries from infection of *U. maydis* in maize cobs. Similarly, du Toit & Pataky (1999) underscored that

Table 3- Yield of the cultivars in control, pre and post-pollination inoculation

	2010				*2011*				*Mean (Cultivar × disease)*			
Cultivar (host)	*Yield control (kg ha^{-1})*	*Yield inoculated (kg ha^{-1}) (PrePI)[1]*	*Yield inoculated (kg ha^{-1}) (PostPI)[2]*	*Mean (cultivar × year)*	*Yield control (kg ha^{-1})*	*Yield inoculated (kg ha^{-1}) PrePI*	*Yield inoculated (kg ha^{-1}) PostPI*	*Mean (cultivar × year)*	*Yield control (kg ha^{-1})*	*Yield inoculated (kg ha^{-1}) PrePI*	*Yield İnoculated (kg ha^{-1}) PostPI*	*Mean cultivar*
Ada-523	11260*	6220	7530	8340	11360	4540	5170	7020	11310	5380	6350	7680
Karaçay	11230	8170	8880	9430	9430	3380	3990	5600	10330	5780	6440	7520
Pioneer-3394	10140	6730	7330	8070	10260	4260	5240	6590	10200	5500	6290	7330
Side	10510	6910	7940	8450	9730	3140	3720	5530	10120	5030	5830	6990
K. Yıldızı	10420	6580	7770	8260	8100	2760	3540	4800	9260	4670	5660	6530
Merit	4010	2410	2530	2980	3870	2280	3060	3070	3940	2350	2800	3030
Antcin-98	3900	2560	2930	3130	4050	1930	2520	2830	3980	2250	2730	2990
Vega	3940	2300	2710	2980	3300	2160	2620	2690	3620	2230	2670	2840
Mean (year × treatment)	8180	5240	5950		7510	3060	3730		7850	4150	4850	5610
Mean	6460				4770							
Year LSD (0.01)= 236; Cultivar LSD (0.01)= 473; Disease LSD (0.01)= 290; Year × cultivar LSD (0.01)= 669												
Year × disease LSD (0.01)= 410; Cultivar × disease LSD (0.01)= 820; Year × cultivar × disease LSD (0.01)= 1160												

PrePI[1], pre-pollination inoculations; PostPI [2], post-pollination inoculations; *, data are means of three replications

Table 4- Mean yield losses of the cultivars tested

Cultivar (host)	*2010*		*2011*		*Mean*	
	Yield loss (%) PrePI[1]	*Yield loss (%) PostPI*[2]	*Yield loss (%) PrePI*	*Yield loss (%) PostPI*	*Yield loss (%) PrePI*	*Yield loss (%) PostPI*
Ada-523	44.7	33.1	60.0	54.4	52.4	43.8
Pioneer-3394	33.6	27.7	58.4	48.9	50.2	42.3
Side	34.2	24.4	67.7	61.7	44.0	37.6
Karaçay	27.2	20.9	64.1	57.6	46.0	38.3
Karadeniz Yıldızı	36.8	25.4	65.9	56.2	49.5	38.8
Merit	39.9	36.9	41.1	20.9	40.3	28.9
Vega	41.6	31.2	34.5	20.6	43.4	31.4
Antcin-98	34.3	24.8	52.3	37.8	38.4	26.2
Mean	36.5	28.0	55.5	44.7	45.5	35.9
	32.2		50.1		40.7	

PrePI[1], pre-pollination inoculations; PostPI [2], post-pollination inoculations

maize cobs were susceptible to the smut fungus from the beginning of silk arising until the two week after silking period of maize. In the course of this vulnerability period, amount of infected cobs reduced with silk aging. In addition, Snetselaar & Mims (1993) postulated that unpollinated cobs were more vulnerable to *U. maydis* infection than pollinated ones.

Vulnerability of maize to fungi colonizing cobs via silks alternates depending on silk maturity e.g., *Fusarium graminearum,* causes ear rot in maize, was highest if silks were inoculated soon after beginning of silk arising and diminished drastically with silk maturity (Enerson & Hunter 1980; Reid et al 1992; du Toit & Pataky 1999). Furthermore, several researchers (Marsh & Payne 1984; Headrick et al 1990) revealed similar results regarding *Fusarium moniliforme* and *Aspergillus flavus*, another cob infecting fungi. Considering all of these, the findings of the present study and aforementioned authors have been suggested that occurrence of maize pollination prior to *U. maydis* infection in cobs renders ovaries more resistant to the fungus.

Of all maize varieties, the highest DS in the PrePI and PostPI was found in Karadeniz Yıldızı (flint corn variety), whereas the lowest DS was in Antcin-98 (popcorn variety) (Table 2). As it had more severe smut gall on its cobs, Karadeniz Yıldızı was more vulnerable to infection of *U. maydis* than the other varieties. However, the cobs of Antcin-98 were less affected by the fungus than the others. In the present study, among the varieties, the DS values were ranked according to host's gall size (from largest to smallest) as follows: Flint, dent, sweet, and popcorn, respectively. It can be concluded that the bigger the cobs, the higher the development of large smut galls on them; flint and dent corn varieties showed higher DS values than the others. In addition, DI values in the study were similar to DS values. These results supported the earlier findings of Pataky & Snetselaar (2006). These authors reported that incidence of smut infection was greater than 50% in 1976 in several areas of Germany where hybrids derived from European flint corn were prevalent. Similarly, in a study conducted by Bojanowski (1969) in Poland, U12 (a flint corn inbred) was identified as susceptible to corn smut. Pataky (1991) stated that extremely susceptible genotypes may exist among dent, flint, floury and other types of corn. In addition, Aydoğdu (2015) was reported that flint and dent corn cultivars were susceptible to *U. maydis* infection.

The average DS, DI and yield losses of all the hosts in 2011 were higher than those in 2010 (Tables 1, 2, 3 and 4). Year-to-year variation found in the present study could be explained as follows: it is

known that environmental conditions can influence the development of diseases, in particular during penetration and infection of the host. Tunçdemir & Iren (1980) reported that the most favorable temperature for development of maize smut ranges between 18 °C and 21 °C. In this regard, in 2010, the average daily temperature on the inoculation day of PrePI and PostPI were 30.2 °C and 31.6 °C, whereas in 2011, they were 27.2 °C and 27.3 °C, respectively (Table 5). Therefore, those temperatures may have adversely affected germination and penetration of the fungus in 2010. However, mean relative humidity (RH) of the August in 2010 was 59.1%, while it was 50.0% in 2011. In addition, the environmental conditions in 2010 may have been favorable for the host. Because, in 2010, average yield of all the hosts in the control plots was 8180 kg ha^{-1}, while in 2011, it was 7510 kg ha^{-1} (Table 3). Depending on these factors, in 2010, average yield loss of all the maize varieties was 32.2%, but in 2011, it was 50.1% (Table 4). Kyle (1929) emphasized that when environmental factors are in favor of the host during the maize growing season, smut infection levels are reduced. In a two-year study conducted in Germany, Görtz et al (2008) stated that in 2006, the frequency of kernel infected by *Fusarium* spp. ranged from 0.7% to 99.7%, while in 2007, the highest incidence of *Fusarium* ear rot was 64%. The authors expressed that the year-to-year variability in the overall infection rate may be explained by significant differences in temperature and precipitation during the growth periods.

Apart from these factors, plant nutrition can affect smut infection. Aydoğdu & Boyraz (2011) reported that nitrogenous and organic fertilization may affect the severity of the disease. Additionally, physiology and morphological structure of the host have an impact on colonization of *U. maydis*. Since maize cultivars tested have specific physiology and morphological features, different disease values

Table 5- Daily mean temperature of the research area during inoculations

Inoculation	*Inoculation date 2010*	*Daily average temperature (°C)*	*Inoculation*	*Inoculation date 2011*	*Daily average temperature (°C)*
PrePI	10 August	30.2	PrePI	15 August	27.2
PostPI	20 August	31.6	PostPI	25 August	27.3

Source: Regional Meteorology Station, Antalya

were determined in the present study. Pataky & Richter (2007) emphasized that leaves surrounding cobs may influence silks and accordingly infection of *U. maydis*. Pataky & Chandler (2003) also emphasized that gall size induced by *U. maydis* varies depending on maize genotypes, virulency of the pathogen, and climatic conditions. A cultivar-trial in the Columbia Basin in the U.S showed marked differences in susceptibility to corn smut between field corn hybrids (Mohan et al 2013).

4. Conclusions

Corn smut is a devastating disease of maize when environmental conditions are favourable for the fungus. In our study, for the first time, yield losses on the cobs, stemming from *U. maydis* infection, were revealed perceptibly based on interactions between maize pollination and *U. maydis*. The present study also suggested that pollination has an influence on *U. maydis* infection in cobs and correspondingly yield losses. Considering maize phenology and this host-pathogen interaction in cobs during maize pollination, new control methods can be developed. Taking into account all, further studies are needed to establish management strategies against the fungus.

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Effect of Row Spacing on Yield, Yield Components and Crude Oil of Autumn and Spring Sowed Mustard (*Sinapis arvensis* L.) in Eight Locations of Turkey

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ABSTRACT

This study was conducted to evaluate the effect of autumn and spring sowing and row spacings (20, 30, 40, 50 and 60 cm) on yield, yield components and crude oil percentage of mustard (*Sinapis arvensis* L.) at eight locations lying in different ecological zones (Ankara, Aydın, Erzurum, Eskişehir, Isparta, Şanlıurfa, Tekirdağ and Tokat) during 2013-14 and 2014-15 growing seasons. The experiment was designed according to the "Split Plots on Randomized Complete Block" with four replications. Autumn and spring sowing were main plots, row spacings were sub-plots in each location. The results showed that, autumn and spring sowing and row spacing significantly affected yield, yield components and crude oil yield across locations. In general, increasing row spacing reduced seed yield and crude oil percentage. The plants from autumn sowing increased crude oil yield compared to the plants from spring sowing. The maximum seed yield and crude oil yield (2525.5 and 695.3 kg ha^{-1}, respectively) was obtained from Tokat during autumn sowing at 50 cm row spacing. It was determined that autumn sowing was more suitable for Aydın, Tekirdağ, Tokat and Şanlıurfa locations. Autumn sowing could also be possible at Ankara, Eskişehir and Isparta locations if plants enter to winter at the right time (8-10 leaves rosette stage). Agronomic performances of mustard at Erzurum was not promising. For autumn and spring sowing, the most suitable row spacing must be 20-30 cm in Ankara, Aydın, Erzurum, Eskişehir, Isparta, Tekirdağ and Şanlıurfa; 50 cm in Tokat in autumn sowing.

Keywords: Autumn and spring sowing; *Sinapis arvensis* L.; Crude oil percentage; Mustard; Row spacing; Yield; Yield components

1. Introduction

Many *Brassica* species are used for oil or biodiesel production in the world. Two species of mustard *Sinapis alba* L. and *Sinapis arvensis* L. family Cruciferae, grow widely in Turkey under natural conditions as weed. Mustard is not cultivated for the production of vegetable oil or biodiesel production in our country.

Mustard oil is not suitable for consuming as edible oil due to high erucic acid but it is convenient for industrial use (İlisulu 1973; Jham et al 2009). Therefore, mustard has been preferred on account of its capacity of providing raw material for biodiesel energy industry (Khan et al 2011; Ahmad et al 2012; Kayaçetin et al 2016).

It has been known that mustard is grown in mild winter regions as late fall and in hard winter regions as late spring crop (İlisulu 1973; Thurling 1974). Limited water and high temperature are two significant factor restricting crop productivity (Angadi et al 2003). Mustard plant might be effected differently under autumn and spring sowing and environment conditions that are based on temperature and cold prevailing during the crop life cycle.

Different mustard varieties are grown in spring and autumn all over the world (Jankowski & Budzyn'ski 2003; Demirel & Cranshaw 2006; Wu et al 2011). The optimum sowing space favorably affects the absorption of nutrients and the exposure of the plant to light. While Christensen & Drabble (1984) in canola, Arif et al (2012) in mustard reported that more uniform distribution of seeds per unit area decreased competition among plant populations so the seed yield of plant was higher in closer row spacings. Kazemeini et al (2010) obtained higher seed yield wider row spacings in canola. Kaur & Sidhu (2006) and Keiwanrad & Zandi (2014) suggested that the crude oil yield of mustard was higher in closer row spacings.

Yield and its development process of mustard like other crops depend on genetic, environmental and agronomic factors (like row spacing, irrigation, seed rate, fertilizer) as well as the interaction between them (Zukalova et al 1988; Shekhawat et al 2012; Keiwanrad & Zandi 2014). Establishment of optimum population density per unit area is a prerequisite for an increased grain yield. Population density is known to influence yield and yield components of mustard in positive or negative way (Johnson et al 2003). Successful adaptation of a plant to the environment involves reducing unfavorable risk factors and increasing favorable factors like optimum radiation, temperature and moisture (Mendham & Salisbury 1995). The aim of the study is to evaluate the effect of row spacing on yield, yield components and crude oil percentage of autumn and spring sowed mustard in eight different locations of Turkey.

2. Material and Methods

2.1. Experimental conditions

The experiments were conducted during the growing seasons of 2013-14 and 2014-15 at eight locations (Ankara, Erzurum, Eskişehir, Isparta, Tekirdağ, Tokat and Şanlıurfa) of Turkey lying at altitudes from 29 to 1893 m above sea level (Table 2). The seeds of mustard were collected from the plants growing under wild conditions in Konya province. Thereafter, these were multiplied for use in the experiment in Central Research Institute for Field Crops in 2012-13.

The experiment was set up in "Split Plots on Randomized Complete Block" design with four replication. Autumn and spring sowing were done in main plots using row spacing of 20, 30, 40, 50, and 60 cm in sub-plots at eight locations. Each plot was 5 m long and consisted of 15 rows (20 cm), 10 rows (30 cm), 7 rows (40 cm), 6 rows (50 cm), 5 rows (60 cm). Seeds were sown at 1-2 cm depth. Nitrogen, phosphorus and sulphur fertilizers were applied at the rate of 100, 50 and 35 kg ha^{-1} in the form of diammonium phosphate, ammonium nitrate and ammonium sulfate respectively (Pyare et al 2008). The total quantity of phosphorus and sulphur fertilizer was applied at the time of sowing. The total nitrogen fertilization was applied in two equal doses, at the time of sowing and rosette formation. No irrigation was done to the experimental plots during the study period of two years.

According to autumn and spring sowing, location and row spacing ratio of winter survival

(%), sowing, emergence, flowering and harvest date of mustard are shown in Table 1. Sowing and harvest date of mustard were made at optimum time under locations ecological conditions. The data could not be obtained due to cold damage at Ankara, Eskişehir and Isparta locations in the first year. Significant cold damage losses didn't observed in the second year as plants entered to winter at 8-10 leaves rosette stage. Ratio of winter survival were 79%, 66% and 83%, at Aydın, Tekirdağ, Tokat and Şanlıurfa locations respectively in the second year. Erzurum ecological conditions, emergence could not be achieved despite irrigation for both years due to high coldness (Table 1).

2.2. Meteorological data of the experimental area

Monthly meteorological data during mustard development in the experimental areas are shown in Table 2.

The total rainfall in 2013-2014 ranged 257.4 to 647.9 mm. The maximum rainfall was found at Tekirdağ location while the lowest rainfall was at Eskişehir location. The total rainfall during 2014-2015 ranged 406.7 to 791.0 mm. While the maximum rainfall were found at Aydın location, the lowest rainfall was at Tokat location. Şanlıurfa location had the maximum average temperature and Erzurum location had the lowest average temperature (Table 2).

Table 1- According to autumn and spring sowing, location and row spacing sowing, ratio of winter survival (%), emergence, flowering and harvest date of mustard

Year	*Autumn and spring sowing*	*Location*	*Ratio of winter survival (%)*	*Sowing date*	*Emergence date*	*Flowering date*	*Harvest date*
2013-14	Fall sowing	Ankara	-	02-Oct-2013	28-Oct-2013	Cold damage	Cold damage
		Aydın	100	25-Oct-2013	15-Nov-2013	06-Mar-2014	10-Jun-2014
		Erzurum	-	12-Oct-2013	-	-	-
		Eskişehir	-	01-Oct-2013	18-Oct-2013	Cold damage	Cold damage
		Isparta	-	10-Oct-2013	29-Oct-2013	Cold damage	Cold damage
		Şanlıurfa	100	31-Oct-2013	12-Nov-2013	25-Mar-2014	22-May-2014
		Tekirdağ	100	02-Oct-2013	24-Oct-2013	21-Mar-2014	17-Jun-2014
		Tokat	100	08-Oct-2013	25-Oct-2013	06-Apr-2014	09-Jun-2014
	Spring sowing	Ankara	-	16-Apr-2014	29-Apr-2014	14-Jun-2014	4-Aug-2014
		Aydın	-	20-Mar-2014	28-Mar-2014	20-Apr-2014	15-Jul-2014
		Erzurum	-	24-Apr-2014	18-May-2014	11-Jul-2014	18-Aug-2014
		Eskişehir	-	08-Apr-2014	24-Apr-2014	02-Jun-2014	22-Jul-2014
		Isparta	-	21-Mar-2014	10-Apr-2014	03-Jun-2014	19-Jul-2014
		Şanlıurfa	-	26-Feb-2014	07-Mar-2014	26-Apr-2014	16-Jun-2014
		Tekirdağ	-	04-Apr-2014	19-Apr-2014	22-May-2014	26-Jul-2014
		Tokat	-	05-Mar-2014	21-Mar-2014	07-May-2014	7-Jul-2014
2014-15	Fall sowing	Ankara	79	14-Oct-2014	02-Nov-2014	26-May-2015	15-Jul-2015
		Aydın	100	03-Nov-2014	22-Nov-2014	18-Feb-2015	26-Jun-2015
		Erzurum	-	16-Oct-2014	-	-	-
		Eskişehir	66	14-Oct-2014	27-Oct-2014	08-May-2015	30-Jun-2015
		Isparta	100	23-Oct-2014	05-Nov-2014	07-May-2015	13-Jul-2015
		Şanlıurfa	83	27-Oct-2014	11-Nov-2014	10-Apr-2015	1-Jun-2015
		Tekirdağ	100	16-Oct-2014	02-Nov-2014	20-Apr-2015	8-Jul-2015
		Tokat	100	14-Oct-2014	25-Oct-2014	04-May-2015	29-Jun-2015
	Spring sowing	Ankara	-	01-May-2015	12-May-2015	22-Jun-2015	10-Aug-2015
		Aydın	-	17-Apr-2015	24-Apr-2015	7-Jun-2015	09-Jul-2015
		Erzurum	-	15-May-2015	30-May-2015	30-Jun-2015	01-Sep-2015
		Eskişehir	-	04-Mar-2015	06-Apr-2015	11-Jun-2015	10-Jul-2015
		Isparta	-	17-Mar-2015	10-Apr-2015	1-Jun-2015	17-Jul-2015
		Şanlıurfa	-	27-Feb-2015	12-Mar-2015	3-May-2015	09-Jun-2015
		Tekirdağ	-	16-Apr-2015	30-Apr-2015	10-May-2015	15-Jul-2015
		Tokat	-	28-Feb-2015	17-Mar-2015	30-May-2015	06-Jul-2015

Table 2- Montly meteorological data of mustard during growing seasons in experimental areas

Location	*Altitude (m)*	*Climatic factors*	*Years*	*Months*												*Total or average*
				September	*October*	*November*	*December*	*January*	*February*	*March*	*April*	*May*	*June*	*July*	*August*	
Ankara	925	Precipitation (mm)	Long term	16.1	35.5	37.5	41.7	37.6	35.6	39.9	49.3	49.1	34.2	16.0	10.2	402.7
			2013-14	1.8	26.0	1.4	4.8	33.8	8.0	47.6	32.2	69.2	58.6	2.4	21.2	307.0
			2014-15	56.8	38.4	26.5	39.5	54.3	39.0	92.1	25.0	67.2	133.7	5.1	25.4	603.0
		Relative humidity (%)	Long years	49.2	61.1	70.8	76.1	71.4	70.3	63.4	59.7	56.6	52.1	46.3	45.4	60.2
			2013-14	44.4	51.1	64.7	74.2	90.4	59.9	60.6	53.1	59.0	56.5	38.7	48.7	55.5
			2014-15	55.1	68.4	69.3	84.0	77.8	70.4	66.3	53.1	63.4	65.8	39.1	43.9	63.1
		Average temperature (ºC)	Long years	18.8	12.8	6.6	2.3	0.6	2.0	5.8	11.3	15.9	20.1	23.5	23.5	11.9
			2013-14	16.9	10.2	6.9	-2.9	1.6	4.4	6.7	11.9	15.0	18.3	24.2	23.6	11.4
			2014-15	19.3	13.3	7.7	5.6	1.2	3.3	7.1	9.5	14.9	18.4	24.9	24.7	12.5
		Maximum temperature (ºC)	Long years	26.0	19.4	12.0	6.1	4.5	6.6	11.3	17.1	22.1	26.6	30.0	30.4	30.0
			2013-14	30.9	26.0	20.6	12.3	14.3	18.1	20.6	26.4	27.7	32.6	34.6	36.9	34.6
			2014-15	34.9	24.7	18.3	14.2	12.3	16.4	21.3	24.0	32.1	28.1	38.0	34.7	38.0
		Minimum temperature (ºC)	Long years	11.9	7.5	2.3	-0.5	-2.3	-1.8	0.9	5.7	9.7	13.1	16.3	16.4	-2.3
			2013-14	5.7	-1.6	-4.7	-13.4	-6.1	-8.7	-6.4	0.2	5.4	8.6	13.2	13.7	-13.4
			2014-15	5.6	-0.3	-2.4	-2.8	-16.0	-8.2	-5.1	-2.6	5.7	11.0	13.2	11.6	-16.0
Aydın	86	Precipitation (mm)	Long years	10.2	39.9	84.3	110.7	97.8	91.0	70.8	55.5	36.8	10.7	2.5	2.8	613.0
			2013-14	18.8	38.8	81.6	12.4	45.8	14.2	72.0	42.0	41.2	41.0	0.0	0.0	407.8
			2014-15	5.2	21.8	117.6	216.9	135.1	126.1	102.8	28.2	25.4	8.9	3.0	0.0	791.0
		Relative humidity (%)	Long years	55.2	61.5	68.2	72.5	68.7	67.8	64.4	62.1	55.8	48.1	48.3	52.5	60.4
			2013-14	45.1	55.7	65.0	63.2	78.5	71.8	64.8	63.9	57.1	52.5	44.9	47.5	59.2
			2014-15	57.9	61.8	72.4	81.9	73.9	68.0	70.9	53.4	57.1	52.5	46.6	51.9	62.4
		Average temperature (ºC)	Long years	23.3	18.3	12.9	9.1	8.0	8.9	11.6	15.8	20.9	26.0	28.5	27.6	17.6
			2013-14	23.6	16.1	12.8	6.2	9.5	9.9	12.6	16.9	21.6	25.8	29.6	30.2	17.9
			2014-15	23.8	19.6	13.6	11.7	8.1	9.2	12.1	14.6	22.1	24.2	29.4	29.1	17.4
		Maximum temperature (ºC)	Long years	32.1	26.4	19.3	14.2	13.2	14.4	17.8	22.5	28.4	33.5	36.4	35.8	36.4
			2013-14	37.5	31.2	28.5	17.4	19.1	22.1	27.3	38.1	35.3	40.6	40.4	43.3	43.3
			2014-15	35.8	32.8	25.6	20.6	20.3	23.1	25.1	31.6	35.3	37.4	41.2	39.8	41.2
		Minimum temperature (ºC)	Long years	17.0	11.0	10.0	3.0	7.0	6.0	8.0	11.0	15.0	18.1	20.6	20.5	3.0
			2013-14	11.1	5.6	1.2	-3.4	-0.7	-1.2	1.8	5.1	9.7	11.7	18.7	19.1	-3.4
			2014-15	13.5	10.3	3.0	2.0	-5.1	-1.5	1.9	3.9	12.7	13.6	19.5	19.7	-5.1
Erzurum	1893	Precipitation (mm)	Long years	19.7	48.2	30.5	22.0	17.7	22.1	32.8	56.7	68.9	40.5	24.4	16.6	400.1
			2013-14	13.6	16.8	19.6	8.3	11.3	8.0	35.7	31.6	88.6	21.6	27.8	42.8	325.7
			2014-15	42.8	45.8	13.4	19.0	13.2	33.6	25.8	61.6	69.8	73.3	13.6	56.0	467.9
		Relative humidity (%)	Long years	53.0	65.4	74.1	78.9	76.1	77.8	74.2	66.8	63.8	58.7	53.4	50.5	66.1
			2013-14	49.8	59.6	74.1	78.6	83.9	80.4	70.2	64.5	68.7	54.9	46.9	52.0	65.3
			2014-15	52.0	71.9	77.2	86.6	82.6	86.7	79.4	67.3	66.6	58.7	46.4	45.4	68.4
		Average temperature (ºC)	Long years	13.7	7.3	0.0	-6.9	-9.8	-8.6	-2.3	5.2	10.0	14.5	18.9	18.9	5.1
			2013-14	13.6	6.0	2.3	-13.4	-10.1	0.3	2.3	7.5	11.3	15.3	20.5	14.6	5.9
			2014-15	14.6	8.4	0.2	-0.8	-8.1	-7.4	-1.6	4.9	10.2	15.7	20.0	20.5	6.4
		Maximum temperature (ºC)	Long years	23.1	15.5	6.3	-1.4	-3.8	-2.4	3.0	11.4	16.8	22.1	26.9	27.6	27.6
			2013-14	28.8	23.7	16.6	1.2	4.0	13.8	14.4	20.8	23.9	28.8	33.4	30.9	33.4
			2014-15	30.9	20.0	13.2	7.8	5.8	6.3	11.1	19.5	23.4	29.8	33.1	34.8	34.8
		Minimum temperature (ºC)	Long years	4.2	0.3	-5.3	-12.1	-15.5	-14.4	-7.7	-0.5	2.9	5.7	9.7	9.5	-15.5
			2013-14	-3.4	-9.0	-11.9	-27.1	-28.5	-18	-16.9	-10.2	-3.2	0.8	6.0	-3.7	-28.5
			2014-15	-3.7	-3.6	-11.0	-14.1	-23.6	-24.6	-23.7	-7.2	-3.4	1.3	7.1	9.2	-24.6
Eskişehir	788	Precipitation (mm)	Long years	13.9	32.5	35.3	42.1	33.1	27.8	30.7	42.4	40.6	25.7	13.0	7.7	344.8
			2013-14	3.0	51.0	19.6	1.6	15.2	3.4	13.1	51.4	31.2	63.7	4.2	0.0	257.4
			2014-15	41.4	34.5	18.2	77.4	54.3	39.0	92.1	25.0	67.2	133.7	5.1	25.4	613.3
		Relative humidity (%)	Long years	57.8	65.4	71.6	77.0	75.1	72.2	66.3	63.1	60.7	56.9	53.5	54.4	64.5
			2013-14	49.5	59.0	66.9	72.6	79.7	60.6	63.2	57.4	61.5	62.7	55.6	57.5	62.2
			2014-15	69.7	77.9	81.2	89.8	89.9	76.7	72.3	59.7	54.6	72.8	55.0	60.3	71.7
		Average temperature (ºC)	Long years	16.8	11.3	5.4	1.3	0.0	1.1	4.5	9.8	14.7	18.7	21.6	21.4	10.6
			2013-14	17.6	10.9	8.5	-1.0	3.8	6.2	8.1	13.2	16.6	20.2	23.8	24.0	12.7
			2014-15	18.4	13.4	7.6	5.5	-0.1	3.4	6.5	9.2	17.5	17.9	23.4	23.4	12.2
		Maximum temperature (ºC)	Long years	25.3	18.9	11.7	5.3	3.9	6.3	11.1	16.4	21.7	25.7	28.9	29.2	29.2
			2013-14	33.2	25.5	21.6	10.6	18.0	22.3	23.4	27.2	28.5	35.4	37.2	36.4	37.2
			2014-15	33.3	25.5	17.5	14.4	12.9	18.1	21.0	27.7	31.8	29.1	36.9	33.4	36.9
		Minimum temperature (ºC)	Long years	8.6	4.5	0.2	-1.8	-3.3	-3.2	-0.8	3.3	7.2	10.7	13.5	13.3	-3.3
			2013-14	6.4	-2.0	-0.6	-8.9	-2.8	-6.4	-2.8	1.3	6.6	8.9	13.6	15.4	-8.9
			2014-15	6.4	0.3	-1.0	-2.8	-13.1	-6.7	-3.6	-2.4	6.6	9.6	12.6	13.6	-13.1

Table 2 (Continue)- Montly meteorological data of mustard during growing seasons in experimental areas

Location	*Altitude (m)*	*Climatic factors*	*Years*	*Months*												*Total or average*
				September	*October*	*November*	*December*	*January*	*February*	*March*	*April*	*May*	*June*	*July*	*August*	
Isparta	1050	Precipitation (mm)	Long years	15.5	35.5	47.7	69.3	59.4	55.8	54.3	56.9	48.9	26.6	17.5	14.7	502.1
			2013-14	3.0	104.0	67.6	29.4	61.3	23.4	78.6	44.8	107.0	42.8	0.8	3.2	565.9
			2014-15	100.0	57.2	37.4	104.2	82.5	100.8	79.0	24.3	10.3	0.7	0.2	0.0	596.6
		Relative humidity (%)	Long years	16.5	19.5	20.1	21.3	19.0	22.8	21.0	20.5	20.4	17.3	15.8	15.0	19.1
			2013-14	43.3	54.0	65.5	64.2	76.7	60.8	63.3	59.5	60.3	49.8	43.5	44.2	57.1
			2014-15	60.2	64.9	69.3	76.6	73.8	68.5	65.2	60.0	60.1	63.5	43.9	51.0	63.1
		Average temperature (ºC)	Long years	18.4	12.5	6.8	3.0	1.7	2.7	5.8	10.4	15.3	20.3	23.5	23.1	12.0
			2013-14	18.9	10.7	8.7	1.0	3.7	5.2	7.3	11.7	15.1	20.0	24.5	24.9	12.6
			2014-15	18.1	13.1	7.0	5.9	1.9	3.3	6.7	9.0	14.8	18.3	24.2	23.8	12.2
		Maximum temperature (ºC)	Long years	26.5	20.3	13.5	7.8	6.3	7.6	11.5	16.4	21.8	26.7	30.4	30.5	30.5
			2013-14	33.1	25.3	22.4	17.1	13.8	18.7	18.9	25.6	27.3	35.4	34.9	36.9	36.9
			2014-15	31.2	25.2	19.7	15.7	12.9	14.2	17.6	22.7	29.2	28.7	36.1	34.9	36.1
		Minimum temperature (ºC)	Long years	10.2	6.0	1.8	-0.8	-2.0	-1.2	0.7	4.4	8.2	12.1	15.2	14.9	-2.0
			2013-14	4.8	-2.2	-3.3	-9.5	-5.3	-7.4	-3.0	-0.6	4.2	8.3	12.4	11.5	-9.5
			2014-15	6.8	0.1	-2.7	-4.7	-13.8	-7.0	-2.5	-3.2	4.8	8.4	11.7	12.8	-13.8
Tekirdağ	29	Precipitation (mm)	Long years	39.7	69.9	68.3	81.0	58.3	56.6	55.3	41.2	35.9	36.9	24.3	12.4	579.8
			2013-14	10.9	95.8	41.3	3.9	44.4	6.0	73.6	46.8	72.1	69.6	107.7	75.8	647.9
			2014-15	0.8	106.3	3.1	69.8	56.5	90.6	28.9	59.2	0.4	52.6	0.5	0	468.7
		Relative humidity (%)	Long years	74.2	79.3	82.4	82.7	80.1	80.8	80.0	78.3	76.8	73.5	69.7	71.4	77.4
			2013-14	60.3	76.2	78.8	73.7	89.8	84.7	81.3	82.2	78.1	75.0	72.2	73.2	77.1
			2014-15	77.8	79.6	84.9	89.2	82.2	78.8	81.8	74.8	74.9	73.3	67.8	67.1	77.7
		Average temperature (ºC)	Long years	19.9	15.3	10.4	6.5	4.8	4.9	7.3	11.7	16.7	21.2	24.0	23.8	13.9
			2013-14	21.8	14.2	12.9	6.1	8.0	8.5	9.8	13.6	17.8	22.0	24.9	25.3	15.4
			2014-15	20.7	15.9	11.0	9.4	5.6	6.5	8.5	11.3	18.5	21.3	25.6	26.4	15.1
		Maximum temperature (ºC)	Long years	24.3	19.5	14.1	9.7	8.4	8.7	11.0	15.7	20.4	25.1	28.1	28.3	28.3
			2013-14	33.1	22.4	22.4	15.3	20.4	20.6	24.0	22.8	27.0	36.9	34.6	33.4	36.9
			2014-15	30.0	27.7	19.6	17.5	16.7	21.1	18.3	24.6	27.9	33.3	33.9	32.1	33.9
		Minimum temperature (ºC)	Long years	15.8	11.8	7.3	3.7	2.2	2.1	4.0	8.0	12.3	16.5	19.1	19.4	2.1
			2013-14	13.6	4.2	1.0	-2.3	-2.5	1.4	1.8	4.9	8.9	12.7	17.1	16.1	-2.5
			2014-15	10.5	5.3	1.3	-1.2	-8.8	-5.2	0.0	1.7	8.9	13.4	16.7	18.2	-8.8
Tokat	571	Precipitation (mm)	Long years	16.8	49.2	52.7	44.4	37.6	36.2	40.8	57.2	62.9	37.9	12.8	7.4	455.9
			2013-14	12.3	45.0	13.8	40.3	14.5	12.6	55.1	12.7	29.2	61.5	7.3	1.1	305.4
			2014-15	39.0	51.6	63.1	39.4	38.4	25.8	57.0	34.5	34.8	35.6	0.2	7.6	425.0
		Relative humidity (%)	Long years	58.3	64.9	69.8	70.5	66.2	63.3	58.7	58.0	60.4	58.3	56.4	56.5	61.8
			2013-14	54.1	58.3	64.5	74.1	67.3	49.6	50.7	43.0	55.0	54.5	46.8	46.2	55.3
			2014-15	54.5	68.5	73.0	75.6	68.0	62.1	65.9	57.6	56.3	62.1	52.7	52.4	62.4
		Average temperature (ºC)	Long years	18.7	13.5	7.3	3.2	2.0	3.0	7.0	12.5	16.2	19.6	22.1	22.5	12.3
			2013-14	18.0	11.7	8.7	-1.0	4.4	8.0	11.1	16.2	17.7	20.5	24.6	25.4	13.8
			2014-15	19.9	14.1	7.1	7.0	2.4	5.2	8.1	10.0	17.2	20.1	22.5	25.4	13.3
		Maximum temperature (ºC)	Long years	26.6	20.4	12.8	7.3	6.0	8.0	12.7	19.1	23.1	26.6	28.9	29.9	29.9
			2013-14	35.3	30.7	21.6	14.7	17.1	21.4	24.9	29.2	34.2	34.4	40.6	40.5	40.6
			2014-15	37.3	24.5	22.1	18.2	15.0	18.8	22.6	26.9	36.4	30.9	37.4	36.4	37.4
		Minimum temperature (ºC)	Long years	12.0	8.1	2.9	0.0	-1.4	-1.0	2.0	6.5	9.5	12.8	15.4	15.6	-1.4
			2013-14	6.7	0.5	-0.8	-10.7	-5.5	-6.0	-4.4	-0.3	8.2	10.6	13.3	15.7	-10.7
			2014-15	5.8	0.8	-2.0	-2.1	-15.3	-7.2	-1.1	-1.0	4.4	9.6	10.7	10.9	-15.3
Şanlıurfa	410	Precipitation (mm)	Long years	4.2	27.5	50.5	68.9	79.7	69.5	61.1	42.1	25.4	4.7	0.9	1.2	435.7
			2013-14	0.0	0.0	19.5	76.7	44.3	20.8	91.6	33.3	6.0	20.6	0.0	1.0	313.8
			2014-15	28.8	25.8	79.4	55.2	82.5	100.8	79.0	24.3	10.3	0.7	0.2	0.0	487.0
		Relative humidity (%)	Long years	39.2	49.4	62.3	69.4	67.4	65.7	59.8	56.2	45.8	34.8	32.4	36.7	51.6
			2013-14	29.3	26.7	57.8	53.4	66.0	45.1	51.7	44.6	29.8	24.4	25.8	27.3	40.2
			2014-15	40.5	49.1	54.1	79.5	68.4	74.4	58.8	50.1	35.6	33.6	26.2	37.0	50.6
		Average temperature (ºC)	Long years	26.6	20.1	12.4	7.3	5.6	6.9	10.9	16.3	22.5	28.3	31.9	31.1	18.3
			2013-14	26.2	19.0	14.6	6.2	8.6	9.9	13.7	18.4	24.3	28.7	32.6	32.3	19.5
			2014-15	26.3	20.1	12.2	9.5	6.2	7.7	11.9	15.7	23.7	28.2	33.5	31.9	18.9
		Maximum temperature (ºC)	Long years	33.8	26.7	18.0	11.7	10.1	11.8	16.5	22.6	28.8	34.8	38.8	38.3	38.8
			2013-14	38.2	32.0	27.0	16.9	18.0	22.1	24.7	30.8	38.7	40.1	43.4	43.5	43.4
			2014-15	40.6	31.9	22.8	17.0	17.2	17.4	24.8	29.9	36.9	38.4	42.8	43.1	36.1
		Minimum temperature (ºC)	Long years	20.2	14.8	8.3	3.9	2.5	3.0	6.2	10.9	15.9	21.0	24.8	24.2	2.5
			2013-14	17.0	10.8	5.9	-2.5	2.4	-1.1	2.2	3.6	12.4	15.3	20.3	20.2	-2.5
			2014-15	14.5	9.5	4.8	2.5	-3.1	-0.6	2.5	4.7	11.8	16.7	21.4	22.1	-3.1

2.3. Soil properties

Soil samples features belonging to experimental areas are shown in Table 3. Soil of experimental areas were low in organic matter except for Eskişehir, clay loamy or loamy and in alkali (Table 3).

2.4. Yield and its components and crude oil percentage

In this study, plant height, number of lateral branches, number of pods per plant, pod length, number of seeds per pod, thousand-seed weight, seed yield, crude oil percentage and crude oil yield was determined as described by Öğütçü (1979).

The oil percentage was determined by grinding 10 g of powdered mustard seed samples and extracting by hexane were use with Gerhardt 2000 soxhlet apparatus.

2.5. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using the MSTAT-C computer Statistical software. The significant differences between the group means were separated using Duncan's test.

3. Result and Discussion

Plant height, number of lateral branches, number of pods per plant, pod length, number of seeds per pod are presented in Table 4; thousand-seed weight, seed yield, crude oil percentage and crude oil yield are presented in Table 5.

In autumn sowing, statistically significant differences were found between the two consecutive years in terms of plant height, number of lateral branches, number of pods per plant, thousand-seed weight, seed yield, crude oil percentage, crude oil yield of mustard. However, no such differences were found in the pod length and number of seeds per pod during both years. The seed yield (1751.5 kg ha^{-1}) of the second year was higher compared to the yield of the first year (1205.4 kg ha^{-1}) (Table 4). This difference was due to higher rainfall during the growing period of plants in the second year.

The effect of location on the seed yield and yield components, including plant height, number of lateral branches, number of pods per plant, pod length, number of seeds per pod, thousand-seed weight and crude oil percentage and crude oil yield were found statistically significant for both years. The maximum plant height (218.97 and 235.96 cm) and the maximum number of lateral branches per plant (10.63 and 14.78) were determined at Şanlıurfa and at Aydın in both years respectively. The highest number of pods per plant (394.58 and 380.78, respectively) was obtained at Aydın during 2013-14 and at Tekirdağ during 2014-15. The maximum pod length (3.50 and 3.87 cm, respectively) were determined at Aydın during 2013-14 and at Eskişehir during 2014-15. The maximum number of seeds per pod (15.50 and 13.48, respectively) was obtained at Aydın during 2013-14 and at Isparta during 2014-15 (Table 4). The maximum thousand-seed weight (3.48 and 4.34 g, respectively) was obtained at Tekirdağ during 2013-14 and at Isparta during 2014-15. The maximum seed yield (1895.4 and 3674.2 kg ha^{-1}, respectively) was obtained at Aydın during 2013-14 and at Tokat during 2014-15. Although Aydın location had higher rainfall during the growing period of plants in the second year (791.0 mm) compared to the first year (407.8 mm), seed yield might be low due to diseases (white rust and mildew), causing significant losses in mustard (Sangeetha & Siddaramaiah 2007). Rainfall during the growing period of plants in the second year (406.7 mm) was higher compared to the first year (224.8 mm) at Tokat location. At flowering and ripening during 2014-2015, relatively low temperatures led to prolonged vegetation period as confirmed in previous studies (Schuster & Taghizadeh 1981; Kondra et al 1983). High seed yields resulted from higher rainfall due to regular and sufficient rainfall during second year (406.7 mm) compared to the first year (305.2 mm) at Tokat locations. Long drought period before flowering causes decreases in seed yield which is similar to previous study results (İptaş & Kolsarıcı 1988). Walton et al (1999) indicated that a longer ripening period affected seed yield positively. Results of the previous studies support that differences in yield

Table 3- Soil samples features belonging to experimental areas

Location	*Year*	*Depth (cm)*	*Texture*	*The ratio of saturation (%)*	*Total salt (%)*	*pH*	*Lime (%)*	*Phosphorus (P)*	*Potassium (K)*	*Organic substance (%)*
Ankara	2013-14	0-20	Clay loamy	64.00	0.041	7.79	28.12	6.63	162.04	1.31
		20-40	Clay loamy	63.00	0.035	7.85	27.40	4.87	149.86	1.31
		Average		63.50	0.038	7.82	27.76	5.75	155.95	1.31
	2014-15	0-20	Clay loamy	63.00	0.028	7.75	31.45	7.35	234.55	0.90
		20-40	Clay loamy	63.00	0.037	7.76	24.82	7.81	219.99	1.49
		Average		63.00	0.033	7.76	28.14	7.58	227.27	1.20
Aydın	2013-14	0-20	Loam	49.00	0.017	8.00	14.23	22.29	52.61	0.53
		20-40	Loam	49.00	0.017	8.06	13.98	17.17	50.13	1.16
		Average		49.00	0.017	8.03	14.11	19.73	51.37	0.85
	2014-15	0-20	Clay loamy	51.00	0.028	7.90	13.29	19.17	77.10	1.30
		20-40	Clay loamy	51.00	0.029	7.96	16.41	15.86	63.00	1.45
		Average		51.00	0.029	7.93	14.85	17.52	70.05	1.38
Erzurum	2013-14	0-20	Loam	50.00	0.018	7.93	5.94	11.23	105.60	0.64
		20-40	Clay loamy	51.00	0.018	7.98	6.20	12.77	92.44	0.53
		Average		50.50	0.018	7.96	6.07	12.00	99.02	0.59
	2014-15	0-20	Clay loamy	54.00	0.450	7.80	5.99	9.61	109.02	0.97
		20-40	Clay loamy	52.00	0.254	7.84	5.17	9.68	86.15	1.20
		Average		53.00	0.352	7.82	5.58	9.65	97.59	1.09
Eskişehir	2013-14	0-20	Clay loamy	61.00	1.000	8.08	10.99	8.59	132.00	3.45
		20-40	Clay loamy	60.00	0.836	7.99	8.06	8.51	136.00	3.87
		Average		60.50	0.918	8.04	9.525	8.55	134.00	3.66
	2014-15	0-20	Clay loamy	58.00	0.043	7.57	22.55	7.96	105.60	1.53
		20-40	Clay loamy	58.00	0.039	7.71	20.17	8.25	102.24	1.71
		Average		58.00	0.041	7.64	21.36	8.11	103.92	1.62
Isparta	2013-14	0-20	Loam	45.00	0.011	7.88	31.19	7.08	40.74	0.26
		20-40	Clay loamy	53.00	0.014	7.83	30.44	5.04	89.27	0.14
		Average		49.00	0.013	7.86	30.82	6.06	65.005	0.20
	2014-15	0-20	Loamy	43.00	0.011	7.88	30.55	3.69	145.90	0.67
		20-40	Loamy	42.00	0.008	7.93	32.93	4.68	149.86	0.99
		Average		42.50	0.005	7.91	31.74	4.19	147.88	0.83
Tekirdağ	2013-14	0-20	Clay loamy	53.00	0.022	7.88	8.10	5.18	92.44	0.13
		20-40	Clay loamy	52.00	0.022	7.83	8.21	4.05	40.74	0.25
		Average		52.50	0.022	7.86	8.16	4.62	66.59	0.19
	2014-15	0-20	Clay loamy	57.00	0.032	7.30	0.74	7.32	57.70	1.61
		20-40	Clay loamy	56.00	0.016	7.62	0.74	6.57	52.61	1.37
		Average		56.50	0.024	7.46	0.74	6.95	55.16	1.49
Tokat	2013-14	0-20	Loamy	46.00	0.015	7.74	11.85	7.44	43.01	0.40
		20-40	Loamy	46.00	0.018	7.79	11.28	5.16	34.21	0.55
		Average		46.00	0.020	7.77	11.57	6.30	38.61	0.48
	2014-15	0-20	Clay loamy	51.00	0.022	7.64	11.65	8.05	65.72	1.27
		20-40	Loamy	49.00	0.023	7.57	15.41	5.39	32.14	1.18
		Average		50.00	0.023	7.61	13.53	6.72	48.93	1.23
Şanlıurfa	2013-14	0-20	Clay loamy	69.00	0.045	7.68	30.0	6.01	160.8	1.74
		20-40	Clay loamy	68.00	0.053	7.73	30.0	2.63	72.0	1.49
		Average		68.50	0.049	7.71	30.0	4.32	116.4	1.62
	2014-15	0-20	Clay loamy	54.00	0.023	7.98	32.93	4.05	102.24	0.75
		20-40	Clay loamy	55.00	0.026	8.02	32.78	1.85	71.31	0.25
		Average		54.50	0.025	8.00	32.56	2.95	86.78	0.50

Table 4- Effects of autumn and spring sowing, location and row spacing on morphologic characteristics of mustard

Fall sowing

Location	*Row spacing (cm)*	*Plant height (cm)*		*Number of lateral branches (branch plant^{-1})*		*Number of pods per plant (pod plant^{-1})*		*Pod length (cm)*		*Number of seeds per pod (seed pod^{-1})*	
		2013-14	*2014-15*	*2013-14*	*2014-15*	*2013-14*	*2014-15*	*2013-14*	*2014-15*	*2013-14*	*2014-15*
Ankara	20	-	147.95	-	5.45	-	120.23	-	2.91	-	10.60
	30	-	156.33	-	6.05	-	143.93	-	2.86	-	10.40
	40	-	156.33	-	6.25	-	133.95	-	3.05	-	11.16
	50	-	165.58	-	6.40	-	158.82	-	3.03	-	12.35
	60	-	155.83	-	6.70	-	162.88	-	2.79	-	10.35
Aydın	20	209.68	131.05	10.60	14.53	400.63 ab	104.63	3.48	2.80	15.35	10.97
	30	210.93	128.35	10.68	12.68	388.85 a-c	81.50	3.55	2.83	14.78	10.13
	40	211.33	131.30	10.75	17.45	393.25 ab	118.85	3.55	2.95	14.90	11.38
	50	217.20	129.45	10.53	14.85	406.30 a	117.63	3.45	3.03	15.48	10.93
	60	206.10	118.88	10.58	14.38	383.85 a-c	119.90	3.45	2.85	15.50	11.53
Eskişehir	20	-	155.13	-	6.93	-	87.76	-	3.88	-	12.33
	30	-	140.13	-	5.68	-	77.80	-	3.85		13.60
	40	-	138.00	-	5.73	-	82.63	-	3.92		12.11
	50	-	150.88	-	6.00	-	80.73	-	3.90		12.49
	60	-	143.25	-	6.40	-	91.95	-	3.80		11.74
Isparta	20	-	118.88	-	4.45	-	72.58	-	3.60		12.23
	30	-	135.33	-	5.73	-	95.70	-	3.61		13.10
	40	-	126.68	-	6.10	-	89.80	-	3.71	-	13.60
	50	-	138.30	-	6.08	-	105.98	-	3.86	-	14.38
	60	-	126.73	-	6.33	-	113.70	-	3.90	-	14.08
Tekirdağ	20	188.50	152.75	7.50	8.00	351.00 b-e	383.00	3.15	3.48	11.50	11.50
	30	211.00	151.50	7.75	7.25	317.50 d-g	380.00	3.45	3.53	10.25	10.75
	40	214.25	153.25	7.75	7.50	351.50 b-e	381.75	3.35	3.60	10.75	10.50
	50	214.50	151.75	7.25	7.75	337.00 c-f	379.00	3.38	3.53	11.75	11.50
	60	199.25	153.50	7.75	7.75	352.50 b-d	380.00	3.05	3.50	11.00	11.00
Tokat	20	125.48	179.95	7.28	6.73	98.90 j	242.43	2.18	2.79	9.98	12.20
	30	123.43	183.70	6.93	6.30	90.70 j	233.95	2.23	2.68	10.60	13.38
	40	121.30	182.90	7.45	6.58	94.95 j	207.60	2.18	2.65	9.93	13.58
	50	120.73	184.28	7.35	4.78	91.48 j	200.18	2.10	2.72	10.55	14.15
	60	115.60	171.15	7.03	5.63	80.35 j	172.20	2.23	2.76	9.63	11.73
Şanlıurfa	20	219.90	244.53	7.80	8.70	267.33 gh	232.15	2.58	2.65	12.85	12.65
	30	221.95	235.90	9.25	6.35	285.83 f-h	167.63	2.73	2.73	11.55	11.80
	40	215.23	231.13	8.03	6.60	206.25 i	189.78	2.48	2.55	12.10	12.80
	50	219.88	228.80	7.28	6.53	300.25 e-h	168.25	2.63	2.70	11.55	12.55
	60	217.88	239.45	7.30	7.15	251.20 hi	166.75	2.70	2.68	11.70	12.78
F value$_{LxR,S,}$		1.00	1.24	1.61	1.35	2.37*	1.48	1.35	1.28	1.47	1.48
Years		189.20 a	174.18 b	8.30 b	8.87 a	272.48 a	221.36 b	2.89	2.91	12.08	11.89
F value$_{Year}$		76.01**		4.74**		146.93**		0.16		1.72	
Location	Ankara	-	156.40 c	-	6.17 c	-	143.96 c	-	2.93 d	-	10.97 c
	Aydın	211.05 ab	127.81 e	10.63 a	14.78 a	394.58 a	108.50 d	3.50 a	2.89 d	15.20 a	10.90 c
	Eskişehir	-	145.48 d	-	6.15 c	-	84.17 d	-	3.87 a		12.45 b
	Isparta	-	129.18 e	-	5.74 c	-	95.55 d	-	3.74 b	-	13.48 a
	Tekirdağ	205.50 b	152.55 cd	7.60 bc	7.65 b	341.90 b	380.75 a	3.28 b	3.53 c	11.05 c	11.05 c
	Tokat	121.31 c	180.40 b	7.21 c	6.00 c	91.28 d	211.27 b	2.18 d	2.72 e	10.14 d	13.01 ab
	Şanlıurfa	218.97 a	235.96 a	7.93 b	7.07 bc	262.17 c	184.91 b	2.62 c	2.66 e	11.95 b	12.52 b
F value$_{Location}$		282.94**	242.69**	106.72**	86.38**	462.35**	198.77**	240.18**	236.13**	164.51**	20.01**
Row spacing	20	185.89	161.46	8.29	7.83	279.46	177.54	2.84	3.16	12.42	11.66 b
	30	191.83	161.60	8.65	7.15	270.72	168.64	2.99	3.15	11.79	12.06 ab
	40	190.53	159.94	8.49	8.03	261.49	172.05	2.89	3.20	11.92	12.10 ab
	50	193.08	164.15	8.10	7.48	283.76	172.94	2.88	3.25	12.33	12.71 a
	60	184.71	158.40	8.16	7.76	266.98	172.48	2.86	3.18	11.96	11.80 b
F value$_{Row\ spacing}$		1.49	1.11	1.87	1.37	1.74	0.26	1.69	2.14	2.04	4.09**
CV (%)		6.41	6.68	8.06	20.22	10.10	18.92	6.02	4.62	6.36	8.72

Table 4 (Continue)- Effects of autumn and spring sowing, location and row spacing on morphologic characteristics of mustard

Spring sowing

Location	Row spacing (cm)	Plant height (cm)		Number of lateral branches (branch plant^{-1})		Number of pods per plant (pod plant^{-1})		Pod length (cm)		Number of seeds per pod (seed pod^{-1})	
		2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2013-14
Ankara	20	137.93	141.18	3.48 m-q	3.63 fgh	72.83 g	93.38	2.91 f-n	3.02 gh	14.09 c-g	12.81 c-i
	30	136.98	136.20	3.43 m-r	4.03 e-h	88.43 efg	89.33	2.82 f-o	3.19 fg	14.11 c-g	13.74 a-g
	40	140.65	138.58	3.18 n-r	4.20 e-h	107.63 d-g	111.30	2.71 h-o	3.16 fg	14.50 c-f	15.53 a
	50	146.03	130.50	3.61 k-p	4.63 ef	129.33 de	118.88	2.97 f-m	3.19 fg	14.78 cde	14.22 a-d
	60	143.13	131.10	4.23 j-n	4.92 de	146.20 d	137.76	2.85 f-n	3.42 d-g	15.58 bcd	14.62 abc
Aydın	20	119.25	76.30	4.93 g-m	3.73 e-h	111.90 d-g	71.48	3.13 c-i	3.85 bcd	8.65 nop	10.38 j-m
	30	117.80	82.23	4.78 h-m	4.58 ef	91.45 efg	100.18	2.85 f-n	4.13 abc	9.75 k-p	11.00 h-m
	40	119.68	90.05	5.08 g-k	4.50 efg	127.03 def	114.35	3.05 e-l	4.43 a	9.88 k-p	11.85 e-k
	50	114.98	88.98	4.58 i-n	4.40 eh	86.00 efg	138.55	3.08 d-k	4.18 abc	7.75 p	11.25 h-m
	60	117.88	81.53	5.00 g-l	3.85 eh	114.80 d-g	94.40	3.10 d-j	4.18 abc	11.50 h-l	11.50 f-l
Erzurum	20	62.30	112.33	5.89 e-i	6.03 c	21.08 h	102.50	2.59 i-o	3.47 d-g	12.00 f-k	16.04 a
	30	69.99	106.95	7.49 ncd	5.93 cd	28.48 h	99.70	2.52 j-n	3.26 fg	12.70 e-j	14.24 a-d
	40	71.61	109.40	7.84 bc	6.45 bc	25.24 h	105.98	2.34 no	3.52 d-g	11.92 g-k	14.92 abc
	50	64.14	108.90	6.35 d-g	7.28 ab	25.18 h	110.08	2.45 mno	3.51 d-g	11.93 g-k	14.49 abc
	60	73.42	103.20	6.78 b-e	6.08 c	27.28 h	115.98	2.50 k-o	3.38 d-g	12.99 e-i	13.84 a-f
Eskişehir	20	118.65	103.21	2.05 r	7.04 abc	79.88 g	113.25	3.88 ab	3.28 efg	19.65 a	9.79 klm
	30	123.00	111.29	2.05 r	7.80 a	78.85 g	102.68	3.65 a-d	3.29 efg	17.95 ab	9.03 m
	40	120.95	93.05	2.10 qr	6.36 bc	82.30 fg	66.28	3.65 a-d	3.76 cde	18.00 ab	10.70 i-m
	50	117.85	107.43	2.20 pqr	6.23 bc	82.05 fg	98.26	3.55 a-e	3.49 d-g	16.05 bc	10.93h-m
	60	126.35	107.66	2.40 o-r	6.16 bc	92.45 efg	101.51	3.58 a-e	3.41 d-g	16.55 bc	9.41 lm
Isparta	20	163.00	94.60	6.65 c-f	3.30 gh	289.43 c	35.08	2.94 f-m	3.48 d-g	13.30 d-h	10.85 h-m
	30	157.70	93.40	6.58 c-f	3.55 fgh	310.40 c	37.40	3.40 b-f	3.55 def	14.50 c-f	11.40 g-i
	40	160.00	92.43	6.73 cde	3.88 e-h	404.95 b	39.60	3.23 c-h	4.02 abc	15.03 cde	12.58 c-j
	50	156.08	90.78	8.13 b	4.55 ef	409.23 b	43.30	4.03 a	4.02 abc	16.08 bc	12.78 c-i
	60	159.58	91.68	10.20 a	4.90 de	469.53 a	45.83	4.04 a	4.26 ab	15.95 bc	13.98 a-e
Tekirdağ	20	119.25	83.75	3.75 j-o	3.25 h	81.25 fg	62.00	3.30 c-g	3.23 fg	10.50 i-o	7.00 n
	30	125.25	81.50	4.25 j-n	3.50 fgh	80.00 g	64.00	3.55 a-e	3.35 efg	9.25 l-p	6.75 n
	40	121.25	85.25	4.75 h-m	3.50 fgh	75.75 g	61.00	3.70 abc	3.30 efg	8.50 nop	6.75 n
	50	123.75	82.25	5.25 f-j	3.25 h	77.25 g	60.75	3.20 c-h	3.30 efg	8.25 op	6.50 n
	60	124.00	82.25D	3.50 l-q	3.50 fgh	74.25 g	62.50	3.33 b-g	3.30 efg	8.75 m-p	6.75 n
Tokat	20	91.53	139.85	6.15 d-h	5.88 cd	91.05 efg	138.93	2.93 f-m	3.18 fg	11.28 k-m	16.00 a
	30	77.83	153.63	6.15 d-h	6.25 bc	91.90 efg	175.40	2.68 h-o	3.41 d-g	10.50 i-o	15.30 ab
	40	82.85	137.78	6.25 d-g	6.55 bc	91.20 efg	169.50	2.80 g-o	3.09 fgh	11.28 h-m	13.15 b-h
	50	89.23	137.00	6.13 d-h	6.28 bc	90.13 efg	187.53	2.70 h-o	3.52 d-g	10.20 j-p	13.10 b-h
	60	93.08	140.55	6.28 d-g	6.23 bc	88.78 efg	197.85	2.70 h-o	3.08 fgh	10.78 h-o	14.43 abc
Şanlıurfa	20	108.45	104.13	4.28 j-m	4.23 e-h	69.58 g	59.35	2.50 k-o	2.68 hi	11.75 g-l	11.68 e-l
	30	111.25	101.73	4.35 j-m	3.90 e-h	73.70 g	52.15	2.48 l-o	2.53 i	11.00 h-n	11.50 f-l
	40	113.35	99.88	4.18 j-m	3.75 e-h	68.75 g	50.00	2.43 mno	2.33 i	11.35 h-m	11.75 e-l
	50	113.50	105.60	3.90 j-m	4.20 e-h	75.65 g	55.90	2.25 o	2.45 i	11.35 h-m	11.70 e-l
	60	109.00	107.25	4.30 j-m	4.65 fg	72.45 g	56.38	2.40 mno	2.50 i	11.55 h-l	12.08 d-k
F value$_{LxR,S,}$		0.85	43101.00	4.03**	1.78*	6.98**	11324.00	2.65**	2.70**	2.46**	1.63*
Years		116.06a	106.63b	4.98	4.20	117.59 a	93.51b	3.02 a	3.39 b	12.54a	11.91 b
F value$_{Year}$		104.57**		0.62		103.49**		3649.91**		18.73**	
Location	Ankara	140.94 b	135.51 a	3.58 f	4.28 c	108.88 b	110.13 b	2.85 c	3.19 e	14.61 b	14.18 a
	Aydın	117.92 c	83.82 d	4.87 d	4.21 c	106.24 bc	103.79 b	3.04 c	4.15 a	9.51 e	11.20 b
	Erzurum	68.29 f	108.16 b	6.87 b	6.35 a	25.45 f	106.85 b	2.48 d	3.43 cd	12.31 c	14.70 a
	Eskişehir	121.36 c	104.53 b	2.16 g	6.72 a	83.11 de	96.40 b	3.66 a	3.45 c	17.64 a	9.97 c
	Isparta	159.27 a	92.58 c	7.66 a	4.04 c	376.71 a	40.24 d	3.53 ab	3.86 b	14.97 b	12.32 b
	Tekirdağ	122.70 c	83.00 d	4.30 e	3.40 d	77.70 de	62.05 c	3.42 b	3.30 cde	9.05 e	6.75 d
	Tokat	86.90 e	141.76 a	6.19 c	6.24 a	90.61 cd	173.84 a	2.55 d	3.25 de	10.81 d	14.40 a
	Şanlıurfa	111.11 d	103.72 b	4.20 e	4.15 b	72.03 e	54.76 cd	2.41 d	2.50 f	11.40 cd	11.74 b
F value$_{Location}$		249.87**	120.67**	139.90**	64.69**	533.68**	57.08**	61.63**	99.15**	124.02**	73.49**
Row spacing	20	115.04	106.92	4.65 b	4.63	102.12 b	84.49 b	6.14	3.27 b	12.65	11.82
	30	114.97	108.36	4.88 ab	4.94	105.40 b	90.10 ab	6.17	3.34 ab	12.47	11.62
	40	116.29	105.80	5.01 ab	4.90	122.86 ab	89.75 ab	6.03	3.45 a	12.56	12.15
	50	115.69	106.43	5.02 ab	5.10	121.85 a	101.66 a	6.36	3.46 a	12.05	11.87
	60	118.30	105.65	5.34 a	5.04	135.72 a	101.52 a	6.26	3.44 a	12.96	12.07
F value$_{Row\ spacing}$		0.91	0.48	4.23**	2.01	13.99**	3.08*	0.38	0.38	4.55**	2.43
CV (%)		6.97	8.36	13.82	14.55	17.76	26.59	8.98	6.48	9.53	11.74

*, P<0.05; **, P<0.01 significantly different according to the Duncan. Data was the means of 4 replications

Table 5- Effects of autumn and spring sowing, location and row spacing on morphologic characteristics of mustard

Fall sowing

Location	*Row spacing (cm)*	*Thousand-seed weight (g)*		*Seed yield (kg ha^{-1})*		*Crude oil percentage (%)*		*Crude oil yield (kg ha^{-1})*	
		2013-14	*2014-15*	*2013-14*	*2014-15*	*2013-14*	*2014-15*	*2013-14*	*2014-15*
Ankara	20	-	3.77 d-h	-	3074.8 e	-	26.08	-	802.0 f
	30	-	3.93 c-f	-	3225.5 d	-	26.21	-	847.8 e
	40	-	3.98 cde	-	3096.6 e	-	25.17	-	782.7 g
	50	-	3.86 c-g	-	2701.7 f	-	23.63	-	641.8 i
	60	-	3.97 c-f	-	2711.7 f	-	25.39	-	688.5 h
Aydın	20	2.65	3.01 k-n	1661.8 b-d	691.5 v	21.16 e-g	21.02	352.9 b-d	145.2 u
	30	2.75	3.63 e-i	1962.5 ab	922.8 qrs	19.82 fg	18.07	398.6 a-c	165.9 t
	40	2.68	3.47 g-j	1811.3 a-c	970.0 pq	19.07 g	18.88	345.2 cd	184.5 s
	50	2.66	3.73 e-h	1960.3 ab	848.0 tu	24.79 c-f	20.49	483.9 a	177.5 st
	60	2.65	3.17 j-m	2081.3 a	1057.3 o	22.52 d-g	19.25	469.1 ab	207.9 r
Eskişehir	20	-	3.54 e-j	-	1376.7 j	-	26.14	-	362.8 l
	30	-	3.48 g-j	-	1267.5 l	-	24.16	-	306.6 n
	40	-	3.45 g-k	-	1207.1 m	-	24.37	-	290.2 o
	50	-	3.35 h-k	-	1002.7 p	-	24.23	-	239.5 q
	60	-	3.28 i-l	-	924.2 qrs	-	26.56	-	244.2 q
Isparta	20	-	3.93 c-f	-	1128.3 n	-	27.49	-	310.2 n
	30	-	4.18cd	-	1328.0 k	-	27.44	-	364.1 l
	40	-	4.28 bc	-	958.8 pqr	-	27.07	-	259.4 p
	50	-	4.60 ab	-	980.5 p	-	28.25	-	277.0 o
	60	-	4.70 a	-	894.0 st	-	28.27	-	252.9 pq
Tekirdağ	20	3.50	3.53 f-j	1080.0 e-h	1137.5 n	31.86 a	29.33	345.3 cd	330.8 m
	30	3.38	3.53 f-j	876.7 g-j	916.7 rs	30.35 ab	24.12	263.0 d-f	221.2 r
	40	3.55	3.58 e-j	985.7 f-I	1007.5 p	28.60 a-c	24.74	281.9 c-f	247.0 pq
	50	3.40	3.45 g-j	733.3 h-j	830.0 u	27.62 a-d	25.39	204.4 e-g	211.9 r
	60	3.58	3.55 e-j	723.3 h-j	801.7 u	25.54 b-e	26.44	184.9 fg	212.7 r
Tokat	20	2.80	2.65 nop	639.8 ij	3219.9 d	27.91 a-d	26.98	176.8 fg	867.7 d
	30	2.90	2.68 nop	513.3 j	3754.9 b	26.25 b-e	24.26	133.3 g	910.1 c
	40	2.83	2.80 mno	611.0 ij	3492.2 c	29.01 a-c	24.07	176.7 fg	841.6 e
	50	2.90	2.87 lmn	1076.0 e-h	3929.2 a	30.11 a-c	26.62	323.8 c-e	1048.1 b
	60	2.95	2.99 k-n	846.8 g-j	3975.0 a	24.87 c-f	26.64	210.7 e-g	1066.8 a
Şanlıurfa	20	2.21	2.38 pq	1473.5 c	1614.3 g	24.76 c-f	25.41	362.5 b-d	410.1 j
	30	2.32	2.23 q	1235.0 e-g	1516.8 h	21.73 e-g	23.61	268.4 d-f	357.9 l
	40	2.34	2.40 opq	1322.3 def	1439.0 i	22.14 e-g	25.68	293.0 c-f	368.3 l
	50	2.30	2.37 pq	1163.8 e-g	1452.5 i	21.39 e-g	26.64	248.0 d-g	388.1 k
	60	2.24	2.04 q	1351.5 def	1454.0 i	21.08 e-g	25.19	286.8 c-f	367.0l
F value$_{LxR,S}$		1.27	1.88*	3.17**	2.31**	1.95*	0.77	4.40**	1.77*
Years		2.83 b	2.98a	1205.4 b	1751.5 a	25.03 a	24.14 b	290.5 b	436.5 a
F value$_{Year}$		23.59**		228.38**		4.49*		133.36**	
Location	Ankara	-	3.89 b	-	2962.1 b	-	25.29 b	-	752.5 b
	Aydın	2.68 c	3.33 c	1895.4 a	897.9 g	21.47 b	19.54 c	409.9 a	176.2 f
	Eskişehir	-	3.42 c	-	1155.6 d	-	25.09 b	-	288.6 d
	Isparta	-	4.34 a	-	1057.9 e	-	27.71 a	-	292.7 d
	Tekirdağ	3.48 a	3.53 c	879.8 c	938.6 f	28.79 a	26.01 ab	255.0 bc	244.7 e
	Tokat	2.88 b	2.80 d	737.4 c	3674.2 a	27.63 a	25.71 ab	204.3 c	946.9 a
	Şanlıurfa	2.28 d	2.28 e	1309.2 b	1495.3 c	22.22 b	25.31 b	291.7 b	378.3 c
F value$_{Location}$		347.32**	133.44**	118.81**	3362.6**	33.15**	27.70**	373.2**	1859.5**
Row spacing	20	2.79	3.26	1213.7	1749.0	26.42 a	26.06 a	180.2	461.3
	30	2.84	3.38	1146.9	1847.4	24.54 c	23.98 c	152.9	453.4
	40	2.85	3.42	1182.5	1738.7	24.70 bc	24.28 bc	160.0	424.8
	50	2.81	3.41	1233.3	1677.8	25.98 abc	25.04 abc	173.5	426.2
	60	2.85	3.38	1250.7	1688.3	23.50 ab	25.39 ab	161.8	434.3
F value$_{Row\ spacing}$		0.76	1.77	0.60	1.88	2.64*	3.47*	1.80	0.83
CV (%)		4.24	7.77	17.71	14.97	11.54	9.57	22.02	21.75

Table 5 (Continue)- Effects of autumn and spring sowing, location and row spacing on morphologic characteristics of mustard

Spring sowing									
Location	*Row spacing (cm)*	*Thousand-seed weight (g)*		*Seed yield (kg ha^{-1})*		*Crude oil percentage (%)*		*Crude oil yield (kg ha^{-1})*	
		2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
Ankara	20	2.62	0.83 l	1907.8 d	660.0	20.21	21.79	387.0 b	143.2
	30	2.48	1.06 i-l	1831.9 e	585.4	18.73	19.82	343.0 d	115.4
	40	2.64	1.29 hi	1687.5 f	575.3	18.63	18.92	318.6 e	108.8
	50	2.64	1.4 1h	2017.0 c	602.7	19.66	20.19	395.9 a	120.9
	60	2.60	1.36 h	2056.8 b	538.5	18.64	18.87	373.1 c	99.6
Aydın	20	1.03	1.03 i-l	444.5 p	206.8	14.06	12.97	62.4 n	30.3
	30	1.00	1.00 i-l	330.8 r	146.0	12.97	12.45	42.5 o	19.1
	40	0.90	0.90 kl	347.8 qr	155.5	12.75	12.81	45.1 o	21.2
	50	0.96	0.96j kl	240.5 u	201.0	11.29	12.09	27.4 q	27.4
	60	0.95	0.95 jkl	283.5 t	133.0	10.98	13.01	31.0 pq	17.9
Erzurum	20	1.25	3.02 def	86.0 z	631.7	8.12	14.36	7.0 s	91.1
	30	1.49	3.05 de	126.2 yz	624.2	7.69	12.90	9.8 rs	80.8
	40	1.40	3.08 de	103.7 z	626.2	8.15	13.03	8.5 rs	80.9
	50	1.28	2.99 def	70.2 z	600.0	7.49	13.28	5.3 s	79.0
	60	1.30	3.01 def	92.4 z	577.8	7.94	12.61	7.4 s	73.1
Eskişehir	20	2.34	3.48 ab	2178.3 a	284.0	17.65	23.01	391.4 ab	65.1
	30	2.36	3.68 a	1587.0 g	295.4	17.38	22.50	277.5 f	65.8
	40	2.18	3.41 abc	1444.8 i	252.5	15.49	22.80	223.7 h	56.8
	50	2.49	3.23 bcd	1482.3 h	215.7	16.14	22.68	239.6 g	48.6
	60	2.40	3.22 bcd	1462.3 hi	163.2	16.30	22.35	238.4 g	36.7
Isparta	20	2.36	2.83 efg	890.0 k	553.0	26.44	23.03	2353 g	127.6
	30	2.27	3.10 de	768.9 m	574.0	24.77	22.15	190.9 j	128.5
	40	2.43	3.13 cde	957.5 j	473.3	24.54	21.77	234.5 g	103.5
	50	2.40	3.08 de	793.3 l	360.3	26.03	22.97	206.0 i	83.6
	60	2.48	3.15 cd	788.3 lm	346.0	22.90	21.05	179.4 k	73.0
Tekirdağ	20	3.25	3.18 cd	523.3 n	64.7	11.72	14.49	107.6 l	6.8
	30	3.25	3.23 bcd	474.2 o	82.8	9.37	12.14	94.7 m	10.2
	40	3.28	3.23 bcd	526.8 n	55.5	12.22	13.47	92.2 m	7.7
	50	3.25	3.15 cd	357.5 q	45.2	10.40	14.86	66.1 n	6.4
	60	3.33	3.20 bcd	308.3 s	59.3	13.63	14.01	61.5 n	8.4
Tokat	20	1.48	2.55 g	218.3 v	2833.7	6.10	20.23	25.8 q	576.1
	30	1.20	2.70 g	152.5 wx	2862.3	8.92	17.37	14.3 r	496.4
	40	1.40	2.74 fg	294.0 st	2676.5	6.00	18.46	35.8 p	493.3
	50	1.40	2.73 fg	253.5 u	2577.5	5.59	18.98	27.0 q	486.9
	60	1.40	2.70 g	305.5 st	2594.5	7.02	18.32	41.6 o	477.3
Şanlıurfa	20	0.72	1.23 hij	137.0 xy	319.5	20.86	14.81	8.2 rs	74.8
	30	0.72	1.16 h-k	104.8 z	311.5	20.12	14.39	8.9 rs	62.5
	40	0.67	1.12 h-l	162.5 w	255.8	18.13	13.80	10.0 rs	54.9
	50	0.76	1.12 h-l	109.8 z	278.3	19.53	14.58	7.3 s	62.6
	60	0.73	1.12 h-l	119.5 yz	293.0	19.71	14.81	8.5 rs	66.0
F value$_{LxR,S,}$		1.44	2.35**	4.95**	0.82	0.74	0.33	1.85*	0.75
Years		1.88b	2.33a	700.7 a	641.7 b	14.87 b	18.14 a	127.2 a	119.7 b
F value$_{Year}$		967.65**		21.62**		188.46**		5.20*	
Location	Ankara	2.60 b	1.19 e	1900.2 a	592.4 c	19.07 b	19.92 b	363.5 a	117.6 b
	Aydın	0.97 e	0.97 f	329.4 e	168.5 g	12.41 d	12.62 c	41.7 e	23.2 g
	Erzurum	1.34 d	3.03 c	95.7 h	612.0 b	7.88 e	13.24 c	7.6 g	81.0 d
	Eskişehir	2.35 c	3.41 a	1630.9 b	241.6 f	16.59 c	22.67 a	274.1 b	54.6 f
	Isparta	2.39 c	3.06 c	839.6 c	461.3 d	24.94 a	22.20 a	209.2 c	103.2 c
	Tekirdağ	3.27 a	3.20 b	438.0 d	57.5 h	19.67 b	13.80 c	84.4 d	7.9 h
	Tokat	1.38 g	2.68 d	244.8 f	2708.9 a	11.47 d	18.67 b	28.9 f	506.0 a
	Şanlıurfa	0.72 f	1.15 e	126.7g	291.6 e	6.93 e	22.03 a	8.6 g	64.2 e
F value$_{Location}$		909.84**	1133.37**	699.85**	1082.39**	118.95**	68.65**	226.68**	437.51**
Row spacing	20	1.88	2.27 b	798.1 a	691.7 a	15.64	19.18 a	15.31 a	13.94 a
	30	1.85	2.37 a	672.0 b	684.8 ab	14.99	17.37 b	12.27 b	12.23 ab
	40	1.86	2.36 a	690.6 b	633.8 abc	14.49	17.86 b	12.10 b	11.59 b
	50	1.90	2.33 ab	665.5 b	610.1 bc	14.64	18.45 ab	12.18 b	11.44 b
	60	1.90	2.34 a	677.1 b	588.2 c	14.58	17.85 b	11.76 b	10.65 b
F value$_{Row\ spacing}$		0.74	0.91	6.97**	4.87**	42614.00	2.86*	4.10**	4.15**
CV (%)		7.10	5.93	16.90	16.89	17.27	12.77	31.98	28.66

*, P<0.05; **, P<0.01 significantly different according to the Duncan. Data was the means of 4 replications

and yield components among locations could be derived from various years and locations which have different ecological conditions including air temperature, precipitation, agronomic practices and differences in the number of plants per unit area (Saran & Giri 1987; Shafii et al 1992; Walton et al 1999). The maximum crude oil percentage (28.79 and 27.71%, respectively) was determined at Tekirdağ during 2013-14 and at Isparta during 2014-15. The highest crude oil yield (409.9 and 946.9 kg ha^{-1}, respectively) was obtained at Aydın during 2013-14 and at Tokat during 2014-15 (Table 5). The maximum crude oil percentage was determined at Tekirdağ with 28.79% during 2013-14; at Isparta locations with 27.71% during 2014-15. Increase of seed oil content from flowering to ripening (Baydar & Yüce 1996); and the longer growth period due to early flowering could be attributed to increase in crude oil percentage (Walton et al 1999). Ripening low temperature and more rainy days prevent oil formation (Kolsarıcı & Başalma 1988). The minimum crude oil percentage was obtained (21.47% and 19.54%) at Aydın locations during both years (Table 5). The result might be due to Aydın location's soil structure as well as air temperature and precipitation. Different row spacing affect mean seed yield and its components were also examined but these were not statistically different except number of seeds per pod during 2014-15 and crude oil percentage for both years. The maximum number of seeds per pod (12.71 seed pod^{-1}) was obtained from 50-cm row spacings (Table 4). The maximum crude oil percentage (26.42 and 26.06%, respectively) was obtained from 20-cm row spacings during both years (Table 5). Crude oil percentage in 20-cm row spacing increased by approximately 12.43% compared to 60 cm row spacing during 2013-14. Similarly 20 cm row spacing showed an increased of approximately 8.67% compared to 30 cm row spacing during 2014-15. Findings of optimum number of plants per unit and uniform distribution of seeds per unit are important factors that determine yield. Kondra (1975), Morrison et al (1990), Misra & Rana (1992), Öztürk (2000), Heidari et al (2003), Kumar & Singh (2003) and Farsak (2009) agreed that seed yield is decreased by increasing row spacing; whereas, Christensen et al (1985), Pyare et al (2008) suggest that seed yield is increased by increasing row spacing. Optimum row spacing is affected by climatic and soil of ecological conditions (Kolsarıcı & Başalma 1988; Shrief et al 1990). The maximum crude oil percentage was determined at 20 cm row spacing for both years. Crude oil percentage in 20 cm increased compared to the 60 cm row spacing during 2013-14 and crude oil percentage in 20 cm row spacing increased compared to the 30 cm row spacing during 2014-15. The crude oil percentage decreased by increasing row spacing (Patel et al 2004; Kaur & Sidhu 2006). Potter et al (1999) did not find any affect of row spacing on crude oil percentage. Sra (1978), Saran & Giri (1987) and Zukalova et al (1988) reported that crude oil percentage might be affected by years and locations ecological conditions including air temperature, precipitation, and soil fertility. In general, the optimum density strengthened the optimal use of environmental condition for the crop and it reduced inter plant competition and results in production of appropriate seeds with more gain in seed weight. However, the excessive numbers of plants caused severe inter plant competition and reduction in thousand-seed weight. These results are in accordance with the findings of Mamun et al (2014) on rapeseed and mustard.

The effect of locations and row spacing on number of pods per plant, crude oil percentage were statistically significant during 2013-14 and thousand seed weight was statistically significant during 2014-15. Seed yield and crude oil yield were statistically significant for both years. The maximum number of pods per plant (406.30 pod $plant^{-1}$) was determined at Aydın and 50-cm row spacing (Table 4). The highest crude oil percentage (31.86%) was determined at Tekirdağ and 20 cm row spacing during 2013-14 (Table 5). The maximum thousand seed weight (4.70 g) was determined at Isparta and 60 cm row spacing during 2014-15. The highest seed yield (2081.3 and 3975.0 kg ha^{-1}, respectively) was obtained at Aydın and 60 cm row spacing and at Tokat and 60 cm row spacing. The maximum crude oil yield (483.9 and 1066.8 kg ha^{-1}, respectively)

were obtained at Aydın with 50 cm row spacing and Tokat with 60 cm row spacing (Table 5).

In spring sowing, statistically significant differences were found between the two consecutive years in terms of plant height, pod length, number of seeds per pod number of pods per plant, thousand-seed weight, seed yield, crude oil percentage, crude oil yield of mustard. However, no such differences were found for number of lateral branches during both years. The seed yield (700.7 kg ha^{-1}) of the first year was higher compared to the seed yield (641.7 kg ha^{-1}) of the second year (Table 5). In spring sowing, especially mean seed yield and crude oil yield were higher during first year compared to their seed and crude oil yield during second year. This differences could have resulted from higher precipitation during the vegetative growing period of plants during the second year.

The effect of location on the seed yield and yield components was found statistically significant for both years. The maximum plant height (159.27 and 141.76 cm, respectively) was detected at Isparta during 2013-14 and at Tokat during 2014-15. The maximum number of lateral branches, number of pods per plant (7.66 and 6.35, respectively) was determined at Isparta during 2014 and at Erzurum during 2015. The highest number of pods per plant (376.71 and 173.84 pod $plant^{-1}$, respectively) was determined at Isparta during 2014 and at Tokat during 2015. The maximum pod length of 3.66 and 4.15 cm was determined at Eskişehir (2014) and at Aydın (2015) respectively. The highest number of seeds per pod of 17.64 and 14.70 was determined at Eskişehir (2014) and at Erzurum (2015) respectively (Table 3). The maximum thousand-seed weight of 3.27 and 3.41 g was determined at Tekirdağ (2014) and at Eskişehir (2015) respectively. The highest seed yield (1900.2 and 2708.9 kg ha^{-1}, respectively) at Ankara in 2014 and at Tokat in 2015. The maximum crude oil percentage (24.94 and 22.67%, respectively) was determined at Isparta in 2014 and at Eskişehir in 2015. The highest crude oil yield 363.5 and 506.0 kg ha^{-1} was detected at Ankara (2014) and at Tokat (2015) respectively (Table 5). The seed yield and its components, including plant height, number of lateral branches, number of pods per plant, pod length, number of seeds per pod, thousand-seed weight, seed yield, crude oil percentage and crude oil yield affected locations for both years. The precipitation of 69.2 and 58.6 mm during flowering at Ankara location, began and continued until May and June until the maturity of capsules after fertilization that positively affected them during 2014. Amirnia et al (2012) reported that the altitude of growth location has a significant effect on ecophisiological parameters of mustard. Erzurum had the highest altitude among locations where vegetative and generative growth showed weak. These plants were not able to complete there vegetative growth and entered generative phase at an earlier stage of growth that resulted in non development of their morphological features before generative maturity, therefore this affected complete formation of grains and yield. So the grains were quite weak and feeble. Long period of drought before flowering caused reduction in seed yield (İptaş & Kolsarıcı 1988), a longer ripening period due to earlier flowering affected seed yield positively (Walton et al 1999). The maximum crude oil percentage was determined at Isparta with 24.94% during 2014; at Eskişehir locations with 22.67% increase during 2014**.** The minimum crude oil percentage was obtained at Erzurum with 7.88% during 2014; at Aydın locations with 12.62% during 2015 (Table 5). Water stress during flowering and ripening resulted in reduction in crude oil percentage of seeds (Hocking et al 1997). From flowering to ripening increase in oil percentage of seed (Baydar & Yüce 1996) and the longer period of vegetative growth due to early flowering could be attributed to the crude oil percentage increase (Walton et al 1999); especially during ripening at low temperature and more rainy days that prevented oil formation (Kolsarıcı & Başalma 1988). The maximum crude oil yield was determined at Isparta with 363.5 kg ha^{-1} at Ankara locations during 2014; at Tokat locations with 506.0 kg ha^{-1} during 2015. The minimum crude oil yield was obtained at Erzurum with 7.6 kg ha^{-1} during 2014; at Tekirdağ locations with 7.9 kg ha^{-1} during 2015 (Table 5). According to these results, these differences among locations could be

due to the effects of growing ecological conditions including temperature, precipitation, agronomic practices (Sra 1978; Christensen et al 1985).

The effect of row spacing on the number of lateral branches was found statistically significant for 2014 year. Differences among pod length, thousand seed weight, crude oil percentage were found statistically significant for 2015. Whereas, number of pods per plant, seed yield and crude oil yield showed statistically significant during both years. The highest number of lateral branches (5.34) was obtained from the 60 cm row spacing. The maximum pod length (3.46 cm) was obtained from the 50 cm row spacing (Table 4). The maximum thousand seed weight (2.37 g) was determined in 30 cm row spacing. The highest crude oil percentage (19.18%) was determined in 20 cm row spacing (Table 5). The maximum number of pods per plant 135.72 and 101.66 pod plant^{-1} were obtained from the 60 cm and 50 cm row spacing respectively (Table 4). The highest seed yield 798.1 and 691.7 kg ha^{-1} was detected from the 20 cm row spacing respectively. The maximum crude oil yield (153.1 and 139.4 kg ha^{-1}, respectively) was obtained from the 20 cm row spacing during both years (Table 5). Different row spacings affected mean yield components but did not statistically affect number of seeds per pod, seed yield and crude oil for both years. Some research showed that seed yield decreased by increasing row spacing (Heidari et al 2003; Arif et al 2012); seed yield increased by increasing row spacing (Christensen et al 1985; Pyare et al 2008); optimum row spacing as affected by climatic and soil conditions of respective ecologies (Kolsarıcı & Başalma 1988; Shrief et al 1990). The maximum crude oil percentage was determined at 20 cm row spacing for both years. The crude oil percentage increased by increasing row spacing (Patel et al 2004; Kaur & Sidhu 2006); in another study, the number of seeds per pod and the crude oil percentage were not affected by row spacing (Angadi et al 2003). The higher seed yield implicates that such plant density facilitated maximum utilization of nutrients and increased dry matter production which ultimately enhanced seed yield, by reducing inter and intra plant competition, due to efficient nutrient uptake, during photosynthesis increased the yield (Cheema et al 2001; Mamun et al 2014). Moreover, using this plant density resulted in creation of more suitable green canopy per unit area with the least inter competition, solar radiation that effectively helped in production of economic yield (Kazemeini et al 2010).

The effects of locations and row spacing was statistically significant for number of pods per plant, seed yield and crude oil yield during 2014, and for thousand seed weight during 2015. Number of lateral branches, pod length and number of seeds per pod were significantly for both years. The highest number of pods per plant (469.53 pod plant^{-1}) was determined at Isparta and 60 cm row spacing (Table 4). The maximum seed yield (2178.3 kg ha^{-1}) was detected at Eskişehir and 20 cm row spacing. The highest crude oil yield (391.4 kg ha^{-1}) was determined in Eskişehir and 20 cm row spacing during 2014 (Table 5). The maximum thousand seed weight (3.68 g) was determined at Eskişehir and 30 cm row spacing during 2015. The highest number of lateral branches, number of pods per plant (10.20 and 7.80, respectively) was obtained at Isparta and 60 cm row spacing at Eskişehir and 30 cm row spacing. The maximum pod length (4.04 and 4.43 cm, respectively) was determined at Isparta and 60 cm row spacing and at Aydın for 40 cm row spacing. The highest number of seeds per pod (19.65 and 16.00, respectively) was determined at Eskişehir and 20 cm row spacing and at Tokat and for 20 cm row spacing for both years (Table 4).

4. Conclusions

Autumn sowing; The results could not be obtained due to cold damage at Ankara, Eskişehir and Isparta locations during 2013-14. Cold damage losses were not observed during 2014-15 as plants entered to winter at 8-10 leaves rosette stage. So autumn sowing could be also possible at Ankara, Eskişehir, and Isparta locations if plants enter winter at right time (8-10 leaves rosette stage). It was determined that autumn sowing is suitable at Aydın, Tekirdağ, Tokat and Şanlıurfa locations. Average of two years

mean, for Tokat ecological conditions showed that mustard should be sown in 50 cm row spacing, for Aydın, Tekirdağ and Şanlıurfa, mustard should be sown in 20-30 cm row spacing to obtain higher seed yield. According to the one year, Ankara, Eskişehir and Isparta ecological conditions, mustard might be sown in 20-30 cm row spacing to obtain higher seed yield. Appropriate emergence could not be achieved in spite of irrigation Erzurum ecological conditions for both years due to high coldness. In gerenal, the crude oil percentage content increased in the wider row spacings. The results of the study emphasise new studies for cold locations after screening of local populations to breed cold resistant cultivars.

Spring sowing; According to the two years mean, it is considered inappropriate for spring sowing because of low yield depending on limited rainfall at Aydın, Tekirdağ and Şanlıurfa locations and; due to differences in the amount and distribution of rainfall at Tokat. The seed yield of the second year was lower compared to the first year under high temperature and low humidity Ankara, Erzurum, Eskişehir and Isparta locations. Because of spring sowing times shortened the development period of mustard or the period of high temperature and low humidity; it is considered that spring sowing is not appropriate because of low yield at Ankara, Erzurum, Eskişehir and Isparta locations. Growing mustard at Erzurum ecological conditions is not promising in terms of seed yield and crude oil yield. In general, 20-30 cm row spacing is recommended for all ecological locations tested in this study.

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Colour Change Kinetics of the Inner and Outer Surface of Brussels Sprouts during Microwave Drying Process

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ABSTRACT

The effect of microwave output powers on colour change kinetics of the inner and outer surface of brussels sprouts was investigated during microwave drying process. The colour changes of the materials were quantified by means of the CIELab scale parameters like L*, a*, and b*. The total color change (ΔE), chroma (C*), hue angle (h*), and browning index (BI) were also calculated by using these values. As expected, microwave drying process changed the colour parameters at different rates depending on the output power used because of browning. The values of a*, ΔE, and BI on both surfaces of the brussels sprouts increased, other values decreased during drying. The mathematical modeling study of color change kinetics indicated that all colour parameters fitted to a zero-order kinetic model and the 460 W output power occured the lowest change rates of all colour parameters. According to the values of activation energy calculated by colour change kinetic parameters, more colour change on the outer surface of brussels sprouts happened by the increase in microwave output power.

Keywords: Browning index; Brussels sprouts; Colour change kinetics; Microwave drying

1. Introduction

Fruits and vegetables are essential food for a balanced diet, but fresh ones can be quickly deteriorated due to high moisture contents of about 80% (Orsat et al 2006; Karam et al 2016). One of the oldest known methods of preserving food is probably drying (Cohen & Yang 1995; Pittia & Antonello 2016). Drying process is used to extend the shelf-life of the products by decreasing the water activity to a low level enough to inhibit enzymatic reactions, microorganisms' growth and other deteriorative reactions (Law et al 2014). This process specially causes changes in some physical properties, such as colour, texture, size and in chemical structure, such as losses of flavor and nutrients (Krokida & Marinos-Kouris 2003; Adak et al 2017). These changes in the functional properties can negatively affect the quality of food products (Kammoun Bejar et al 2011; Dehnad et al 2016). In the conventional drying techniques, the long drying times at relatively high temperatures at the falling rate periods often cause undesirable thermal degradation of final products (Mousa & Farid 2002; Zhang et al 2006). Conversely, microwave drying process reduces the browning and improves the final

quality of dried products with a shorter drying time (Zhang et al 2006).

Among the quality attributes of the fruits and vegetables, colour is one of the most important quality indicators influencing the degree of acceptability of the foodstuffs by the consumer. It is an indicator of the inherent good qualities in related with the marketing values of the product (Doymaz 2012; Sabarez 2014). The variations in the product colour during drying process is the result of many browning reactions (i.e., enzymatic and non-enzymatic), pigment degradation and oxidation reactions (Barreiro et al 1997; Lozano & Ibarz 1997; Lee & Coates 1999; Maskan 2001; Serratosa et al 2011; Sabarez 2014). The kinetic modeling of colour changes in fruits and vegetables during drying process can be used to predict the evaluation of colour with time (Devahastin & Niamnuy 2010; Sabarez 2014). The ability to predict the changes in the colour during drying can be useful in optimizing and controlling the parameters of drying process that gives the desired maximum colour attributes (Sabarez 2014).

Numerous studies have reported in order to evaluate the performance of microwave drying and quality of the dried product. While these studies have examined, it has been seen that some fruits and vegetables, such as potatoes (Bouraoui et al 1994), raisins (Kostaropoulos & Saravacos 1995), pears (Kiranoudis et al 1997), apples (Feng & Tang 1998), bananas (Maskan 2000), kiwifruits (Maskan 2001), carrots (Wen et al 2003) and asparagus (Nindo et al 2003; Duenãs et al 2008) had dried and analyzed and the some characteristics of final products had determined (Karam et al 2016). While there are lots of studies on the change kinetics of fruits and vegetables, only four kinetic studies related to colour change during microwave drying are found in the literature. These studies are related to the drying of bamboo shoot slices (Bal et al 2011), okra (Dadali et al 2007a), basil (Demirhan & Özbek 2009) and spinach (Dadali et al 2007b) by microwave at different output powers. In the literature, no information on the colour degradation of brussels sprouts under different output power conditions during the microwave drying process is available.

The objective of this work is to study the kinetics of colour degradation on the inner and outer surface of brussels sprouts during the microwave drying processes whose theoretical output power is at 460, 600, and 700 W, and to calculate the activation energies for colour change kinetic parameters using the exponential expression based on Arrhenius's equation. Brussels sprouts are one of the popular consumed vegetables due to their rich nutritional content. But the consumption of brussels sprouts is limited to winter months. In this study, optimization of the microwave process conditions to be based on the stability of the color parameters is performed in order to enable the consumption of brussels cabbages every month of the year. This study is important in terms of good understanding of the colour changes during microwave drying. Moreover, this study can be helpful for engineering design and optimization of the microwave drying systems, and obtaining the final products that has optimum colour attributes.

2. Material and Methods

2.1. Material

Fresh brussels sprouts were purchased from a local supplier in Izmir, Turkey and were stored at a temperature of 4±1 ºC until the experiments were carried out. Prior to drying experiments, brussels sprouts divided perpendicular to the fruit axis into approximately equally-sized two part by knife. To determine the initial moisture content, 5 g of samples were dried in an oven (Dedeoğlu, Turkey) at 105 ºC until the weight did not change any more (Miglio et al 2008). The initial moisture content of brussels sprouts was calculated as 6.71±0.34 (g water g^{-1} dry matter).

2.2. Method

2.2.1. Drying equipment and drying procedure

In the microwave drying experiments, a programmable microwave oven (Arçelik, MD 674, Turkey) with a maximum output power of 700 W was used. The dimensions of microwave cavity were 452 mm×312 mm×262 mm. The technical properties of microwave oven were ~230 V, 50 Hz, and 2650 W, a frequency of 2450 MHz (a wavelength of 12.24

cm). The microwave oven was run by a control terminal that controlled both microwave power level and emission time. Drying experiments were applied with 460, 600 and 700 W microwave output power levels to determine the effects of microwave output power on colour change of brussels sprouts. Samples (38±0.5 g) were placed at the center of a rotating glass plate in the microwave oven. Moisture loss of the samples was recorded every 0.75 min until no discernible weight change was observed. Three replications of each experiment were performed, and the data given were as an average of these results. In the experiments, the reproducibility was found in the range of ± 5%. The microwave output power was applied until the final moisture content of samples was equal to 0.03 (g water g^{-1} dry matter) as an average of the results obtained.

2.2.2. Colour measurements

The colour of the inner and outer surface of brussels sprouts was measured periodically during the microwave drying processes by a reflectance Minolta colourimeter (CR-400 Model Colourimeter, Konica Minolta Sensing, Inc., Osaka, Japan). Five measurements were taken at random locations by putting the head of colourimeter directly above the sample. L*, a* and b* values were determined at the result of the measurements. The chroma (C*) (Equation 1) and hue (h*) (Equation 2) values were estimated by the a* and b* values. Moreover, the total colour change (ΔE) (Equation 3) and browning index (BI) (Equation 4) were calculated from the values of L*, a*, b*.

$$C^* = \sqrt{\left((a^*)^2 + (b^*)^2\right)} \quad (1)$$

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$\Delta E = \sqrt{\left((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right)} \quad (3)$$

$$\Delta L^* = L_0^* - L^*$$
$$\Delta a^* = a_0^* - a^*$$
$$\Delta b^* = b_0^* - b^*$$

$$BI = \frac{[100(x - 0.31)]}{0.17} \qquad x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)} \quad (4)$$

Where; L_0^*, a_0^*, b_0^* were the colour values of brussels sprouts at the beginning of drying; L^*, a^*, b^* were colour values of brussels sprouts at the pre-specified time.

The L* value expresses lightness (from 0 to 100), the value of a* defines colour of green (-a*) to red (a*), and the value of b* also represents colour of blue (-b*) to yellow (b*) (Mirzabeigi Kesbi et al 2016). The one of the other parameters derived from the values of L,* a*, and b* scale is C* which shows colour saturation and is proportional to its intensity. The h* is widely used to specify colour in food products, especially meats, fruits and green vegetables. The angles of 0º or 360º point out red hue as angles of 90º, 180º, and 270º show yellow, green, and blue hues, respectively. ΔE is utilized in evaluation of food quality during thermal processing (Ahmed & Shivhare 2001). Browning index (BI) shows the purity of brown colour, which is an important colour parameter for drying processes where enzymatic and non-enzymatic browning occur (Barreiro et al 1997; Maskan 2001; Bal et al 2011).

2.2.3. Kinetic considerations

In order to describe how the colour change of foodstuffs as a function of drying time, the general equation that expresses the reaction rate is represented by (Equation 5);

$$\frac{dC}{dt} = -k(C)^n \quad (5)$$

Where; k represents the kinetic rate constant, C is the rate of change in the quality factor at time t, and n is the order of reaction. The zero-order and first-order kinetic models that obtained by integration Equation (5) are given as Equation (6) and (7), respectively. In literature, it seems that both of these kinetic models were used (Maskan 2001; Dadali et al 2007a; Dadali et al 2007b; Demirhan & Özbek 2009; Bal et al 2011) to study the colour change of foods during microwave drying process.

$$C_t - C_0 = \pm k.t \quad (6)$$

$$\frac{C_t}{C_0} = exp(\pm k \cdot t) \quad (7)$$

Where; C_0, indicates the initial value of colour parameters (L*, a*, b*, C*, h*, ΔE and BI) at zero time and C_t is the value at pre-defined time t, *k,* represents the reaction rate (min^{-1}) in the kinetic model. In the equations, the sign of "±" shows formation and degradation of any quality parameter (Maskan et al 2002; Prachayawarakorn et al 2004; Bal et al 2011).

The kinetic parameters (k and C_0) for each colour parameters related with colour change of the inner and outer surface of brussels sprouts were determined by fitting the experimental data to both of Equations (6) and (7) using least square method. The reaction order of all colour parameters, the best fit and its kinetic reaction rate was determined for each process.

The activation energy required for colour change of brussels sprouts depending on the influence of microwave output power was calculated to use the best fit kinetic reaction rates and least square method by the exponential expression based on Arrhenius's equation (Equation 8) (Dadali et al 2007a; Dadali 2007b; Dadali et al 2007c; Demirhan & Özbek 2009):

$$k = k_0 \exp\left(\frac{-E_a \cdot m}{P}\right) \quad (8)$$

Where; E_a is the activation energy (minimum energy needed for colour change during microwave drying process) (W g^{-1}); m is the initial weight of fresh sample before drying (g); P is microwave output power (W), k is the kinetic reaction rate of the quality parameters (min^{-1}) and k_0 is the pre-exponential constant (min^{-1}).

2.2.4. Statistical analysis

The values of kinetic parameters (C_0, k, k_0 and E_a) were calculated by fitting the model to the experimental data utilizing the nonlinear least squares procedure (Microsoft Excel 2010 and Solver Add-In package of Excel). The coefficient of determination (R^2), chi-square (χ^2) (Equation 9), the residual sum of squares (RSS) (Equation 10) and root mean square error (RSME) (Equation 11) were used as main criterias to decide the best fit of the used mathematical model to the experimental data. The higher values of R^2 and the lower values of RMSE and $\chi 2$, especially, pointed out a better model in terms of fitting (Erbay & Icier 2010; Balbay & Şahin 2012; Içier et al 2014).

$$\chi^2 = \frac{\sum_{i=1}^{N}(C_{\exp,i} - C_{pre,i})^2}{N - P} \quad (9)$$

$$\text{RSS} = \sum_{i=1}^{N}(C_{\exp,i} - C_{pre,i})^2 \quad (10)$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N}(C_{\exp,i} - C_{pre,i})^2}{N}} \quad (11)$$

Where; $C_{exp,i}$ is the experimental value of the i^{th} analysis; $C_{pre,i}$ is the predicted value of i^{th} analysis, N is the total number of data experimentally determined and P is the constants' number in a particular kinetic model.

3. Results and Discussion

3.1. Colour changes in dried product

3.1.1. L, a*, b* and total colour change (ΔE)*

To determine the influence of microwave output power to colour change kinetics of the inner and outer surface of brussels sprouts, three microwave output powers that were as 460, 600, and 700 W, were used at drying process. The L*, a*, b* and total colour change (ΔE) values of the inner and outer surface of brussels sprouts dried at different microwave output power are shown in Figures 1a-d.

The change of L* values is presented in Figure 1a. According to microwave output powers used, the values of L* on the outer surface of the brussels

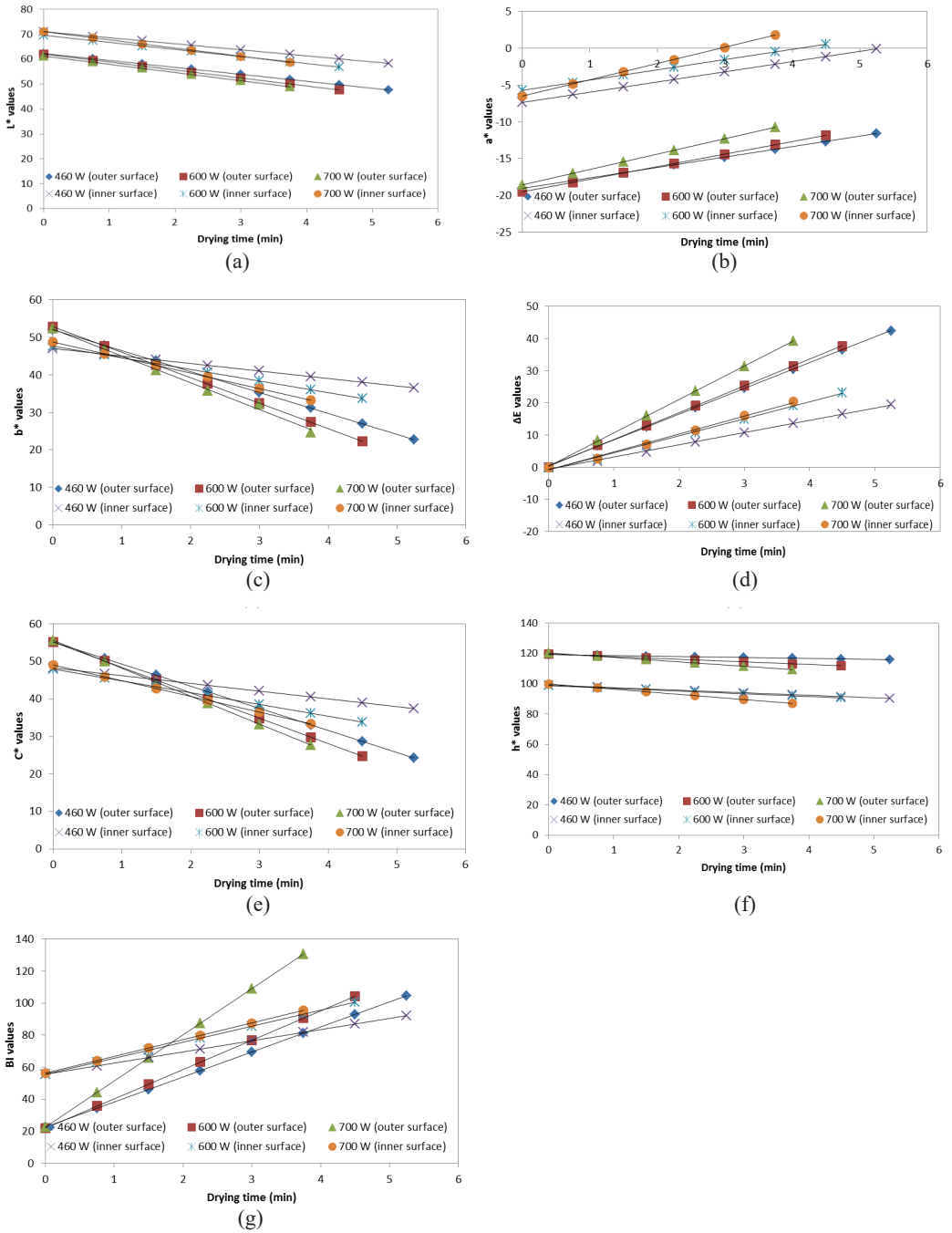

Figure 1- Kinetics of change of the (a), L* values; (b), a* value; (c), b* value; (d), ΔE; (e), C*; (f), h*, and (g) BI as a function of drying time at different microwave output powers comparing experimental curve with predicted model (-)

sprouts changed between 47.70 and 48.90 while the L* values on the inner surface of the brussels sprouts varied between 56.90 and 58.66. It is observed from Figure 1a that the L* values decrease simultaneously with the increase in drying time at all microwave power outputs. The decrease in L* values observed by the using of different microwave output powers between 460-700 W is an indication of the browning on both the inner surface and the outer surface of the brussels sprouts.

The value of a* is a redness/greenness scale. A sharp increase in negative direction was observed in the values of a* (Figure 1b) during microwave drying. The initial colour of both the inner surface and the outer surface of samples showed negative a* values, indicating greenness. At the end of the microwave drying, the inner surface colours of the samples dried at 600 W and 700 W had the positive a* values as 0.58 and 1.79, respectively. As a result of these, it can be said that brussels sprouts samples partially lost their greenness and the colour of samples, especially the inner surface, became redder when dried by microwave.

The value of b* is also a yellowness/blueness scale. Undergoing microwave drying process, the b* values on the outer surface of samples decreased sharply while the b* values of the inner surface decreased slowly. As shown in Figure 1b, the final values of b* changed from 22.31 to 24.68 on the outer surface of samples and from 33.32 to 36.55 in the inner surface of samples depending on the microwave output powers. It can be stated that during the microwave drying process the yellowness reduced in the sample, but this decrease was more pronounced on the outer surface of the samples.

After microwave drying, the lightness and yellowness was decreased and the redness was increased on both the inner surface and the outer surface of the brussels sprouts. On the inner surface of samples, light green-brown colour is more dominant due to a decrease slowly of yellowness and more increasing of redness. The dark green-brown colour on the outer surface of samples is seemed owing to sharp decrease of lightness and yellowness. The samples became redder as they lost their greenness and yellowness when dried. This may be owing to decomposition of chlorophyll and carotenoid pigments (Kostaropoulos & Saravacos 1995; Lee & Coates 1999; Weemaes et al 1999; Maskan 2001) nonenzymatic Maillard browning and formation of brown pigments (Rhim et al 1989; López et al 1997; Maskan 2000; Maskan 2001). Similar trends about changing of the lightness, redness/greenness and yellowness/blueness on colour during microwave drying at different output powers were also reported by Maskan (2001), Dadali et al (2007a), Dadali et al (2007b), Demirhan & Özbek (2009), Bal et al (2011).

As a whole, the total colour change (ΔE) on the outer surface of brussels sprouts increased significantly during microwave drying depending on microwave output power used and ranged from 37.61 to 42.39 (Figure 1d). The ΔE values on the inner surface of brussels sprouts also varied between 19.60 to 23.20 by changing of microwave output power applied (Figure 1d). From these results, it can be understood that the final colour and colour change of brussels sprouts have depended on the output power used in the microwave drying process. The microwave output power chosen is very important for determining the final product's colour that effects the acceptation of product by the consumer. The obtained results about the change of ΔE values depending on the microwave power applied was determined to be in agreement with earlier reported literature by Dadali et al (2007a), Dadali et al (2007b), Demirhan & Özbek (2009), Bal et al (2011).

3.1.2. Chroma (C), hue angle (h*), and browning index (BI)*

It is seen that C* values of both the inner and the outer surface of brussels sprouts decreased slightly during the microwave drying process and closely followed the b* values (Figure 1c and Figure 1e). This indicates that yellow colour is more stable in inner surface than outer surface of brussels sprouts

due to the fact that the C* value shows the degree of colour's saturation and is commensurate with the strength of the colour (Maskan 2001). The final C* values changed slightly (24.23-27.68 in the inner surface of brussels sprouts and 33.34-37.48 in the outer surface of brussels sprouts) according to the microwave output powers applied. The similar observations have been reported in several studies published in the literature by Maskan (2001), Dadali et al (2007a), Bal et al (2011).

The value of h* also slightly decreased from 119.79 to 109.41-116.03 in the outer surface of brussels sprouts and from 99.30 to 86.95 (at 700 W)-90.71 in the inner surface of brussels sprouts during microwave drying (Figure 1f). This explains that the colour of brussels sprouts, except the inner surface colour of product dried with 700 W, did not lose greenness exactly but greenness decreased. (90 ºC < h* < 180 ºC). The inner surface colour of brussels sprouts dried with 700 W started to turn orange-red (h* < 90 ºC). It is also observed that the values of h* were influenced by the microwave output power used. In the literature, several authors have reported similar observations about the change of h* value depending on the microwave output power (Dadali et al 2007a; Dadali et al 2007b; Demirhan & Özbek 2009; Bal et al 2011).

The BI values of outer surface of dried brussels sprouts varied between 104.34 and 130.51 while the BI values of inner surface of dried brussels sprouts changed between 92.38 and 100.74 (Figure 1g). The BI values increased during microwave drying process and varied depending upon microwave output powers applied. The results supporting this phenomenon are also found in different studies (Maskan 2001; Dadali et al 2007a; Dadali et al 2007b; Demirhan & Özbek 2009; Bal et al 2011).

3.2. Kinetic considerations of colour parameters

Experimental data related with changes of all colour parameters in the outer and inner surface of brussels sprouts were fitted to a zero-, and first-order kinetics model by using nonlinear least squares procedure. The values estimated from the fittings and statistical parameters are given in Tables 1-4. The kinetic constants obtained from different models for the colour degradation were seen to have different values owing to microwave output power in the same surface. For example, in the using 700 W as a microwave output power, the kinetic reaction rates for ΔE value of inner surface of brussels sprouts calculated from zero- and first-order kinetics models were 5.8878 min^{-1} and 0.4871 min^{-1}, respectively (Table 1). Similar trends were also observed in the other colour parameters of brussels sprouts for processes in which different microwave output powers are used. The kinetic reaction rates (k) of zero-order model kinetics were higher in whole cases than those of first-order model. These results are in agreement with the findings of Maskan (2001) and Bal et al (2011).

It was observed that the zero-order kinetic model was an appropriate representation for defining the changes in L*, a*, b*, ΔE, C*, h* and BI values with higher R^2 and lower RMSE and $\chi 2$ values. The kinetic reaction rates in both outer and inner surface colour for the values of all colour parameters increased while the microwave output power increased from 460 to 700 W (Table 1). As observed in the studies of Dadali et al (2007a), Dadali et al (2007b), Demirhan & Özbek (2009) and Bal et al (2011), these findings means that the degradation rate of colour in the both of two surfaces with the increasing microwave output power, was faster due to high energy transferred into the food material. It is guessed that the products are exposed to high temperature with the increasing power levels undergoing microwave drying (Chua & Chou 2005; Alibas 2007). High temperature could cause the replacement of magnesium in the chlorophyll with hydrogen, thereby converting chlorophylls to pheophytins exhibits a grey–brown colour (Rudra et al 2008; Therdthai & Zhou 2009). For this reason, the product colour is adversely affected.

3.3. Estimation of activation energy

To determine the effect of microwave output powers on the outer and inner surface's colour of brussels sprouts, the activation energies that are energies

Table 1- The estimated kinetic parameters and the statistical values of zero-order and first-order models for the values of L*, a*, b* and ΔE on the outer surface of brussels sprouts at the different microwave output powers applied

Kinetic model types	*Power (W)*	*Quality parameters*	*Kinetic parameters*		*Statistical parameters*			
			k (min^{-1})	*C_0*	*R^2*	*RSS*	*RMSE*	*χ^2*
Zero order	460	L*	-2.7945	62.3714	0.9999	9.0546E-07	3.3643E-04	1.5091E-07
		a*	1.4192	-19.0412	0.9998	1.0926E-06	3.6957E-04	1.8211E-07
		b*	-5.5742	52.0666	0.9999	6.8404E-07	2.9241E-04	1.1401E-07
		ΔE	7.9345	0.7380	0.9999	2.3193E-08	5.3843E-05	3.8654E-09
	600	L*	-3.1741	62.0165	0.9998	1.2095E-06	4.1568E-04	2.4190E-07
		a*	1.7081	-19.5193	0.9999	6.3035E-07	3.0008E-04	1.2607E-07
		b*	-6.7821	52.8276	0.9999	1.0629E-06	3.8967E-04	2.1258E-07
		ΔE	8.2036	0.6911	0.9999	6.8750E-08	9.9103E-05	1.3750E-08
	700	L*	-3.3228	61.3598	0.9999	3.3972E-07	2.3795E-04	8.4931E-08
		a*	2.0918	-18.5960	0.9999	1.1995E-09	1.4139E-05	2.9987E-10
		b*	-7.1393	52.2190	0.9995	2.8190E+00	6.8545E-01	7.0476E-01
		ΔE	10.2525	0.7344	0.9997	6.7821E-06	1.0632E-03	1.6955E-06
First order	460	L*	-0.0517	62.8585	0.9985	2.7273E-01	1.8464E-01	4.5456E-02
		a*	-0.0957	-19.4981	0.9949	n.c	n.c	n.c
		b*	-0.1562	54.9809	0.9863	5.1137E+00	7.9951E-01	8.5228E-01
		ΔE	0.3095	8.7987	0.7610	1.1393E+03	1.1934E+01	1.8988E+02
	600	L*	-0.0591	62.5104	0.9986	2.2716E-01	1.8014E-01	4.5432E-02
		a*	-0.1132	-20.0299	0.9946	n.c	n.c	n.c
		b*	-0.1917	56.2628	0.9847	1.2165E+01	1.3183E+00	2.4330E+00
		ΔE	0.3551	7.9668	0.7676	8.6559E+02	1.1120E+01	1.7312E+02
	700	L*	-0.0616	61.7663	0.9989	1.3226E-01	1.4847E-01	3.3064E-02
		a*	-0.1501	-19.2265	0.9933	n.c	n.c	n.c
		b*	-0.1944	54.8183	0.9751	9.6590E+00	1.2688E+00	2.4148E+00
		ΔE	0.4172	8.5117	0.7754	9.3410E+02	1.2477E+01	2.3352E+02

n.c, not calculated

required for the change of colour parameters was calculated with the exponential expression based on Arrhenius's equation (Table 5). The reaction kinetic constants for all colour parameters calculated from a zero-order kinetic model fitted to Equation (8). Arrhenius model described well the effect of microwave output power changes on colour of brussels sprouts due to high R^2 values, low RMSE and $\chi 2$ values for each colour parameters. The values of activation energy changed within the range of 6.1579 (L* value) - 55.6175 (h* value) in the outer surface of the brussels sprouts while they were between 11.3315 (L* value) and 28.1590 (h* value) in the inner surface of the brussels sprouts (Table

Table 2- The estimated kinetic parameters and the statistical values of zero-order and first-order models for the values of L*, a*, b* and ΔE on the inner surface of brussels sprouts at the different microwave output powers applied

Kinetic model types	*Power (W)*	*Quality parameters*	*Kinetic parameters*		*Statistical parameters*			
			k (min^{-1})	*C_0*	*R^2*	*RSS*	*RMSE*	*χ^2*
Zero order	460	L*	-2.4112	71.0729	0.9997	1.2204E-07	1.2351E-04	2.0341E-08
		a*	1.3760	-7.3221	0.9997	2.8515E-07	1.8879E-04	4.7524E-08
		b*	-2.0047	47.0704	0.9999	2.2335E-06	5.2838E-04	3.7225E-07
		ΔE	3.9575	-1.1805	0.9999	8.6879E-10	1.0421E-05	1.4480E-10
	600	L*	-2.8118	69.5510	0.9998	5.5312E-09	2.8110E-05	1.1062E-09
		a*	1.3985	-5.7093	0.9998	6.2046E-08	9.4147E-05	1.2409E-08
		b*	-3.0850	47.6298	0.9999	7.0501E-08	1.0036E-04	1.4100E-08
		ΔE	5.5103	-1.5983	0.9999	1.6973E-08	4.9242E-05	3.3947E-09
	700	L*	-3.3320	71.1560	0.9999	9.5735E-12	1.2632E-06	2.3934E-12
		a*	2.2160	-6.5220	0.9999	8.5368E-12	1.1928E-06	2.1342E-12
		b*	-4.1317	48.8157	0.9999	8.0631E-08	1.1592E-04	2.0158E-08
		ΔE	5.8878	-1.7036	0.9999	2.8288E-08	6.8663E-05	7.0720E-09
First order	460	L*	-0.0377	71.3799	0.9992	1.3383E-01	1.2934E-01	2.2305E-02
		a*	-0.3978	-9.0726	0.9987	n.c	n.c	n.c
		b*	-0.0488	47.4002	0.9987	1.2486E-01	1.2493E-01	2.0809E-02
		ΔE	0.3493	3.3248	0.8940	5.0645E+01	2.5161E+00	8.4409E+00
	600	L*	-0.0452	69.8855	0.9992	1.4232E-01	1.4259E-01	2.8464E-02
		a*	-0.5964	-7.8302	0.9974	n.c	n.c	n.c
		b*	-0.0779	48.2636	0.9975	3.7343E-01	2.3097E-01	7.4685E-02
		ΔE	0.4061	3.9336	0.9067	5.4795E+01	2.7978E+00	1.0959E+01
	700	L*	-0.0523	71.5017	0.9992	1.0635E-01	1.3314E-01	2.6588E-02
		a*	-1.0291	-11.1873	0.9967	n.c	n.c	n.c
		b*	-0.1044	49.6770	0.9969	5.5339E-01	3.0370E-01	1.3835E-01
		ΔE	0.4871	3.4281	0.9211	3.1183E+01	2.2797E+00	7.7957E+00

n.c, not calculated

5). When Table 5 is examined, it is seen that the activation energies of all colour parameters, except h* and BI value, for the outer surface of brussels sprouts were lower than for the inner surface of brussels sprouts. This may indicate that there is more colour change on the outer surface of brussels sprouts as microwave output power increased. It also means that the outer surface of the brussels sprouts is sensitive to the color change and an increase of microwave output power. The results of activation energy and *k* values related with the colour change in the outer and inner surface of the brussels sprouts at different microwave output power are parallel to each other. The results obtained in this study related with activation energy values are generally lower than the results determined in the studies of spinach (Dadali et al 2007b) and basil (Demirhan & Özbek 2009) and are similar to the results obtained in the study of okra (Dadali et al 2007a), except the values of h* and BI. The reasons of these differences are

Table 3- The estimated kinetic parameters and the statistical values of zero-order and first-order models for the values of C*, h* and BI on the outer surface of brussels sprouts at the different microwave output powers applied

Kinetic model types	*Power (W)*	*Quality parameters*	*Kinetic parameters*		*Statistical parameters*			
			k (min^{-1})	*C_0*	*R^2*	*RSS*	*RMSE*	*χ^2*
Zero order	460	C*	-5.9049	55.2322	0.9999	3.2036E-07	2.0011E-04	5.3394E-08
		h*	-0.6111	119.2349	0.9998	1.8950E-04	4.8670E-03	3.1584E-05
		BI	15.5916	22.7588	0.9998	1.6660E-05	1.4431E-03	2.7767E-06
	600	C*	-6.7777	55.2072	0.9999	3.5156E-07	2.2411E-04	7.0313E-08
		h*	-1.7565	119.7728	0.9998	5.3468E-05	2.7637E-03	1.0694E-05
		BI	18.2969	22.0052	0.9999	2.7206E-07	1.9714E-04	5.4411E-08
	700	C*	-7.4608	55.6629	0.9999	1.3539E-07	1.5022E-04	3.3848E-08
		h*	-2.9214	120.3698	0.9999	4.0470E-07	2.5971E-04	1.0117E-07
		BI	28.7383	22.7393	0.9998	6.2784E-06	1.0229E-03	1.5696E-06
First order	460	C*	-0.1559	58.3132	0.9863	1.4437E-01	1.3434E-01	2.4062E-02
		h*	-0.0052	119.2454	0.9998	3.9933E-02	7.0651E-02	6.6554E-03
		BI	0.2198	34.1552	0.9546	3.3820E+02	6.5019E+00	5.6367E+01
	600	C*	-0.1796	58.4231	0.9867	4.2090E-01	2.4521E-01	8.4180E-02
		h*	-0.0153	119.8432	0.9998	2.2743E-02	5.7000E-02	4.5486E-03
		BI	0.2565	33.9370	0.9519	3.3699E+02	6.9384E+00	6.7397E+01
	700	C*	-0.1908	58.5346	0.9897	5.6655E-01	3.0729E-01	1.4164E-01
		h*	-0.0257	120.5177	0.9998	8.7610E-02	1.2084E-01	2.1902E-02
		BI	0.3230	39.9671	0.9392	7.2100E+02	1.0962E+01	1.8025E+02

Table 4- The estimated kinetic parameters and the statistical values of zero-order and first-order models for the values of C*, h* and BI on the inner surface of brussels sprouts at the different microwave output powers applied

Kinetic model types	*Power (W)*	*Quality parameters*	*Kinetic parameters*		*Statistical parameters*			
			k (min^{-1})	*C_0*	*R^2*	*RSS*	*RMSE*	*χ^2*
Zero order	460	C*	-2.0673	48.3318	0.9999	1.7268E-07	1.4692E-04	2.8780E-08
		h*	-1.7287	99.3145	0.9999	1.1434E-06	3.7806E-04	1.9057E-07
		BI	7.0180	55.5369	0.9999	2.1399E-08	5.1719E-05	3.5664E-09
	600	C*	-3.1585	48.0465	0.9999	1.0822E-06	3.9320E-04	2.1645E-07
		h*	-1.7982	98.8033	0.9999	8.8303E-07	3.5517E-04	1.7661E-07
		BI	10.0219	55.6442	0.9998	3.9774E-07	2.3837E-04	7.9548E-08
	700	C*	-4.2033	49.1011	0.9999	1.0053E-07	1.2944E-04	2.5133E-08
		h*	-3.4189	99.7759	0.9999	2.1864E-07	1.9089E-04	5.4659E-08
		BI	10.4175	56.3496	0.9998	4.2392E-06	8.4056E-04	1.0598E-06
First order	460	C*	-0.0490	48.6734	0.9987	1.4437E-01	1.3434E-01	2.4062E-02
		h*	-0.0184	99.4215	0.9998	5.3796E-02	8.2003E-02	8.9659E-03
		BI	0.0913	57.6944	0.9947	6.3104E+00	8.8815E-01	1.0517E+00
	600	C*	-0.0793	48.7071	0.9974	4.2090E-01	2.4521E-01	8.4180E-02
		h*	-0.0191	98.8937	0.9998	7.8815E-03	3.3555E-02	1.5763E-03
		BI	0.1218	58.8101	0.9927	1.2119E+01	1.3158E+00	2.4239E+00
	700	C*	-0.1058	49.9898	0.9968	5.6655E-01	3.0729E-01	1.4164E-01
		h*	-0.0371	100.0271	0.9996	7.7133E-02	1.1338E-01	1.9283E-02
		BI	0.1302	58.9637	0.9941	6.5248E+00	1.0428E+00	1.6312E+00

Table 5- The activation energies determined for the color degradation on inner and outer surface of brussels sprouts for quality parameters

	Quality parameters	*Kinetic parameters*		*Statistical parameters*			
		k_0 *(min^{-1})*	*Ea (W g^{-1})*	R^2	*RSS*	*RMSE*	χ^2
Outer surface	L*	4.6593	6.1579	0.9995	6.6742E-05	4.7167E-03	6.6742E-05
	a*	4.2889	13.7157	0.9977	5.1017E-04	1.3041E-02	5.1017E-04
	b*	11.6468	8.8213	0.9991	1.2327E-03	2.0270E-02	1.2327E-03
	ΔE	15.5721	8.6364	0.9991	2.0750E-03	2.6300E-02	2.0750E-03
	C*	11.5411	8.1849	0.9992	9.7668E-04	1.8043E-02	9.7668E-04
	h*	59.7515	55.6175	0.9590	1.0982E-01	1.9133E-01	1.0982E-01
	BI	95.1603	23.3136	0.9931	5.8934E-01	4.4322E-01	5.8934E-01
Inner surface	L*	6.0210	11.3315	0.9984	6.4261E-04	1.4636E-02	6.4261E-04
	a*	5.2813	17.6528	0.9961	1.2603E-03	2.0497E-02	1.2603E-03
	b*	16.7957	26.1339	0.9913	1.9989E-02	8.1627E-02	1.9989E-02
	ΔE	12.8590	13.9977	0.9976	4.7924E-03	3.9968E-02	4.7924E-03
	C*	16.6122	25.6086	0.9916	1.9321E-02	8.0251E-02	1.9321E-02
	h*	14.2967	28.1590	0.9898	1.4953E-02	7.0600E-02	1.4953E-02
	BI	22.8652	13.9101	0.9976	1.4950E-02	7.0592E-02	1.4950E-02

that the texture, colour and chemical composition of analyzed food samples are different to each other (Dadali et al 2007b).

4. Conclusions

This study aims to explain the colour change kinetics of brussels sprouts in the both of two surfaces using colour parameters such as L*, a*, b* during microwave drying at 460, 600 and 700 W microwave output powers. It was determined that all colour parameters relating to the outer and inner surface of brussels sprouts influenced by microwave drying and microwave output power applied. All colour parameters, except ΔE and BI values, on both two surfaces of the brussels sprouts decreased while the values of ΔE and BI increased irrespective of the microwave output power used. While the decrease of brightness (L* values) accepts as an indicator of browning in the drying process, the loss of b* value explains that the yellowness of samples decreased due to decomposition of several pigments such as chlorophylls and carotenoids, formation of nonenzymatic Maillard browning, and brown pigments. The decrease in C* values shows the stability of decreasing yellowness during microwave drying. With the decreasing h* values, greenness starts to disappear and even, greenness in the inner surface of product that were dried at 700 W, turns to orange-red colour. The BI values proved that microwave drying process produced more brown colour and the increase of a* values supported this result. When the colour parameters are examined together, it is observed that the change in the colour on both the inner surface and outer surface of brussels sprouts increases as the microwave output power increases. Zero-order and first-order kinetic models were utilized to explain the colour change kinetics of the inner surface and outer surface in the brussels sprouts and it was observed that the changes of all colour parameters related to inner and outer surface of brussels sprouts during microwave drying were fitted to zero-order reaction kinetics. The obtained values of R^2, $\chi 2$ and RSME supported that the colour changes during microwave drying process took place at the zero order reaction kinetics. By examining the reaction kinetic rates of all colour parameters, the fastest colour change was observed at 700 W microwave output power while

the slightest colour change was determined at 460 W microwave output powers. Although some darkening occurred, microwave drying at 460 W maintained a good colour close to that of the fresh brussels sprouts. The Arrhenius model described well the microwave output powers dependence of the kinetic parameters for all the colour parameters, which was used for the calculation of activation energy for colour change kinetic parameters in both the inner surface and outer surface of brussels sprouts. According to the results of activation energies related with colour parameters, the colour change on the outer surface of brussels sprouts has been found to be more sensitive to the increase in microwave output power. The colour criteria assessments shows that drying of brussels sprouts at 460 W occured the lowest change rates on the brightness, greenness and yellowness parameters. It was found that 460 W among the microwave output powers used is the optimum microwave power level in the microwave drying of brussels sprouts with respect to colour criteria. When the microwave output powers used are compared, the use of low output powers such as a 460 W in the microwave drying process allows the production of well quality dried brussels sprouts in terms of colour that is a very important criteria for product acceptability and consumer satisfaction.

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Improvement Storability of 'Angeleno' Plum with the Combination of 1-Methylcyclopropene Treatment and Controlled Atmosphere Storage

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ABSTRACT

The combined effects of postharvest 1-methylcyclopropene (1-MCP) treatment and controlled atmosphere (CA) storage on storage life and quality of 'Angeleno' (*Prunus salicina* Lindl.) plum were investigated after being treated with 1-MCP (625 ng g^{-1}) at room temperature for 12 h. Following the treatment, fruit were stored in normal atmosphere (NA) and CA (1% O_2-3% CO_2) at 0 ºC and 90±5% relative humidity for 120 d. Fruit firmness, weight loss, respiration rate, ethylene production, soluble solid content, titratable acidity, fruit skin color and sensorial evaluation (external appearance, taste and chilling injury) were performed at harvest date and one month intervals during storage. Only 1-MCP treatment delayed the change in properties related to fruit ripening such as fruit softening, decrease in titratable acidity and increase in soluble solid content. Nevertheless, these effects were significantly higher when 1-MCP-treated plums were stored in CA conditions. The highest color change was found in NA conditions. 'Angeleno' plum stored in CA conditions gave the best results in terms of some quality parameters during storage. The combination of 1-MCP and CA storage prolonged storage life of 'Angeleno' plum compared to other treatments. These result demonstrated that the postharvest 1-MCP treatment can be used in the cold storage of Angeleno plums.

Keywords: Firmness; Postharvest treatment; *Prunus salicina*; Quality; SmartFresh

1. Introduction

Plum is a highly perishable climacteric fruit (Khan & Singh 2009) and postharvest softening is a major factor limiting the storage period (Menniti et al 2006). Fruit softening, induced by ripening, is partially controlled by endogenous ethylene biosynthesis (Abdi et al 1997). Ethylene can profoundly affect quality of harvested fruit and these effects can be beneficial or deleterious depending on fruit (Watkins 2006).

Removing ethylene from storage rooms is generally beneficial in maintaining fruit quality and extending storage life. As in other climacteric species, ethylene triggers softening, an increase in soluble solids content, a decrease in titratable acidity (Larrigaudière et al 2009). Climacteric fruit such as plums ripen quickly and decay before they reach their market. Some of the strategies reported to retard metabolic changes and to improve storage life and quality in plum, includes: lowering the storage temperature, modifying air composition, stored at controlled atmosphere (CA) conditions (reducing O_2 and increasing CO_2 concentrations) and inhibition of ethylene production (Khan & Singh 2009). However these strategies may be limited because of the appearance of storage

disorders. Apricots and plums develop two main types of physiological storage disorders, internal browning and gel breakdown (Jooste & Taylor 1999). Menniti et al (2006) indicated that CA storage is beneficial in extending the postharvest life of plums, but its benefits may be limited by the physiological disorders such as internal breakdown and gel breakdown, attributed to chilling injury (CI). Chilling injury manifests itself as browning of the flesh due to the enzyme oxidation of polyphenols and tannins (Dodd 1984). Plums with CI show no visible external changes, making the disorder difficult to detect. On account of these problems, treatments with 1-MCP and CA storage or combination of these are exciting strategies for controlling ethylene production and thus extending post-harvest storage life and reducing quality losses of fruit (Menniti et al 2006; Watkins 2006). 1-MCP and CA generally are more effective when used in combination.

At standard temperature and pressure 1-MCP is a gas (Blankeship & Dole 2003) with a molecular weight of 54 and a formula of C_4H_6 (Özkaya et al 2007). This material is nontoxic, odorless, and compared with ethylene; 1-MCP is active at much lower concentrations (Jeong et al 2003). 1- MCP is thought to interact with ethylene receptors and thus block ethylene-dependent responses (Serek et al 1994). The affinity of 1-MCP for the receptor is approximately 10 times greater than that of ethylene (Blankenship & Dole 2003). The effect of 1-MCP on postharvest life of fruit is bidirectional. First, it provides the potential to maintain fruit quality after harvest. Second, 1-MCP provides a powerful tool to gain insight into the fundamental processes that are involved in ripening and senescence (Watkins 2006). Many trials have been carried out to determine the effects of 1-MCP on postharvest behavior of different fruit and quality losses. 1-MCP has been found to inhibit the action of ethylene and thereby extend the storage life of a range of fruit such as plum (Abdi et al 1998) and apple (Fan et al 1999) and apricot (Fan et al 2000). 'Angeleno' plums are harvested in the late season and have a short postharvest life compared to other climacteric fruit such as apples and pears. Improving postharvest life would increase the marketing period of this late season cultivar. The objective of this research was to determine the combined effect of 1-MCP treatment and CA storage on the storage life and quality of 'Angeleno' plums during storage.

2. Material and Methods

2.1. Plant material, storage conditions and 1-MCP treatments

The experiment was conducted on 8-year-old plum (*Prunus salicina* Lindl. cv. Angeleno) trees in Isparta-Turkey. Trees were grafted on Myrobalan (*Prunus cerasifera* Ehrh.) rootstock. Uniform trees free from pests and diseases were selected and all the experimental trees have similar cultural practices. Fruit were harvested according to fruit firmness and soluble solids content and carried to laboratory immediately. Plums were selected for uniformity of size and mass, and precooled by forced cold air. After precooling, plums were exposed to four different treatments (100 kg plums in each group): 1. Fruit without 1-MCP treatment were stored in normal (air) atmosphere (NA) conditions (NA/Control); 2. Fruit were treated with 1-MCP (625 ng g^{-1}) at room temperature for 12 h and then stored in NA conditions (NA/1-MCP); 3. Fruit without 1-MCP treatment were stored in CA (1% O_2-3% CO_2) conditions (CA/Control); 4. Fruit were treated with 1-MCP (625 ng g^{-1}) at room temperature for 12 h and then stored in CA (1% O_2-3% CO_2) conditions (CA/1-MCP). For the 1-MCP treatment, the plastic boxes were covered with a 1 m^3 polyethylene bag in a gas-tight chamber. The treatment was carried out using the device provided by SmartFresh Company that consists of a small ventilator and reaction mixture. To obtain 625 ng g^{-1} (commercial dose on most of the commodities) 1-MCP in polyethylene bag, the reaction mixture contained 15 mL activator solution, 2 tablets of activator and 2 tablets of 1-MCP. Fruit were exposed to 625 ng g^{-1} 1-MCP at room temperature for 12 h. After 1-MCP treatment, the polyethylene bag was opened and ventilated. Fruit in all treatments were stored at 0 ºC and 90±5% relative humidity for 120 d, and the following analyses were performed initially and one month intervals during storage.

2.2. Chemical and physical analysis

Fruit flesh firmness was measured over 15 fruit in each replicate. Fruit firmness was determined using a digital texture machine (Lloyd Instruments LF Plus) and measured via compression using a 50 N load cell and a stainless steel, 5.1 mm diameter cylindrical probe (Martinez-Romero et al 2003; Serrano et al 2003; Luo et al 2009) with a constant speed of 100 mm min^{-1} at harvest date and during storage period. The maximum force generated during the probe travel was used for data analysis. The results were expressed as Newton (N).

Weight loss of plums was measured over 15 fruit in each replicate and expressed as the percentage of loss of weight with respect to the initial weight. Weight loss was determined by the Equation;

Weight loss = [(First weight - Last weight) / First weight] × 100 (1)

Respiration rate and ethylene production were measured in 750-800 g of fruit samples for each replicate. Fruit were weighed and placed in 4 L airtight jars for 24 h at 20 ºC. Then gas sample was taken from jars and injected into gas chromatographs. Measurements were made in split/splitless (S/SL) of inlet in split mode with gas sampling valve with 1 mL gas sample by using fused silica capillary column (GS-GASPRO, 30 m × 0.32 mm I.D., U.S.A), with thermal conductivity detector (TCD) for respiration rate measurements and flame ionization detector (FID) for ethylene production measurements by Agilent GC-6890N (U.S.A and Canada) model gas chromatography (GC) and Chemstation A.09.03 [1417] software. Carrier gas (helium) flow was 1.7 mL min^{-1} in stable flow mode. The temperature of the oven, TCD and FID detectors were 250 ºC and 250 ºC, respectively. Results were expressed as µL kg^{-1} h^{-1} for ethylene production and mL CO_2 kg^{-1} h^{-1} for respiration rate. Total soluble solids (TSS) content was measured using a digital refractometer (Atago Pocket PAL-1) and expressed as percentage. Titratable acidity (TA) was determined by a digital pH meter (Hanna Instruments HI 9231) and titrimeter (Digitrat, Isolab), and expressed as percentage of grams of malic acid equivalent per kg fresh weight (g kg^{-1}). Fruit skin color was determined using a Minolta CR-300 colorimeter (Minolta Ramsey, NJ, USA) over 15 fruit in each replicate. Minolta color measurement apparatus was calibrated according to the standard white calibration plate (Y= 92.3, x= 0.3136 and y= 0.3194). The values were expressed by the CIE L^* (brightness-darkness), a^* ($+a^*$: red, $-a^*$: green) and b* ($+b^*$: yellow, $-b^*$: blue) system and the values were evaluated as L^*, chroma (C^*) and hue angle (h^o).

$h^o = \tan^{-1} (b^*/ a^*)$ $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$ (2)

External appearance was rated on a hedonic scale of 1-9 (≤ 1-4: poor, ≥ 5: marketable, 7-8: good, 9: excellent) and taste was rated on a hedonic scale of 1-5 (1: very poor, 2: poor, 3: mild, 4: good, 5: excellent). The internal translucency and browning symptoms which affect fruit quality visually were recorded, and expressed as an index of chilling injury (CI). The scale of CI was defined visually according to the percentage of affected pulp and where: Grade 1 was 25%, grade 2 was 25 to 50%, grade 3 was 50 to 75% and grade 4 was 75% (Candan et al 2006).

CI ındex = ∑ (Grade of intensity × Number of fruit at this grade) / total fruit (3)

The experiment was set up according to the completely factorial randomized design with 3 replications. Main effects and interactions were analyzed and means were compared by Tukey's Tests at a significance level of 0.05 (Table 1). All analyses were performed with SPSS software package v.18.0 for Windows by General Linear Model (GLM) univariate test.

3. Results

3.1. Effect of treatments on weight loss during storage

The weight losses of plums during storage period were illustrated in Figure 1. The interaction effects between treatments and storage period for weight loss were significant ($P<0.05$). The weight loss of plums increased steadily with prolonged storage period in

Table 1- Anova for dependent variables for treatments, storage period and their interactions

	WL	*FF*	*EP*	*RR*	*TSS*	*TA*	*L**	*C**	*h°*	*T*	*EE*	*CI*
Tr	**	**	**	**	**	**	**	NS	NS	**	**	**
SP	**	**	**	**	**	**	*	*	NS	**	**	**
Tr × SP	*	**	**	*	*	*	NS	NS	NS	**	**	**

NS, represents non-significance at P<0.05; **, represents significance at the 0.01 level; *, represents significance at the 0.05 level; Tr, ,reatments; SP, storage period; WL, weight loss; FF, fruit firmness; EP, Ethylene production; RR, respiration rate; TSS, total soluble solid; TA, titratable acidity; C*, Chroma; h°, hue angle; T, taste; EE, external appearance; CI, chilling injury

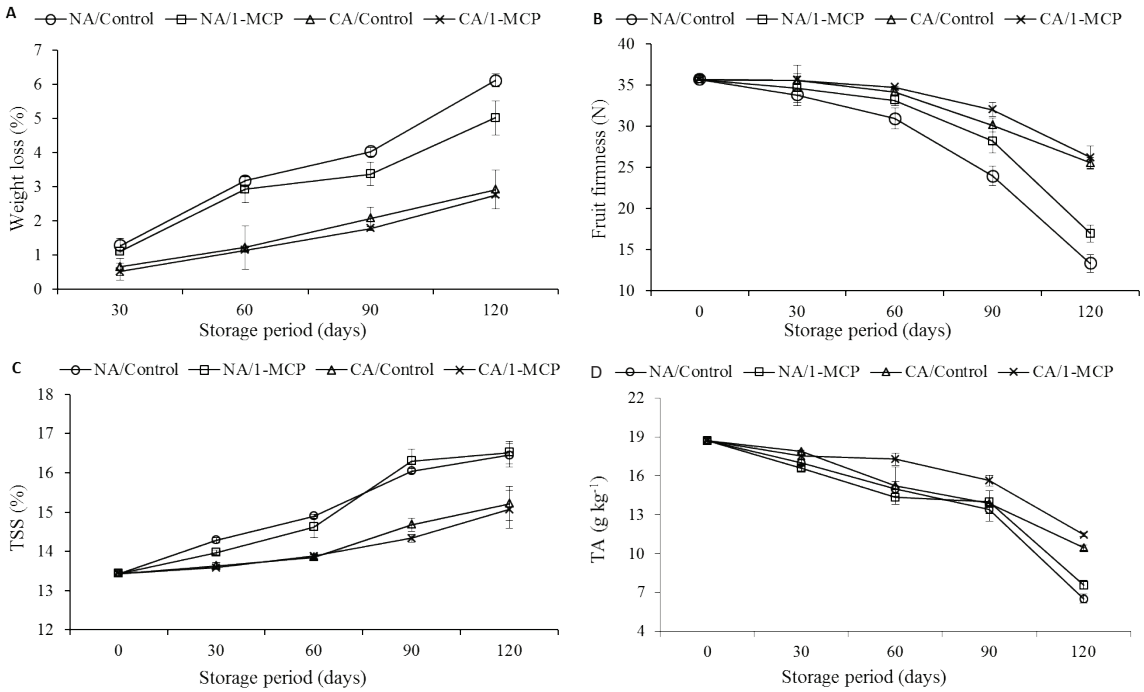

Figure 1- Weight loss (A), fruit firmness (B) total soluble solids content (TSS) (C) and titratable acidity (TA) (D) of plum cv. Angeleno during storage

all treatments. Weight loss in 1-MCP-treated fruit was less compared to the control fruit in NA and CA conditions. The combination of 1-MCP and CA gave the lowest (2.75%) weight loss after 120 d cold storage whereas the highest weight loss (6.11%) was obtained from control fruit stored in air.

3.2. Effect of treatments on fruit firmness during storage

The changes of fruit firmness observed in treated and control fruit during the storage period were given on the Figure 1. The interaction effects between treatment and storage period for fruit firmness were significant (P<0.01). In all treatments, the firmness of plums decreased with increasing storage periods. The amount of decrease in 1-MCP-treated fruit was less than that of non-treated fruit. The combination of 1-MCP and CA was more effective to maintain fruit firmness compared to other treatments. While fruit firmness at harvest was 35.68 N, the control group fruit in NA displayed the highest firmness loss (13.29 N), followed by NA+1-MCP (16.93 N),

CA/Control (25.52 N) and CA/1-MCP (26.19 N) treatments, respectively at the end of the storage period (Figure 1).

3.3. Effect of treatments on TSS content and TA during storage

The TSS and TA of plums during the storage period were illustrated in Figure 1. All treatment displayed an increase in TSS of plums during storage. Control group in NA had the highest TSS (15.02%) while the lowest value (14.06%) was obtained from fruit treated with 1-MCP, and stored in CA. The TSS of 1-MCP-treated plums was lower than those of non-treated plums in both conditions. On the contrary of the TSS values, the TA of 1-MCP-treated plums (14.25 g kg^{-1} in NA-16.13 g kg^{-1} in CA) was higher than those of control groups (14.13 g kg^{-1} in NA-15.23 g kg^{-1} in CA). The best results in terms of TA also were obtained from the combination of 1-MCP and CA.

3.4. Effect of treatments on ethylene production and respiration rate during storage

For the plums stored in CA as well as in NA, the ethylene production increased with increasing storage periods. The effect of the 1-MCP treatments was more effective than that of the control groups. In NA conditions, the ethylene production of control fruit was 1.18 µL kg^{-1} h^{-1} while this value was obtained as 0.65 µL kg^{-1} h^{-1} for the 1-MCP-treated plums. Generally, fruit under CA had lower ethylene production than those of NA. Plums treated with 1-MCP and stored in CA had the lowest ethylene production (0.37 µL kg^{-1} h^{-1}) followed by control group of CA (0.43 µL kg^{-1} h^{-1}).

The respiration rates of plums were higher at the end of storage compared to initial values in all conditions. 1-MCP-treated fruit had lower respiration rates than those of control groups in NA and CA. Especially, the combination of 1-MCP and CA was determined to be the most effective treatment for the suppression of respiration rate (4.65 mL CO_2 kg^{-1} h^{-1}) as well as ethylene production (Figure 2).

3.5. Effect of treatments on fruit skin color during storage

In all treatments, L^* values of plums decreased at the end of storage period compared to initial values. L^* value, representing brightness, of fruit treated with 1-MCP and stored in CA was the highest (25.30), while the lowest value was obtained from 1-MCP-treated fruit in NA (23.67). Considering the C^* values, 1-MCP-treated plums in CA storage gave higher values than those of the other treatments. Plums stored in CA had limited changes in C^* values according to fruit kept in NA. During storage, hue angle decreased in NA, while these values increased in CA (Table 2).

3.6. Effect of treatments on external appearance, taste and chilling injury during storage

The changes in fruit external appearance, taste and chilling injury observed in 1-MCP-treated and non-treated fruit during the storage period (NA and CA) were given in Table 3. The external appearance

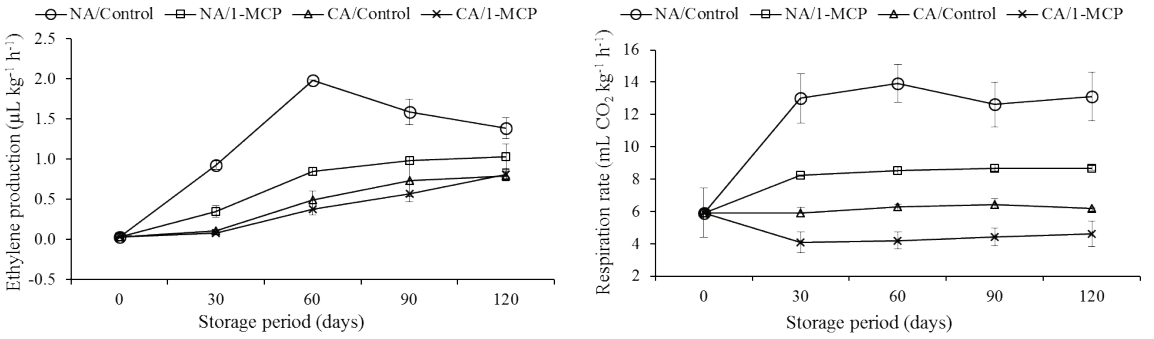

Figure 2- Ethylene production (A) and respiration rate (B) of plum cv. Angeleno during storage

Table 2- L*, chroma (C*) and hue angle (h°) values of plum cv. Angeleno during storage

*L**						
Treatments	*0*	*30*	*60*	*90*	*120*	*Means*
NA/Control	25.35±0.27	24.22±0.16	23.52±0.32	23.07±0.57	22.80±0.11	23.79 *b*[*]
NA/1-MCP	25.80±0.35	23.40±0.33	23.45±0.42	22.32±0.38	23.38±0.17	23.67 *b*
CA/Control	25.59±0.19	25.18±1.13	25.51±0.65	24.90±0.45	25.07±0.49	25.25 *a*
CA/1-MCP	25.65±0.32	25.02±1.95	25.40±0.75	25.30±0.80	25.11±0.37	25.30 *a*
Means	25.60 a[*]	24.45 ab	24.47 ab	23.90 b	24.09 b	
*C**						
NA/Control	11.26±0.20	7.97±0.88	10.14±0.46	9.97±0.93	8.99±0.27	9.66[ns**]
NA/1-MCP	10.68±0.28	8.79±0.46	9.84±0.66	10.03±0.14	8.67±0.53	9.60
CA/Control	10.79±0.50	9.83±0.33	10.43±0.62	10.13±0.45	9.39±0.13	10.11
CA/1-MCP	11.07±0.23	10.09±3.11	10.36±0.36	10.18±0.28	9.79±0.27	10.30
Means	10.95 a[*]	9.17 b	10.19 ab	10.08 ab	9.21 b	
h°						
NA/Control	21.59±0.72	24.23±5.22	19.47±0.47	18.42±0.92	19.90±0.73	20.72[ns]
NA/1-MCP	23.93±5.94	19.31±2.34	18.54±0.39	20.57±0.46	21.70±1.37	20.81
CA/Control	18.76±1.83	21.27±1.43	22.46±2.48	23.09±2.05	20.86±0.77	21.29
CA/1-MCP	19.83±3.55	21.27±7.03	19.73±0.52	19.88±0.32	22.36±1.62	20.61
Means	21.03[ns]	21.52	20.05	20.49	21.20	

[*], values in a same column and row for each effect followed by different letters are significantly different at P<0.05, n= 3, Tukey; [**]ns, non-significant

and taste scores of plums decreased depending on the length of storage period. However, there was a positive effect of 1-MCP treatment on external appearance and taste of fruit compared to control treatments. With the prolonged storage life, there was a continuous increase in CI during the storage.

Table 3- Taste, external appearance and chilling injury of plum cv. Angeleno during storage

Taste (1-5 scale)						
Treatments	*0*	*30*	*60*	*90*	*120*	*Means*
NA/Control	5.00±0.00 a[*]	4.56±0.11 ab	3.28±011 d	1.83±0.17 ef	1.33±0.17 f	3.20
NA/1-MCP	5.00±0.00 a	4.72±0.15 a	3.67±0.10 cd	2.06±0.06 e	1.22±0.05 f	3.33
CA/Control	5.00±0.00 a	4.89±0.06 a	4.44±0.06 ab	3.44±0.06 cd	1.61±0.20 ef	3.88
CA/1-MCP	5.00±0.00 a	5.00±0.00 a	4.83±0.1 a	3.94±0.06 bc	1.50±0.29 ef	4.06
Means	5.00	4.79	4.06	2.82	1.42	
External appearance (1-9 scale)						
NA/Control	9.00±0.00 a[*]	8.89±0.06 a	6.28±0.31 d	4.17±0.10 e	2.06±0.06 f	6.08
NA/1-MCP	9.00±0.00 a	9.00±0.00 a	6.72±0.43 cd	4.17±0.17 e	2.67±0.35 f	6.31
CA/Control	9.00±0.00 a	9.00±0.00 a	7.78±0.11 b	6.56±0.06 cd	4.22±0.11 e	7.31
CA/1-MCP	9.00±0.00 a	9.00±0.00 a	8.17±0.17 ab	7.28±0.31 b	4.06±0.06 e	7.50
Means	9.00	8.97	7.24	5.54	3.25	
Chilling injury (0-4 scale)						
NA/Control	0.00±0.00 g	0.07±0.04 g	0.38±0.15 fg	1.78±0.22 cd	3.58±0.16 a	1.16
NA/1-MCP	0.00±0.00 g	0.04±0.04 g	0.49±0.25 fg	1.40±0.14 de	2.49±0.25 b	0.88
CA/Control	0.00±0.00 g	0.00±0.00 g	0.23±0.03 g	1.16±0.12 de	2.27±0.07 bc	0.73
CA/1-MCP	0.00±0.00 g	0.00±0.00 g	0.09±0.06 g	0.92±0.01 ef	1.37±0.09 de	0.48
Means	0.00	0.03	0.30	1.31	2.43	

[*], means with different letters are statistically significant at P<0.05, n= 3, Tukey. External appearance scale, 1-9 (≤ 1-4: poor, ≥ 5: marketable, 7-8: good, 9: excellent); Taste scale, 1-5 (1: very poor, 2: poor, 3: mild, 4: good, 5: excellent); Chilling injury index, Grade 1 was 25%, Grade 2 was 25 to 50%, Grade 3 was 50 to 75% and Grade 4 was 75%

The amount of increase in 1-MCP-treated fruit (0.88% in NA - 0.48% in CA) was less than those of non-treated fruit (1.16% in NA - 0.73% in CA).

4. Discussion

Weight loss of fruit can lead to shriveling which reduces both market value and consumer acceptability. As expected in this research, higher weight loss in NA condition is related to higher water vapor losses depending on evaporation. Less weight loss in plums treated with 1-MCP and stored in CA may be due to retarded senescence and constant relative humidity of CA compared to NA. In addition, it was thought that the higher weight loss in NA, especially after 60 days of storage, related to lower relative humidity of NA condition. Manganaris et al (2008) reported that 1-MCP treatment could have a modified epicuticular wax metabolism as the cuticle acts as a barrier to vapor movement from inside the cuticle or delay changes epicuticular waxes that occur during senescence.

The textural change is one of the most important features during fruit ripening and affects postharvest life of fruit (Luo et al 2009) and can cause a significant reduction in quality during postharvest life (Salvador et al 2003). The explanation for the firmness maintenance of 'Angeleno' plum in CA (Figure 2) could be related to maintain the integrity of cell wall pectin and low weight losses. Rapid softening of plums in NA (Figure 2) can be due to high ethylene production of fruit in this condition. Similarly, Menniti et al (2004) showed that plum cultivars with high rates of ethylene production softened and ripened faster than low ethylene producing cultivars. The positive effect of the combination of 1-MCP with CA storage on fruit firmness was determined in our study as well. Our results agreed with some previous reports that 1-MCP and CA maintained fruit firmness of plums compared to control groups (Candan et al 2006; Menniti et al 2006). Ethylene production is necessary for a normal ripening process in all plums. Storage techniques, such as CA, which inhibit ethylene production, have been shown to improve post-storage quality of stone fruit (Kader et al 1982). This study showed that the ethylene production was lower in CA than that of NA conditions (Figure 2). Similarly, Pretel et al (1999) and Thompson (2010) reported that fruit had a lower respiration rates and ethylene production during CA storage compared to air storage. The effectiveness of the 1-MCP and CA on the inhibition of ethylene production and respiration rate was clear. Findings of this research are accordance with those of Luo et al (2009) and Valero et al (2004).

Soluble solid content includes reducing and non-reducing sugars, organic acids, and other soluble metabolites (Salisbury & Ross 1985). TSS increased gradually with ripening of plums during storage period. The increase of TSS can be due to reducing water content of plums. A greater increasing in TSS (Figure 1) was determined, as expected, at NA conditions (treated and non-treated plum) because of the higher weight loss (Figure 1). Likewise, according to Kluge et al (1996), sugar loss due to respiration could account for sugar increases with weight loss. As shown in Figure 1, the increase of TSS and the fall in TA were significantly inhibited by 1-MCP and CA. The loss of acidity occurred rapidly in NA conditions compared to CA conditions especially, the combination of 1-MCP with CA. Similar results were found by Manganaris et al (2008) and Diaz-Mula et al (2009). Skin color of plums changed from deep purple to dark black during the storage. This change was delayed in all fruit treated with 1-MCP (Table 2), as has been previously reported by Valero et al (2003) and Manganaris et al (2008) in plums. The positive effect of CA storage on the color difference of fruit was determined in this study, and these findings seem to be a general effect of CA in delaying the fruit ripening process during storage. Results of this study are similar to the findings by Crisosto & Kader (2000), who reported that one of the major benefits of CA storage are delay of color changes. As expected, the external appearance and taste scores of plums declined linearly with storage time. However, one of the most important physiological disorders of Japanese type plums, namely CI, increased with prolonging storage period. Similar

to other quality parameters, the rate of CI was higher when plums were stored in NA conditions. However, CI development was also delayed by the combination with 1-MCP treatment and CA (Table 3). These results show that CA storage was able to delay the normal changes that occur during the postharvest ripening processes. Similarly, Kaynaş et al (2009) indicated that CI increased during storage duration, and the use of CA storage reduces CI (Macheix et al 1990).

5. Conclusions

As a result, according to many quality parameters, the combination of 1-MCP with CA was effective to extend the storability of 'Angeleno' plum compared to other treatment. As a non-phytotoxic, odorless and effective gas, 1-MCP could be a promising candidate for use in prolonging post-harvest life of 'Angeleno' plums.

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Index-based Assessment of Agricultural Drought using Remote Sensing in the Semi-arid Region of Western Turkey

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ABSTRACT

The purpose of the study was to analyze agricultural drought in citrus areas of Seferihisar Kavakdere Plain by calculating NDVI and SAVI values and the surface temperature. The results showed that NDVI and SAVI have negative correlations with surface temperature during irrigation seasons, where significantly increased temperature and decreased rainfall reduced moisture availability for plants. The correlation coefficients between NDVI and surface temperature are -0.893 for 2013 and -0.600 for 2014. The correlation coefficients between SAVI and surface temperature are -0.857 for 2013 and -0.783 for 2014. The combination of NDVI, SAVI and surface temperature provides very useful information for agricultural drought monitoring and an early warning system.

Keywords: Remote sensing; Surface temperature; Vegetation index

1. Introduction

Drought is a complex natural hazard that can cause devastating losses across large regions (Moorhead et al 2015). Agricultural drought is the decrease in the productivity of crops because of irregularities in rainfall, increase in the temperature rate, etc. which cause a decline in the soil moisture (Sruthi & Aslam 2015). The monitoring agricultural drought is imperative for countries whose economic viability is strongly tied to agriculture (Lessel et al 2016). The agriculture sector is much affected by drought as it is highly dependent on the weather, climate, soil moisture, etc. Drought has been a recurrent phenomenon in Turkey for the last several decades. The drought occurrences have been generally closely related to lack of precipitation combined with high temperatures (Mengu et al 2011).

Drought is a severe problem, which influences different aspects of human life. It can cause the degradation of environment and many economic problems, especially in the agriculture sector (Goddard et al 2003; Ebrahimi et al 2010).

Rainfall, soil moisture, increasing temperature and changes in vegetation cover are the primary parameters affecting drought (Ebrahimi et al 2010).

Satellite derived vegetation indices are optical measures of canopy 'greenness,' useful in the assessment of active photosynthesizing and transpiring foliage (Glenn et al 2007). Different vegetation indices are available today, but none of the

major indices is considered inherently outstanding to the rest in all circumstances, and some indices are better suited than others for certain uses. NDVI and SAVI are widely used for vegetation studies at a regional as well as a global level because they are simple to calculate (Sruthi & Aslam 2015).

NDVI and SAVI are calculated from the visible and near-infrared light reflected by vegetation. Healthy vegetation absorbs most of the visible light that hits it, and strongly reflects near-infrared light. Unhealthy or sparse vegetation results more visible light and less near-infrared light (NASA 2017).

NDVI and SAVI are vegetation indices that give information on the development status of plants and they can be determined by remote sensing. These indices are being used to estimate vegetation photosynthesis activity and monitor drought (Koksal 2008; Escribano-Rodríguez et al 2012; Camoglu et al 2013). Values of NDVI are between -1 and +1. While the NDVI of green areas is between 0 and 1, that of water and cloudy areas are less than 0. Vegetated surfaces are described by high NDVI values while soils typically result in low but positive NDVI that can vary somewhat with soil type, wetness, and brightness (NDVI~0.05 to 0.24) (Bausch 1993; Glenn et al 2007).

NDVI is used for description of continental land cover, vegetation classification, and vegetation phenology (Tarpley et al 1984; Tucker et al 1985; Dutta et al 2013), as well as for monitoring agricultural drought. SAVI is another vegetation index which considers soil background reflectance. In addition to NDVI and SAVI, climate variables (precipitation, temperature) are used in agricultural drought assessment (Bunting et al 2017).

This study focuses on the agricultural drought of the Seferihisar Kavakdere Plain through the analysis of three biophysical parameters, namely NDVI, SAVI and surface temperature. With this study, the drought tendency and the period of drought will be determined by examining the change of NDVI/SAVI values with respect to years and the influence of temperature and precipitation. Thus, by determining the periods when there is a trend of drought, effective use of water resources and proper planning of irrigation (how much and when to irrigation) may be considered.

2. Material and Methods

2.1. Material

The study area is located at Seferihisar in Izmir which is on Kavakdere plain in the west of Turkey in 2013 and 2014. Seferihisar Kavakdere Plain is located at latitude 38º 9' 28.8036'' N and longitude 26º 53' 33.7200'' E. The area has a semi-arid climate. The average rainfall is 613.1 mm based on rainfall data for 1981-2010. The major land use is citrus (pink), grape (blue) and olive trees (green) (Figure. 1). The total agricultural area is around 500 hectares. The greatest part of this is planted with 3045 da of citrus (about 61%) and grapes (about 33%), while the rest is dominated by olive-trees.

An aerial photograph was acquired to determine crop pattern. The polygons of citrus areas were determined by screen digitizing method (Bolca et al 2007) by using Microstation-Bentley software (Microstation 1995). The geo-rectification of all geographic data was performed using the UTM projection system, zone 35 European Datum 1950 (Figure 2).

2.2. NDVI and SAVI indices

The data needed for NDVI and SAVI were obtained by using Landsat satellite images (Moorhead et al 2015).

The NDVI is the ratio of the differences in reflectivities for the near-infrared band (NIR) and the red band (RED) to their sum (Waters et al 2002). The NDVI is a sensitive indicator of the amount and condition of green vegetation. Values for NDVI range between -1 and +1. NDVI was computed using Equation (1);

$$NDVI = (NIR\text{-}RED) / (NIR+RED) \quad (1)$$

The SAVI is an index that attempts to "subtract" the effects of background soil from NDVI so that

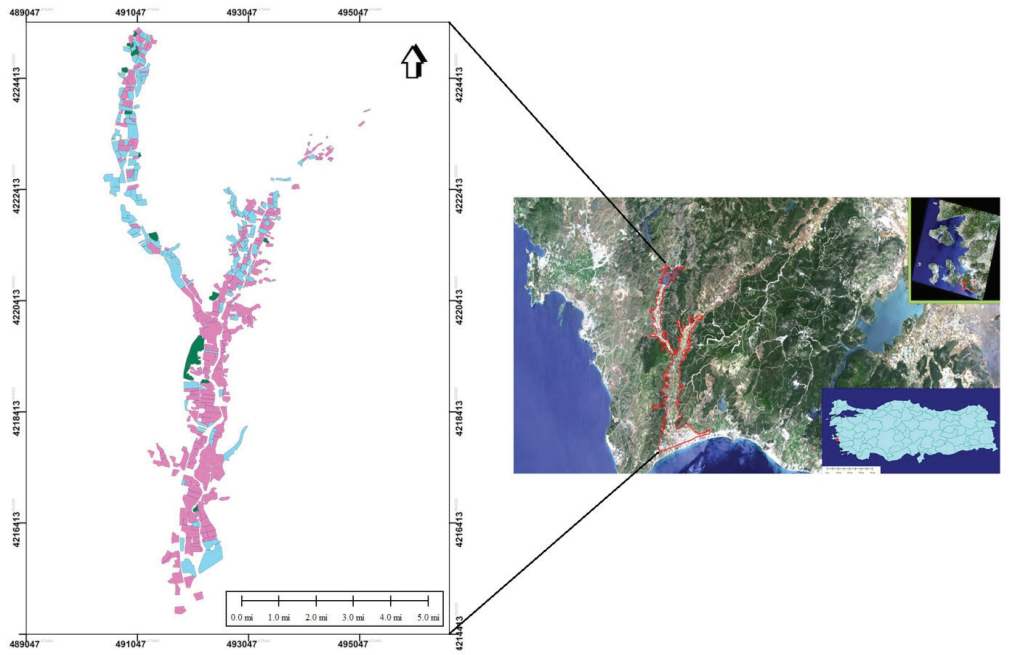

Figure 1- Seferihisar Kavakdere Plain (Pink:Citrus, Blue:Grape, Green:Olive)

Figure 2- Citrus trees in the study area

impacts of soil wetness are reduced in the index. It was computed using Equation (2);

$$SAVI = (1+L)\ (NIR-RED)\ /\ (L+NIR+RED) \quad (2)$$

L is soil brightness correction factor. If L is zero, SAVI becomes equal to NDVI (Waters et al 2002). Using a constant soil adjustment factor (L), the SAVI models the first order of soil-vegetation interactions, and significantly reduces soil background effects across a wide range of vegetation conditions (Huete 1988; Qi et al 1993; Qi et al 1994).

The NDVI and SAVI values of Kavakdere Plain were determined from May to October for citrus growing seasons.

2.3. Surface temperature

The surface temperature of the study area were calculated using thermal bands of Landsat. Surface temperature was calculated using Equation (3) (Waters et al 2002);

$$T_s = \frac{K_2}{\ln\left(\frac{\varepsilon_{NB} K_1}{R_C} + 1\right)} \quad (3)$$

Where; T_s, surface temperature (K); ε_{NB}, narrow band emissivity; R_c, the corrected thermal radiance; K_1, K_2, constants for Landsat images.

Correlation coefficients were calculated to determine association between NDVI/SAVI and surface temperature.

3. Results and Discussion

Figures 3(a) and (b), and Figures 4(a) and (b) represent the line graph obtained for surface temperature and NDVI and SAVI of every month of the growing season for 2013 and 2014.

It can be clearly seen that surface temperature and NDVI/SAVI are inversely proportional to each other. When the temperature is greater, NDVI/SAVI values are less, and this indicates a decrease in the vegetation density (Tucker 1979; Santos & Negri 1996). Reflection values of NIR started to display an increase in June when citrus trees were growing, after that were followed by a decrease in September.

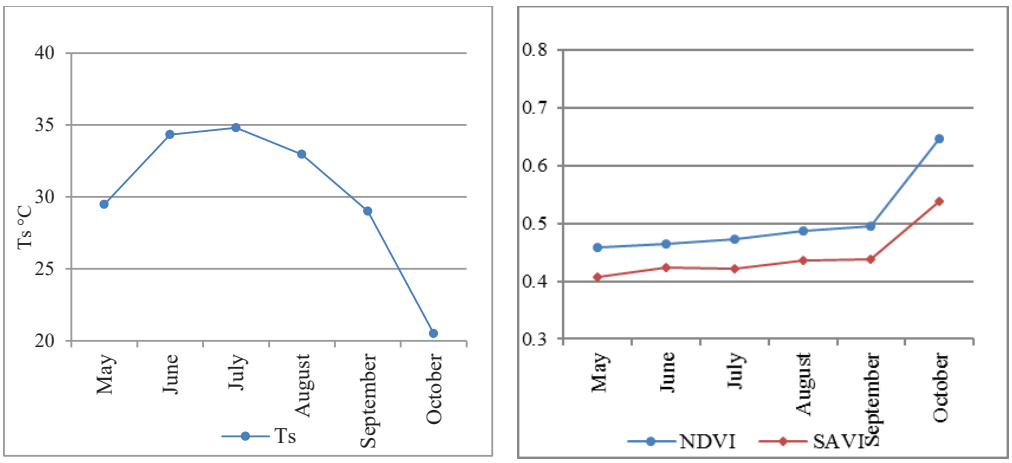

Figure 3- (a), Surface temperature; (b), NDVI and SAVI for Citrus (2013)

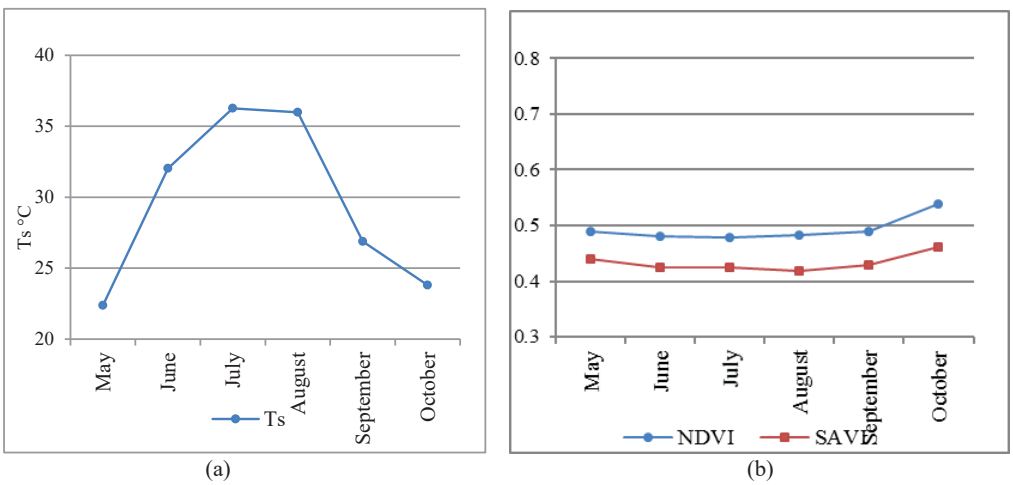

Figure 4- (a), Surface temperature; (b) NDVI and SAVI for Citrus (2014)

In the both years, NDVI/SAVI values were lower during the hotter months of the growing season (June, July and August) whereas they were higher in September and October. The decrease in soil moisture due to irregular or no rainfall together with increased temperature caused the agricultural drought to be severe. By calculating the correlation coefficients between surface temperature and NDVI/SAVI, it can be clearly seen that they show a correlation. The correlation coefficients between NDVI and surface temperature were -0.893 for 2013 and -0.600 for 2014, the same coefficients for SAVI and surface temperature were -0.857 for 2013 and -0.783 for 2014. Negative correlation between NDVI/SAVI and surface temperature was found on the Kavakdere Plain, where significantly increased temperature and decreased rainfall reduced moisture availability for plants. Sruthi & Aslam (2015) conducted an analysis of the correlation between surface temperature and NDVI for 2002 and 2012 in the Raichur region of India and obtained results of -0.635 and -0.586. Ozelkan et al (2011) found a negative correlation between NDVI and surface temperature. Kornieli et al (2009) indicated that when water was the limiting factor for vegetation growth, NDVI-Surface temperature correlation was negative. These values agreed with the results of the present study.

SAVI values were lower than the NDVI values, because SAVI takes into account not only vegetation but also the effects of the soil reflection. Qi et al (1994) stated that the SAVI index measured the vegetation index more sensitively because, differently from other vegetation indices, it included a soil correction factor which took account of reflections from plants and soil.

NDVI and SAVI values for October were higher in 2013 than in 2014, because there were less rainfall and higher temperature in October 2014 than in October 2013 (Table 1). In a study by Zougrana et al (2015) in the south-west of Burkina Faso, a positive correlation was found between NDVI and rainfall. It was found by Bunting et al (2017) that SAVI had a strong correlation with precipitation and negative relationship with temperature in southwestern US. Kornieli et al (2009) indicated that the strongest negative correlations were between NDVI and surface temperature with low precipitation (moisture-limited growth) areas in the western US.

Precipitation is the major responsible factor of vegetation growth that is indicated by NDVI (Dutta et al 2013) and SAVI.

Temperature is an important factor that may affect NDVI (Ji & Peters 2005; Dutta et al 2013) and SAVI variation. It was found that during drought periods, vegetation indices values were relatively low, while surface temperature were relatively high (Kogan 2000; Kornieli et al 2009). Drought resulting from decreased rainfall and increasing temperatures lead a reduction in NDVI and SAVI in areas of vegetation. High summer temperatures are one of the most important factors causing drought. NDVI and SAVI are the most common vegetation indices which are obtained from satellite and used to monitor the areas affected by drought (Escribano-Rodríguez et al 2012).

Table 1- Climate data of study area

		May	*June*	*July*	*August*	*September*	*October*
2013	Rainfall (mm)	50.4	0.0	0.0	1.4	8.4	107.2
	Max.Temperature (°C)	33.0	36.1	37.0	38.0	32.6	28.8
	Min.Temperature (°C)	11.7	13.4	18.3	18.9	12.5	6.4
2014	Rainfall (mm)	4.4	21.8	0.0	0.0	4.0	39.8
	Max.Temperature (°C)	29.6	34.6	35.0	38.1	32.3	29.1
	Min.Temperature (°C)	11.8	12.6	17.6	17.8	12.3	9.4

4. Conclusions

In conclusion, the spatial and temporal drought status of the Kavakdere Plain was evaluated over two years using vegetation indices and temperature data obtained from Landsat images. Irregular or no rainfall and high temperatures in the area caused a fall in NDVI and SAVI values in the citrus growing season. In particular, these indices were lower in the second year of the study, associated with an increase in temperatures and a decrease in rainfall. This indicates an increasing trend to drought in the area. The significant relationship between NDVI/SAVI, temperature, and rainfall in the agricultural areas emphasizes that drought inhibits density of green vegetation. This shows that the lowest NDVI/SAVI values occur in the extreme dry conditions. Therefore, early drought detection is important for planning of the water resources and the irrigation management in Kavakdere Basin, whose economy strongly depends on agriculture.

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Tyrophagus neiswanderi (Acari: Acaridae) as a Pest of Greenhouse Spinach in Antalya, Turkey

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ABSTRACT

Tyrophagus neiswanderi (Johnston & Bruce 1965) generally lives in stored products as saprophyte and on dead bodies of arthropods living in the soil, organic fertilizers, mushroom houses, greenhouses, algae and plant scraps. In this study, however, it was observed in roots and shoots of spinach (*Spinacia oleracea* L.) plants originated from greenhouse commercial crop in 2016-2017, in cultivated areas around Antalya, Turkey. This is the first record of *T. neiswanderi* occurring in spinach plants under greenhouse conditions in Antalya, Turkey.

Keywords: Astigmata; Mite; Antalya; Turkey

1. Introduction

Tyrophagus spp. (Acari: Acaridae) are fungivorous mites live in stored food products and decaying organic materials. Some *Tyrophagus* Oudemans (1924), species can cause economic damage both in ornamental plants and vegetables grown in greenhouses (Fan & Zhang 2007). *T. putrescentiae* (Schrank), *T. perniciosus* Zakhvatkin, *T. similis* Volgin, *T. neiswanderi, T. longior* (Gervais) and *T. palmarum* (Oudemans) are the most common species cause which damages in houses, stored foods and some plants (Griffiths 1979). *T. neiswanderi* is mainly considered a pest of ornamental and horticultural crops (Sánchez-Ramos et al 2007). Eight species of the *Tyrophagus* have been recorded from Turkey (Özer et al 1989; Çobanoğlu 1996; 2009; Kılıç & Toros 2000; Kılıç et al 2012; Kumral & Çobanoğlu 2015). They are; *T. longior* (Gervais 1844); *T. lini* (Oudemans 1924); *T. neiswanderi* (Johnston & Bruce 1965); *T. perniciosus* (Zakhvatkin 1941); *T. putrescentiae* (Schrank 1781); *T. robertsonae* (Lynch 1989) and *T. similis* (Volgin 1949).

Tyrophagus similis Volgin that belong to genus *Tyrophagus*, has been detected in dry apricots and stored products in Turkey (Kılıç & Toros 2000; Çobanoğlu 2009). It has been reported that *Tyrophagus putrescentiae* (Schrank) is detected in *Morchelle* spp. (Ascomycetes), roses and bulbous plants (Çobanoğlu & Bayram 1998; 1999; Bayram & Çobanoğlu 2006). *Tyrophagus perniciosus* Zahvatkin species was detected in the conifer collected in the surveys carried out in different regions of Turkey between 1999 and 2003 (Bayram & Çobanoğlu 2007). In addition, *T. putrescentiae*

and *T. perniciosus* species were detected on fresh onion cultivated in diffrent districts of İzmir, Turkey in between 2006-2008 (Kılıç et al 2012). To our best knowledge, there is no record that *Tyrophagus s*pecies have damage to spinach in Turkey. *T. neiswanderi* the first detected on spinach in Antalya province in the Mediterranean region. There is also report that *T. neisvanderi* is found in some plants (cucumber) grown in the greenhouse (Johnston & Bruce 1965; Fischer 1993; Kadono & Endo 1996).

2. Material and Methods

The research was carried out on the spinach grown greenhouse condition in Antalya Province during 2016-2017. The survey was conducted every two weeks throughout the vegetation periods (September to March). The pest observations were carried out on at least 20 plants in an area of 250 m^2. Plant material was collected from fresh leaves near the root was examined by stereo microscope, and the pests were prepared for identification. All measurements are given in micrometers (µm). The mites were extracted by using Berlese funnel set-up and the mites were cleared in a mixture of lactophenol: Nesbitt 1:1 and mounted in the Hoyer's medium, on microscobic slides. The mites were identified according to Hughes (1976), Griffiths (1985) and Fan & Zhang (2007). All measurements are given in micrometers (µm). The mean of the measurements is given first followed by the range in parentheses. Figures taken by digital image system or drawn by using microscope directly. The voucher specimens of species were deposited in the mite collection of the Department of Plant Protection (Ankara University, Ankara, Turkey). The mean of the measurements is given first followed by the range in parentheses. Species identification was made by Dr. Sultan Çobanoğlu (Ankara University, Ankara, Turkey).

3. Results

Genus (*Tyrophagus* Oudemans, 1924): *Tyrophagus* species is mostly fungivorous and is commonly found in stored food products and decaying organic matter. The species of this genus are well known and common species. *Tyrophagus* (Astigmata, Acaridae) includes about 35 species and is worldwide in distribution (Fan & Zhang 2007).

Female

T. neiswanderi is relativelly large, milky-white colour (Figure 1). Female dorsum (3 females measured) Length: 494-577 µm (Figure 2).

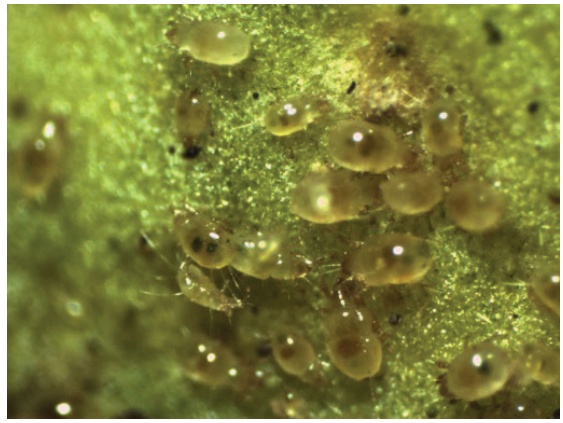


Figure 1- Feeding on spinach leaf

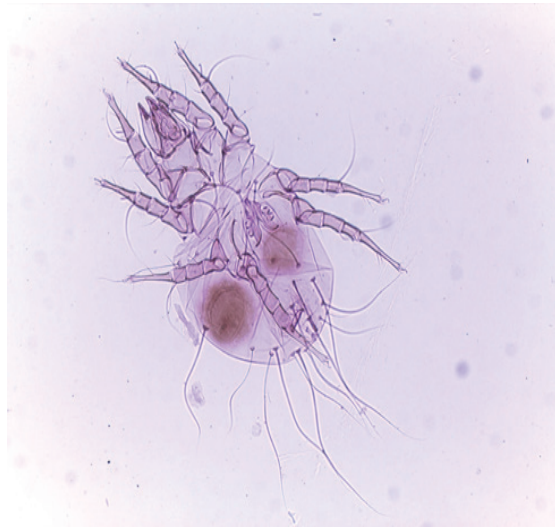


Figure 2- Dorsum

The most important characteristics of this genus are the following: external vertical setae (ve) is placed on anterior of dorsal propodosomal shield

with the half length of internal vertical (vi) (Figure 3a)*;* they rise on the same level. Internal scapular setae (Sci) (Figure 7) are longer than the external scapular setae (Sce) (Figure 7a). Supracoxal setae present. On genu I ($Ϭ_1$) less than three times longer than ($Ϭ_2$) (Figure 3).

Dorsal propodosomal shield with prominent eyespots, Supra coxal setae (Figure 5) widened at base with a few moderate pectination. Hysterosomal setae d1 short, as long as or slightly longer than c1 and antrerior lateral setae (d_2).

Leg I, solenidion Tarsus I (Figure 4) and II (ω) cylindrical, with a round tip. Spermatheca (Figure 6) triangular, funnel-shaped base longer and expanded like funnel shaped. All legs with well developed pretarsus and stalked like claw.

Male

Male (3 males measured) length 384-501 µm (Figures 8). Male is very close to female. Idiosoma is 416 (384-501) µm in lenght and 251 (186-310) µm in width. The shape of idiosoma and dorsal setae and solenidion on tarsus I and II and the genu I (Ϭ1 and Ϭ2) similar as in female. On the ventral surface of the male, one pair of small anal suckers exists on each side of the anus (Figure 9). The two suckers on Tarsus IV (Figure 10) are divided in three part from the base to apex of the segment. The lateral sclerites of supporting aedeagus (Figure 11) are turned outwards, the aedeagus short and bent, tapering from base to tip with straight end.

Distribution:

Tyrophagus neiswanderi (Johnston & Bruce 1965) (Figures 1-11): Material examined: Turkey, Antalya, Tarım district, (36°53'12.80"N, 30°44'44.92), elevation: 47 m, 15♀♀, 10♂♂, 30.09.2016, Kırışık and Topuz.

This is a cosmopolit species. Argentina, Australia, Brazil, China, Germany, England, Endonasia, The Netherlands, Italy, Japan, Mexico, Netherlands, New Zealand, Poland, South Africa, Switzerland, Spain, U.K., U.S.A., Turkey (Fan & Zhang 2007; Cılbırcıoğlu 2017).

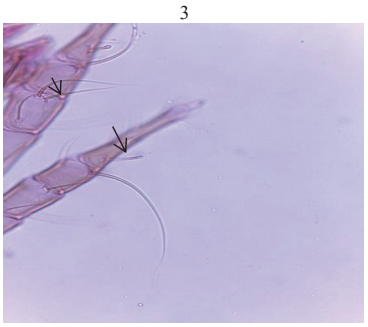
Distribution in Turkey: Kastamonu (Cılbırcıoğlu 2017).

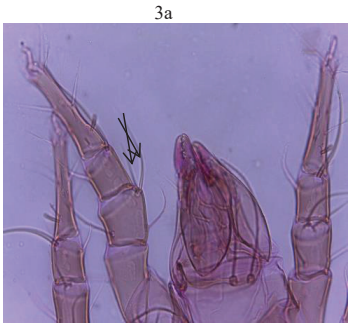
4. Discussion

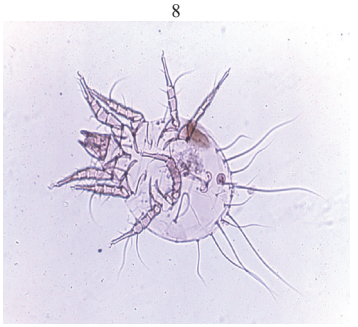
T. neiswanderi was determined in 2016, in commercial spinach greenhouses located in Tarım district of Antalya, Turkey. Field observations started from spinach planting to until the harvesting period. The soil was especially rich in manure and the mites continued devoloping by infecting the spinach plants until to the the next planting season. It has been determined that the pest caused damage to plants during the vegetation period (September to March) in 2016 and 2017.

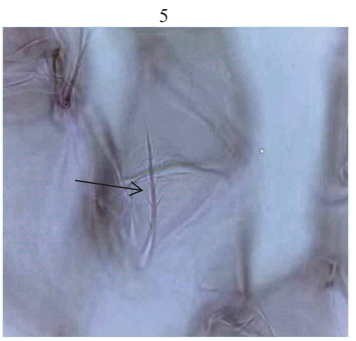
T. similis, T. pernicious and *T. dimidiatus* (Hermann) species have been found to cause damage to spinach (Lange & Bacon 1958; Saito 2016; Kasuga 2005; Nakao 1989; Nakao & Kurosa 1988). However, there is no report that *T. neiswanderi* was harmful to spinach. *T. neiswanderi* is mostly harmful to the cucumber plants. It feeds on the outer part of young cucumber plants, causing morphological disorder and reducing the market value of the product. It also gives damage to plants, causing yellowish spots on the leaves after that they drop such as numerous small holes shown up to 4 mm in diameter (Johnston & Bruce 1965; Nakao & Kurosa 1988; Fischer 1993; Kim et al 2014). *T. neiswanderi* was also detected in orchids grown in New Zealand (Martin & Workman 1985) and cutflowers grown in Japan (Ehara & Gotoh 2000). In surveys caried out in Antalya, it has been observed that high number of *T. neiswanderi* adults was seen on the young leaves and they damaged roots and young shoots of the spinaches and led to further morphological disorders (Figure 12).

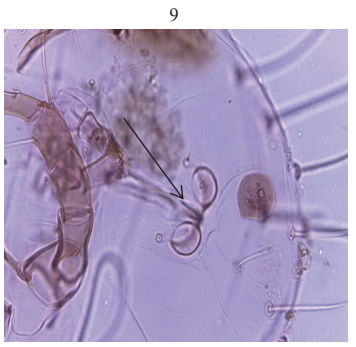
Since the *T. neiswanderi* usually feeds in soil with organic fertilizers, plant detritus and small organisms, it requires strick control measures for soil. Even though chemicals may not be direct solution to control this pest. Indeed, *T. similis* could not be controlled with chemicals applied to soil and spinaches (Kasuga & Amano 2002; 2003). However, a previous study pointed out that *T. similis*

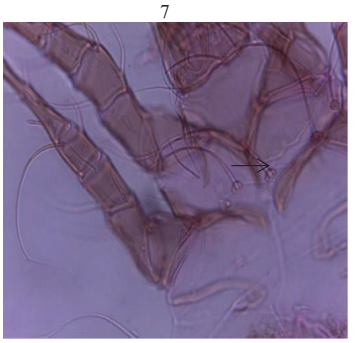


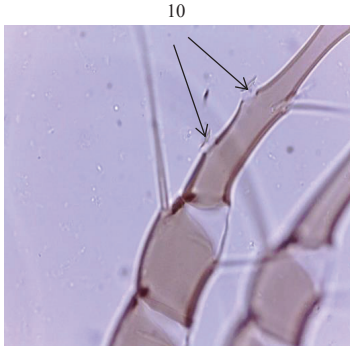


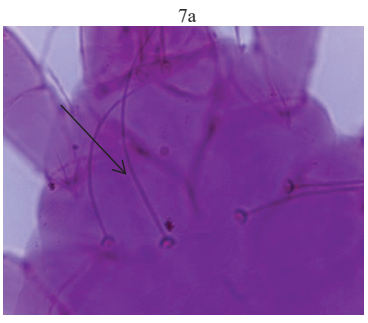


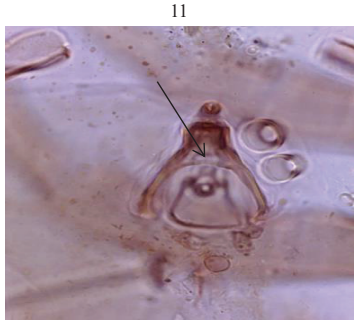












Figures 3-7- *Tyrophagus neiswanderi* female: 3. On genu I (σ_1 and σ_2), 3a. Vertical internal seta(vi) 4. Tarsus I. Solenidion, 5. Supracoxal seate x 100, 6. Spermatheca, 7. Scapular setae sci, 7a. Scapular setae sce

Figures 8-11- Tyrophagus neiswanderi male: 8. Dorsum, 9. Anal sucker, 10. Tarsus IV, sucker, 11. Aedeagus

Figure 12- Damage to spinach

could be controlled by increasing soil temperature at least at 35 °C for 5 hours. Additionally, it was also suggested that reducing organic manure usage and removing plant wastes from plantation area would be useful to decrease pest damage (Kasuga & Amano 2000; Kasuga & Honda 2006).

T. neiswanderi is mostly associated with greenhouse plants. In our case it makes serious damage on the greenhous spinach. It is necessary to investigate control measures and management methods against *T. neiswanderi*.

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Aflatoxin Contamination in Hazelnut Oil Obtained from Hazelnuts Containing High Levels of Aflatoxin

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ABSTRACT

In this study, the issue of whether the aflatoxin in high contaminated hazelnut has been passed to the hazelnut oil during production or not has been investigated. The oil and oil cake of the hazelnut samples that contained aflatoxin at a high level were obtained for the study. The aflatoxin concentrations in hazelnut, hazelnut oil and oil cake were measured, and how much of the aflatoxin in the hazelnut was passed into the oil and oil cake has been determined. Aflatoxin analysis was performed using AOAC (Association of Official Analytical Chemists Method): 991.31 method, which is one of the validated method used in aflatoxin analysis in hazelnuts. The highest aflatoxin concentration in hazelnut oil has been determined as AFB1: 0.93, AFG1: 0.52, AFB2: 0.47 and AFG2: 0.21 μg kg^{-1}. At the end of the study, it was determined that although the hazelnuts of which the hazelnut oil was obtained contained aflatoxin at a very high level, it was passed to the oil at very low levels below the maximum limits defined by the European Union, and almost all of it remained in the oil cake. Aflatoxin in hazelnut is passed to hazelnut oil at very low amount.

Keywords: Aflatoxin; Hazelnut; Hazelnut oil; Hazelnut oil cake

1. Introduction

Hazelnut (*Corylus avellana* L.) is cultivated on the coast of the Black Sea of Turkey and in Southern Europe. Turkey is the biggest producer of hazelnut in the world, accounting for 75% of total world production (Aktaş et al 2011; Baltacı et al 2012). Hazelnut contains sterols, tannins, essentials minerals, free phenolic acids, sugars, organic acids and phenolic compound. Additionally, hazelnut contains tocopherols and other bioactive polyphenols, which exhibit a beneficial effect on human health, reducing oxidative stress and risk of cancer, stroke, inflammation, and other neurodegenerative diseases (Schmitzer et al 2011). It has a rich nutritional source with 65% oil, 14% protein, and 16% carbohydrates. More than 90% of its oil consists of unsaturated fatty acids, especially oleic ($C_{18:1}$, 80%) and linoleic ($C_{18:2}$, 12%) acids (Özkal et al 2005). Hazelnut is also used in production hazelnut oil due to the high fat content. Hazelnut oil is also used for several purposes such as cooking, salad dressings, and flavoring ingredients,

among others. Hazelnut fatty acid composition is very similar to that of olive oil. Oleic (C 18:1) and linoleic (C 18:2) are the main fatty acids in both oils (Parcerisa et al 2000). Hazelnut oil also contained-two to three times more α-tocopherol than olive oil (Benitez-Sánchez et al 2003; Alaşalvar et al 2009).

The contamination of foodstuffs with aflatoxin is a major problem worldwide. Keeping food and feed ingredients away from mould is one of the major difficulties encountered in cultivated areas, especially in humid regions. The Black Sea Region is an area with high rainfall and a climate that is hot in summer and warm in winter. This feature increases the growth of mould in food.

Mycotoxins are those secondary metabolites of fungi which are associated with certain disorders in animals and humans (D'Mello & Macdonald 1997). Aflatoxins (AFs) are considered one of the main types of mycotoxins produced by different species of toxigenic fungi, especially *Aspergillus flavusand, Aspergillus parasiticus* species. The most important aflatoxin types are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1) and M2 (AFM2). Among them, AFB1 shows the highest toxicity (Milhome et al 2014; Özlüoymak 2014; Asghar et al 2016). Mycotoxin contaminated grains and oil seeds are toxic and carcinogenic to humans and animals. The primary target organ of aflatoxin in toxicity and carcinogenicity with acute toxicity, immunosuppressive, mutagenic, teratogenicity and carcinogenic properties is the liver (Binder et al 2007). Informing producers and consumers that aflatoxin causes serious health problems is vital. The European Commission has set maximum permissible limits 10 µg kg^{-1} for total aflatoxin and 5 µg kg^{-1} for AFB1 in hazelnut (EC 2010). Informing producers and consumers that aflatoxin causes serious health problems is vital.

Hazelnut grows in wet and humid climatic conditions. Adverse climatic conditions result in the formation of aflatoxin in hazelnuts during the harvesting, drying and storing processes (Miletic et al 2009; Lavkor & Biçici 2015). Studies into contamination of aflatoxin in nuts and nut products in literature have accelerated with the detection of aflatoxin and aflatoxigenic moulds (Aycicek et al 2005; Gürses 2006; Bircan et al 2008; Basaran & Ozcan 2009; Baltacı et al 2012). Numerous studies have reported high incidence of aflatoxins contamination in edible oils such as olive oil (Daradimos et al 2000; Papachristou & Markaki 2004; Cavaliere et al 2007; Ferracane et al 2007), peanut oil (Elzupir et al 2010; Yang et al 2011), sesame oil (Elzupir et al 2010; Idris et al 2010), blended oil (Yang et al 2011), groundnut (Idris et al 2010; Mariod & Idris 2015), cottonseed oils (Idris et al 2010) and sunflower (Elzupir et al 2010; Mariod & Idris 2015) in worldwide.

Studies on aflatoxin in hazelnut oil are inadequate in Turkey and in other countries. The use of hazelnut oil as edible oil is becoming widespread as an alternative to olive oil in terms of the nutrients it contains. Hazelnuts that are not sold or consumed in the market are generally preferred for oil production. The probability of the existence of aflatoxin is high in these hazelnuts. The purpose of this study is to determine the rate of the aflatoxin that is passed to oil during the production of hazelnut oil, and to determine the amount of aflatoxin that remains in the oil cake after production.

2. Material and Methods

2.1. Materials and reagents

Methanol, acetonitrile (ACN), hexane, nitric acid 65%, potassium bromide and sodium chloride HPLC graded and purchased from Merck (Darmstadt, Germany). Immunoaffinity columns (Aflaprep P07) with 1 mL volume were purchased from R-Biopharm Rhone Ltd. (Darmstadt, Germany). Standard solution of aflatoxin (Aflastandard, R-Biopharm) was used in the preparation of calibration curves and recovery experiments. The stock standard of aflatoxin is sold as a 1000 ng mL^{-1} concentration of a methanol solution. It consists of 250 ng mL^{-1} AFG1, AFG2, AFB1, and AFB2 type aflatoxins. Ultra-pure waters was produced by Sartorius Arium Pro VF (Goettingen, Germany).

2.2. Samples

All of five hazelnut samples containing aflatoxin were taken from Food Control Laboratory, Ordu, Turkey. Hazelnut samples of at least 1 kg were transported to the laboratory in sterile polyethylene bags under cold conditions and preserved at -20 °C until the experimental process could be conducted. All samples were analysed individually (without subsampling) for aflatoxin content tests. Firstly, aflatoxin analyses were carried out on hazelnut samples. The oils and oil cake of these samples were obtained using hexane in the soxhlet apparatus. Amounts of aflatoxin were determined in the hazelnut oil and oil cake. All experiments were performed with at least three replicates.

2.3. Aflatoxin analysis and extraction process

The analysis was performed according to AOAC Official Method 991.31:2000 (AOAC 991:31), which has international validity in aflatoxin analysis (AOAC 1991).

Each of hazelnut, hazelnut oil and oil cake samples (25 g) were taken in a blender jar, 5 g of sodium chloride and 125 mL of ACN/H2O (70:30, v:v) were added to it. After blended for 2 min at high speed, the extract was filtered through Whatman No. 4 filter paper (Whatman International, Maidstone, UK). 15 mL was removed and 30 mL of water was added. It was mixed thoroughly and the extract was filtered through Whatman No. 4 filter paper. Finally, 15 mL of the reconstituted extract were passed through the Immunoaffinity columns (IAC) at a flow rate of 2 mL min^{-1}. After passed to two aliquots of 10 mL ultrapure water through the column, AFs bound to the specific antibody were slowly released using 1 mL of methanol and diluted with 1 mL ultrapure water in HPLC vials. Vital was fully mixed in Vortex and made suitable for the high-performance liquid chromatography (HPCL).

2.4. Instrument and chromatographic conditions

Analysis was performed using a HPLC 1100 series (Agilent Technologies, Barcelona, Spain) fitted with an auto-sampler and a fluorescence detector operated at an excitation wavelength, of 360 nm and emission wavelength of 430 nm. HPLC mobile phase was a mixture of water-acetonitrile-methanol (6:2:3, v:v:v) with a flow rate of 1.0 mL min^{-1}. The chromatographic reverse phase HPLC separation was performed on a ODS-2 column. Column temperature was 25 °C. The injection volume was 100 µL.

2.5. Validation of the analytical method

In the analysis of hazelnut, hazelnut oil and oil cake samples, the method of AOAC 991.31 that is a valid method in aflatoxin analysis was used (AOAC 1991). The retention times of the standard and samples were respectively AFB1, AFB2, AFG1 and AFG2, 14.5 min., 12.2 min., 10.6 min and 9.0 min. with 2% standard deviation. Peaks were found to be quite symmetrical and sharp. There was no interference.

2.5.1. Linearity

Linearity was estimated by diluting the total aflatoxin standard stock solutions at concentrations of 0.10, 0.50, 1.0, 2.5, 5.0, 10.0 and 20.0 ng mL^{-1}. The concentration of the samples is within the range of calibration. Samples exceeded the calibration range were reread by being diluted. Linear correlation coefficient (R^2) was found above 0.999 for all aflatoxin types. The residual standard deviation (RSD) values were below 1%. The values of the calibration curves are shown in Table 1.

Table 1- Summary of calibration curve parameters

Aflatoxin	*Regression equation*	*R^2*	*RSD%*
AFG2	Y= 6.67775x-1.82801e^{-1}	0.99941	0.38583
AFG1	Y= 6.44608x-2.05951e^{-1}	0.99967	0.26338
AFB2	Y= 11.43629x-1.36371e^{-2}	0.99916	0.47764
AFB1	Y= 8.85254x-1.77944e^{-1}	0.99957	0.41328

R^2, linear correlation coefficient; RSD, residual standard deviation

2.5.2. Accuracy and precision

Recovery, repeatability analysis was conducted in a hazelnut oil sample that did not contain toxin by adding standard addition from aflatoxin obtained from R-Biopharm Rhone that were spiked with 0.5, 1.0 and 2.5 µg kg^{-1} of each aflatoxin. All spike

samples were kept at room temperature for at least 1 hour before analysis. Spike samples were studied as three parallels and three injections each (ICH 2006). From this point, the recovery was calculated and the accuracy of method was found according to Equation 1. Repeatability was used for precision, and the relative standard deviation (RSD) of the results was calculated according to Equation 2 and repeatability was found. Recovery rates ranged from 90.7-102.6% as AFG1>AFB1>AFG2>AFB2. These values are within the acceptable values of AOAC and the Codex Alimentarius. The AOAC guideline for the acceptable recovery at the 10 µg kg^{-1} level is 70-125% and the Codex acceptable recovery range is 70-110% for a level of 10-100 µg kg^{-1}, and 60-120% for a level of 1-10 µg kg^{-1} (Codex Alimentarius 1993; AOAC 2013). RSD percentage values were quite low. These results show that this method is suitable for aflatoxin analysis in hazelnut samples.

$$Recovery(\%) = (Recovered\ concentration\ /\ Infected\ concentration) x 100 \quad (1)$$

$$Relative\ standard\ deviation(\%) = (\ Standard\ deviation\ /\ Mean\ ) x 100 \quad (2)$$

2.5.3. Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) were calculated according to signal to noise (S/N) method. LOD and LOQ determined as signal to noise (S/N) ratio of 3 and 10 respectively (Şengül 2016). The results of repeatability, recovery, LOD and LOQ are given in Table 2.

3. Results and Discussion

In this study, five hazelnut samples that contained aflatoxin at high levels were examined. The oil was extracted from each hazelnut sample and the oil, oil cake and hazelnut samples were determined for aflatoxin existence by HPLC. The analyses were made in accordance with the AOAC 991:31, which is a validated method (AOAC 1991). Aflatoxin

Table 2- Validation studies in hazelnut oil samples

Aflatoxin	*Spiking level (µg kg^{-1})*	*Repeatability (mean±SD[a]) (µg kg^{-1})*	*Recovery (%) (mean±SD)*	*RSD_R [b] (%)*	*LOD (µg kg^{-1})*	*LOQ (µg kg^{-1})*
AFG2	0.50	0.443±0.012	88.58±2.42	2.74	0.0492	0.1642
	1.00	0.749±0.013	74.87±1.30	1.74		
	2.50	1.892±0.102	75.68±4.09	5.40		
AFG1	0.50	0.409±0.015	81.87±3.02	3.69	0.0528	0.1761
	1.00	0.710±0.011	70.97±1.15	1.63		
	2.50	1.870±0.026	74.81±1.04	1.39		
AFB2	0.50	0.446±0.017	89.22±3.37	3.77	0.0484	0.1615
	1.00	0.786±0.005	78.63±0.49	0.62		
	2.50	2.193±0.052	87.73±2.07	2.36		
AFB1	0.50	0.366±0.020	73.27±3.96	5.41	0.0590	0.1968
	1.00	0.648±0.007	64.85±0.74	1.15		
	2.50	1.750±0.016	70.00±0.66	0.94		

[a]SD, Standard deviation; [b]RSD_R, Relative standard deviation

results on the samples are shown in Table 3. When the results are examined, it is seen that aflatoxin is passed to the oil at a very low level when the oil was extracted from the hazelnut samples. The highest aflatoxin concentration was detected in the AFG1 among the hazelnut samples analyzed in the study, and AFB1, AFG2 and AFB2 follow this. In the hazelnut oil, the highest concentration was found in AFB1; and AFG1, AFB2 and AFG2 follow this. The rate of the transition of the aflatoxin to the oil is at 5.83% for AFB2, 4.38% for AFB1, 3.44% for AFG2 and 1.88% for AFG1. It was observed that aflatoxin remained in oil cake at a great rate. The maximum aflatoxin concentrations in the analyses of hazelnut oils were as follows; AFB1: 0.93; AFG1: 0.52; AFB2: 0.47; and AFG2: 0.21 µg kg^{-1}. When the amount of the aflatoxin in the oil cake was examined, it was observed that the aflatoxin values in the oil cake were very high. Almost all of the aflatoxin remained in the oil cake. We can claim that the aflatoxin in the hazelnut is not passed to the oil and almost all of it remains in the oil cake.

The aflatoxin limits in foods vary according to countries and their economic conditions. According to the European Union, the maximum aflatoxin limits permitted in vegetables oil are 2 µg kg^{-1} AFB1 and 4 µg kg^{-1} for total aflatoxin (EC 2010). The values that we found in hazelnut oil are below these limit values.

Since no studies were detected in the literature on determining aflatoxin in hazelnut oil, the results were compared with the results of the studies that were conducted to determine aflatoxin in other oils. When the literature results were examined, it is observed that some oil types are contaminated more with aflatoxin.

Table 3- The values aflatoxin in hazelnut, hazelnut oil and hazelnut oil cakes samples

Sample no		*Hazelnut (µg kg^{-1}) mean±SD*	*RSD*	*Hazelnut oil (µg kg^{-1}) mean±SD*	*RSD*	*Hazelnut oil cake (µg kg^{-1}) mean±SD*	*RSD*
1	G2	5.88±0.230	3.98	LOD	-	5.79±0.013	0.22
	G1	20.65±0.360	1.76	0.32±0.006	1.86	18.70±0.082	0.44
	B2	6.37±0.120	1.85	0.36±0.005	1.44	6.17±0.050	0.81
	B1	18.61±0.588	3.18	0.60±0.006	1.05	15.49±0.181	1.17
2	G2	5.83±0.423	7.28	LOD	-	5.58±0.020	0.44
	G1	35.32±0.579	1.66	0.11±0.019	17.20	33.69±0.130	0.40
	B2	3.32±0.191	5.77	0.03±0.001	3.58	3.29±0.050	1.58
	B1	17.63±0.362	2.03	0.20±0.016	8.13	15.34±0.441	2.88
3	G2	9.27±0.251	2.73	0.32±0.006	1.97	7.15±0.106	1.47
	G1	28.07±1.187	4.24	0.52±0.003	0.61	23.33±0.352	1.49
	B2	8.02±0341	4.29	0.47±0.008	1.73	7.66±0.154	2.00
	B1	21.31±0.912	4.25	0.93±0.012	1.30	17.20±0.414	2.39
4	G2	7.13±0.185	2.51	LOD	-	5.70±1.189	20.95
	G1	42.23±2.422	5.73	0.06±0.008	12.14	39.71±7.163	18.04
	B2	3.85±0.021	0.51	0.02±0.004	28.78	3.64±0.680	18.72
	B1	20.46±0.404	1.96	0.09±0.002	2.52	19.63±3.492	17.76
5	G2	6.06±0.090	1.41	0.21±0.061	29.10	4.20±0.332	7.75
	G1	17.84±0.342	1.87	0.34±0.033	9.77	16.30±1.771	10.88
	B2	5.59±0.330	5.91	0.33±0.024	7.36	4.94±0.520	10.57
	B1	14.59±0.871	5.98	0.51±0.075	14.70	14.39±1.512	10.50

Similar findings were observed in olive oil samples. Ferracane et al (2007), found the presence of AFB1 only 3 out of 30 samples were contaminated ranging from 0.54 to 2.50 μg kg^{-1} in olive oil (Ferracane et al 2007). Additionally, Papachristou & Markaki (2004), who studied 50 samples and 60 ng kg^{-1} of AFB1 was found only one of them (Papachristou & Markaki 2004). Daramidos et al (2000) determined AFB1 in 2.8-15.7 ng kg^{-1} of the concentration range in 72% of 50 olive oil samples. However one sample was contaminated with 46.3 ng kg^{-1}. In 14 samples AFB1 was not detectable (Daramidos et al 2000). Cavalier et al (2007) found that thirty-five olive oil samples were analysed and aflatoxins were not detected (Cavalier et al 2007). As it is observed in these studies, Aflatoxin is observed in a small amount in the olive oil samples, and the highest value reported is 2.5 μg kg^{-1}. Aflatoxin is not passed to the oil in olive oil samples, which is the case in hazelnut oil, and exists in very small amounts.

A study was conducted on peanut oil by Yang et al (2011), and the following values were reported for 15 of the 31 samples; AFB1 (0.15-2.72 μg kg^{-1}), and for 6 of them as AFB2 (0.15-0.36 μg kg^{-1}), and for 3 of them as AFG1 (0.01-0.02 μg kg^{-1}). They examined other oils in the same study and AFB1 contamination was recorded in 15 peanut oils, six blended oils and single animal oil (fish oil). However, AFB2 and AFG1 were found only in peanut oil and in no other type of oil (Yang et al 2011). This could indicate that peanut oils are more susceptible to aflatoxin contamination. When compared with our results, AFB1 is the toxin found with the highest level in peanut oil and in the other oils analyzed in this study, as in the case of hazelnut oil. AFG1 was the toxin found at the second highest level in our results. This stems from the fact that the AFG1 amount is high in the hazelnut samples we used in the analyses in our study. It is passed to oil with the lowest level when examined the percentages of passed to oils. AFB types are passed to oil more than other. We may conclude this from the results of the study conducted by Yang et al (2011).

Elzupir et al (2010), conducted a study on a total of 81 vegetable oil samples including peanuts (n= 21), sesame (n= 14), and sunflower (n= 19). The average concentrations in peanut oil were found to AFB1 as 16.3, AFB2 as 1.0, AFG1 as 12.9 and AFG2 as 11.6 μg kg^{-1} (Elzupir et al 2010). These values are extremely high when compared with the results of our study and other studies. AFB1 is the toxin found with the highest level in this study. However, AFG1 and AFG2 values were also determined to be high. Average levels in sesame oil were found AFB1 as 43.6; AFB2 as 0.3: AFG1 as 47.5 and AFG2 as 102.7 μg kg^{-1}. In sunflower oil was detected AFB1 as 24.6, AFB2 as 0.3: AFG1 as 24.5 and AFG2 as 14.8 μg kg^{-1}. These values are very different from the results we found in hazelnut oil.

4. Conclusions

The hazelnuts that are used in hazelnut oil production industry are the ones that are not sold in the market with low quality. For this reason, it is highly probable that aflatoxin exists much in these hazelnuts. However, the results obtained in the analyses show that aflatoxin is passed to the oil at a very low concentration. Aflatoxin is not passed to oil and is accumulated in the oil cake. This reveals another problem that has to be investigated, because the oil cake of the hazelnuts is used as feed in animal feed industry. For this reason, it has to be investigated for aflatoxin before used in feed industry.

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The Effect of Adding Corn Silage at Different Ratios to Orange and Tangerine Wastes on Biogas Production Efficiency

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ABSTRACT

In this study, biogas production efficiencies of mixtures obtained by adding corn silage (CS) to citrus industrial wastes at different ratios were determined. Orange (OJPW) and tangerine processing juice wastes (TJPW) (crusts and shells) were selected as materials in the study. 25%, 50%, 75% of CS was added to these selected wastes. Changes in the obtained mixture chemical properties (dry matter, dry organic matter, crude ash, crude protein, crude oil, Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF)), biogas production and methane content in the biogas were investigated. The results of the study showed that the highest crude protein content was found in 100% TJPW (10%), raw fat percentage in 100% TJPW (5.14%), dry matter content in 100% CS (93.56%), ADF in 100% CS (22.74%) and the NDF in a mixture of 25% OJPW + 75% CS (45.08%).

The highest methane production was determined for a mixture of 100% TJPW and 50% TJPW + 50% OJPW (0.46 m^3 kg^{-1} ODM). Also the highest biogas production was determined in a mixture of 50% OJPW + 50% TJPW (0.90 m^3 kg^{-1} ODM). The mixing of CS in TJPW and OJPW reduced significantly the production of methane and biogas in the mixture. As a result of the statistical analysis, significant differences ($P \leq 0.05$) were found in both methane and biogas production of agricultural wastes.

Keywords: Orange wastes; Mandarin wastes; Corn silage; Biogas; HBT

1. Introduction

Today the gradual decrease of fossil fuel resources has increased the need for renewable energy sources (Üçok et al 2016). In recent years, due to its contribution to the sustainable development of countries, there is increasing interest in the energy obtained from biomass, especially biogas (Mansourpoor & Shariati 2012; Üçok et al 2016; Mojtaba & Ahmad 2012). Wastes emerged from agriculture, and related industry is major sources of biogas production. Application of biogas technology provides not only the disposal of waste but also energy production (Deublein & Steinhauser 2008).

Citrus fruits are among the most grown and consumed fruit group in the world. In this regards, China ranks first with about 32 million tons,

followed by Brazil with 20 million tons and the USA with 10 million tons. Turkey is ranked the eighth with 3.7 million tons (FAO 2015). Approximately 85% of citrus production in Turkey is produced in the Mediterranean region (TUİK 2015). Turkey has an important position in the production of citrus fruits especially regarding exports to the Middle East (Iraq, Saudi Arabia, and Iran), Russia, Ukraine, Romania, Poland, and Bulgaria. However, there are significant problems in the export of products grown in recent years due to political and geopolitical problems among these countries, to which exports have been made. Therefore, most of these products can not be consumed due to excess supply in the domestic market (Çallı 2012).

Processing the citrus fruits into juice, a large part of the separated pulp consisting of crust which is rich in cellulose and pectin can be utilized in bioprocesses with the aid of preprocessing. This may create an opportunity for the fruit juice industry in Turkey for the valorization of wastes (crusts and pulps) existed from the processing of orange and tangerine regarding the production of biogas.

The efficiency of biogas production from fruit processing wastes can be increased by co-fermentation with wastes which have higher biogas potential (Elaiyaraju & Partha 2012). This may encourage the establishment of small-scale biogas plants in the region, leading to the prevention of environmental pollution and the utilization of significant energy resources. In Europe, there are many central biogas plants that are operated successfully, producing biogas from thousands of farm-type and organic household and industrial wastes producing biogas from animal wastes and energy plants (Çallı 2012). Although numerous studies on the usage of fruit pulps and wastes for biogas production have been carried out (Aslanzadeh & Özmen 2009; Elaiyaraju & Partha 2012; Nguyen 2012; Wikandari et al 2014a; Wikandari et al 2014b), there are limited studies on co-fermentation of two plant-based materials to increase biogas production efficiency concerning fruit pulps and wastes.

In this study, it was aimed to determine the biogas production efficiencies of the mixtures obtained by adding corn silage to citrus industrial wastes at different rates. For this aim, corn silage has been added to the orange and mandarin wastes produced during fruit juice industry production activities at different rates. The chemical properties of these obtained mixtures, biogas productions and methane rates in the biogas have been determined.

2. Material and Methods

2.1. Material preparation

In this study, orange juice processing waste (OJPW) and tangerine juice processing waste (TJPW) in the form of pulp were selected as materials, and corn silage (CS) with a high biogas potential was selected as co-fermentation material. The OJPW and TJPW were received from the enterprises producing fruit juice in Çukurova Region, and the CS was obtained from Animal Feeding Center at Çukurova University. The samples were dried at room temperature for three weeks at Biogas laboratory, Department of Biosystem Engineering in Kahramanmaraş Sütçü İmam University. The moisture contents of the samples dried at room temperature were determined as 82.89%, 82.66%, and 64.65% (wet basis, w.b.) for TJPW, OJPW, and CS, respectively, before grinding. The dried samples were ground in a grinder to a grain size of 1 mm (based on the VDI 4630 2006 standard). The ground samples were weighed with a precision of 0.2 g ± 0.1 µg to fit the established experimental conditions.

In this study, the ground materials were taken in quantities appropriate to the standard conditions (0.2 g) and the samples in Table 1 were prepared. Samples were placed in 100 mL glass syringes as three replicates. Dry matter (drying in the oven), crude oil (according to the Soxhlet method), crude protein (according to the Kjeldahl method), crude ash analyses (in the ash furnace) were analyzed based on AOAC (1990), and Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) analyses were conducted according to Van Soest et al (1991).

Table 1- Biogas production materials

Material	*Number of samples*
100% OJPW	3
100% TJPW	3
100% CS	3
75% OJPW+25% CS	3
50% OJPW+50% CS	3
25% OJPW+75% CS	3
75% TJPW+25% CS	3
50% TJPW+50% CS	3
25% TJPW+75% CS	3
50% OJPW+50% TJPW	3

2.2. Biogas and methane measurements

Methane rates in biogas and biogas production were determined by the HBT method (Heffrich & Oechsner 2003). In the study, the prepared samples were placed into 100 mL glass syringes located in the incubator. In the same way, three inoculum syringes, which were prepared using burette, each receiving 30 mL of inoculum for control group samples, were also placed in the sections in the incubator. The syringe piston was removed before the weighed samples were placed in the syringe, and plastic clips were attached to the silicone hoses in the tip of the injectors and used for gas transfer. Vaseline was applied to the pistons of the injectors, leaving a three-finger space at the top and bottom, to prevent gas escape during analysis. Afterwards, the glass syringes were made ready to use by attaching its pistons and closing the clips. The syringes were placed horizontally into the incubator at 37 °C after the inoculum was placed. The methane measurement system used to determine the methane content prior to operations in the incubator was calibrated with a calibration tube (60.5% CH_4) (S-AGM plus 1010 sensor). The purpose of calibration is to verify that the measured gas is at standard conditions (0 °C and 1013 hPa). Experiments lasted for 35 days. Measurements were made every 6 hours for the first 6 days, 8 and 12 hours later on the subsequent days, and the methane efficiency in each sample was determined. While Equation (1) was used to calculate the normal volume of the produced gas in the glass syringes prepared for each sample of the materials studied, Equation (2) was used to determine the methane content of the formed biogas, and Equation (3) was used to calculate cumulative methane over time (VDI 4630 2006).

$$(V_0^{tr}=V\left(\frac{(P-P_W)(T_0)}{(P_0)(T)}\right)) \quad (1)$$

Where; V_0^{tr}, Volume of gas under normal conditions (mLN); V, Volume of gas read (mL); P, Air pressure at the time of reading (hPa); P_w, The steam temperature of the water in the outside (hPa); T_0, Normal temperature (273 K); P_0, Normal pressure (1013 hPa); T, Temperature of the gas which has undergone fermentation in the outside (300 K)

$$(C_{CH_4}^{tr} = C_{CH_4}^{f}\left(\frac{P}{(P-P_W)}\right)) \quad (2)$$

Where; $C_{CH_4}^{tr}$, The volumetric methane content in dry biogas (%); $C_{CH_4}^{f}$, Volumetric methane content in moist biogas (%); P, Gas pressure during reading (hPa); P_w, The steam temperature of the water in the outside (hPa).

$$(M_{CH_4}(t) = M_{CH_4}(0)+\int_{t_1}^{t_2} M_{CH_4}(t)\,dt) \quad (3)$$

Where; $M_{CH_4}(t)$, Cumulative methane production (Nm^3 CH_4 kg^{-1} ODM^{-1} min^{-1}); $M_{CH_4}(0)$, Methane production when t = 0 (Nm^3 CH_4 kg^{-1} ODM^{-1} min^{-1}); t_2-t_1, Time between two measurements (min).

2.3. Preparation of ınoculum

Sludge, which is a mixture of liquid + solid phase, was received from Gaziantep Water and Sewerage Administration (GWSA) central wastewater treatment plant. It was filtered through four layers of cheesecloth and was mixed with 1:2 buffer solution to prepare the inoculum. Buffer solution consisted of 500 mL of distilled purified water, 0.1 mL of solution A, 200 mL of solution B, 200 mL of solution C, 1 mL of resazurin (0.1%, w v^{-1}) solution C, and 40 mL of solution E. Solution A was prepared by dissolving 13.2 g of $CuCl_2 2H_2O$, 10.0 g of $MnCl_2 4H_2O$, 1.0 g of $CoCl_2 6H_2O$, 8.0 g of $FeCl_2 6H_2O$ in distilled water, completing the volume to 100 mL. Solution B was prepared by dissolving 35 g of $NaHCO_3$ and 4 g of NH_4HCO_3 in distilled

water, completing the volume to 100 mL. Solution C was prepared by dissolving 5.7 g of Na_2HPO_4, 6.2 g of KH_2PO_4, 0.6 g of $MgSO_47H_2O$ in distilled water, completing the volume to 1000 mL. Solution D was prepared by dissolving 0.5 g of resazurin in distilled water, completing the volume to 100 mL. Solution E consisted of 95 mL of distilled water, four mL of N-NaOH and 625 mg of $Na_2S_9H_2O$.

2.4. Evaluation of data

The mean and standard deviation values, statistical analyzes and variance analyzes of the measurements made in three replicates were determined, and the obtained values were interpreted by transferring them into the figures and tables.

3. Results and Discussion

3.1. Chemical properties of materials

The chemical properties of the mixes obtained as a result of the analysis are given in Table 2. As can be seen from the Table 2, in the chemical analyses conduced in the samples, the highest crude protein ratio was found for the 100% TJPW (10%); the highest crude fat was determined for the 100% TJPW (5.14%); the highest dry matter content was found for 100% CS (93.56%); the highest ADF ratio was determined for 100% corn silage (22.74%); and the highest NDF rate was determined to be in the mixture of 25% OJPW+75% CS (45.08%).

3.2. Biogas and methane production values of materials

The cumulative specific methane production values of the inoculum and mixes based on time are shown in Figure 1. According to results, the maximum methane production in the studied materials ranged from between 30 and 35 days (Figure 1). In the study, the highest methane production (0.46 m^3 kg^{-1} ODM) was determined for 100% TJPW, and the mixture of 50% TJPW + 50% OJPW, and the highest biogas production (0.90 m^3 kg^{-1} ODM) was found for the mixture of 50% OJPW + 50% TJPW. The cumulative specific methane and biogas productions and methane rates in the biogas are shown in Table 3. Methane and biogas production in mixtures containing CS at different ratios of OJPW and TJPW varied between 0.31-0.46 m^3 kg^{-1} ODM and 0.62-0.90 m^3 kg^{-1} ODM, respectively (Table 3). The change in the cumulative specific methane and biogas production values are given in Figure 2. The results of variance analysis of methane production, biogas production, and biogas methane rates are given in Table 4.

Table 2- Chemical properties of mixes

Material	*Crude protein content (%)*	*Crude oil content (%)*	*Dry matter content (%)*	*Organic matter content (%)*	*ADF (%)*	*NDF (%)*
100% OJPW	7.78±0.09^{de}	2.30±0.06^{d}	88.67±0.02^{bc}	95.56±0.15^{ab}	14.76±0.08^{c}	20.12±0.11^{f}
100% TJPW	10.00±0.13^{a}	5.14±0.11^{a}	88.65±0.19^{c}	95.40±0.18^{ab}	20.21±0.03^{ab}	26.49±0.04^{e}
100% CS	7.55±0.06^{e}	2.43±0.14^{d}	93.56±0.21^{a}	95.02±0.26^{ab}	22.74±0.18^{a}	43.04±0.13^{ab}
75% OJPW+25% CS	7.56±0.04^{e}	2.38±0.09^{d}	90.88±0.05^{abc}	96.11±0.17^{ab}	19.53±0.13^{ab}	34.68±0.27^{d}
50% OJPW+50% CS	7.59±0.09^{e}	2.56±0.13^{d}	91.62±0.46^{ab}	93.71±0.39^{b}	21.36±0.25^{ab}	38.59±0.02^{bc}
25% OJPW+75% CS	7.46±0.02^{e}	2.50±0.07^{d}	92.16±0.31^{ab}	97.56±0.41^{a}	21.11±0.04^{ab}	45.08±0.01^{a}
75% TJPW+25% CS	9.27±0.11^{b}	4.68±0.09^{ab}	89.59±0.15^{bc}	95.25±0.16^{ab}	19.21±0.08^{ab}	37.12±0.02^{bc}
50% TJPW+50% CS	8.72±0.04^{c}	4.07±0.05^{bc}	90.49±0.27^{ab}	95.86±0.04^{ab}	21.09±0.32^{ab}	39.37±0.34^{bc}
25% TJPW+75% CS	8.06±0.15^{d}	3.40±0.08^{c}	92.05±0.34^{ab}	96.75±0.047^{ab}	22.68±0.38^{a}	44.54±0.16^{a}
50% OJPW+50% TJPW	8.81±0.07^{c}	3.85±0.15^{c}	88.70±0.27^{abc}	94.85±0.33^{ab}	17.65±0.07^{bc}	23.44±0.08^{ef}

P≤0.05 a, b, c, d, e**,** the differences between the mean scores of cumulative specific methane and biogas production, represented by different letters in the same column, are significant; P≤0.1 a, b, the differences in the mean scores of the ratio of methane in the biogas indicated by different letters in the same column are significant

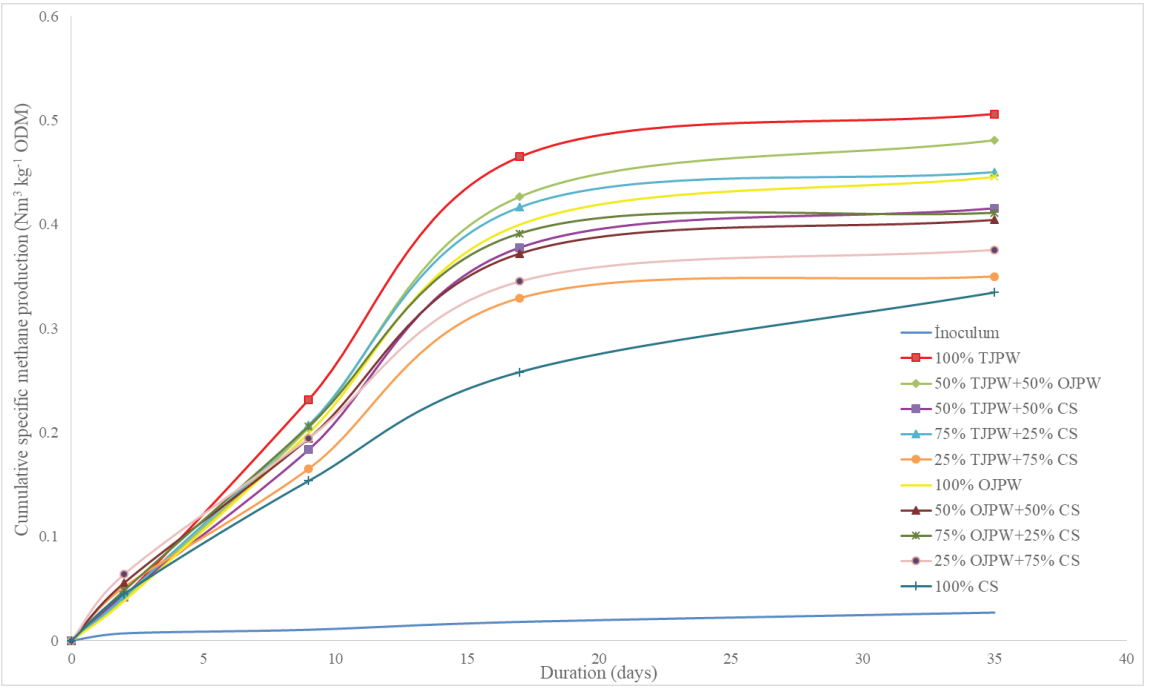

Figure 1- Change in average cumulative methane productions of materials based on time

Table 3- Methane and biogas productions and methane rates in biogas of materials

Material	*Cumulative specific methane production (m^3 kg^{-1} ODM)*	*Cumulative specific biogas production (m^3 kg^{-1} ODM)*	*Methane rate in biogas (%)*
100% OJPW	0.41±0.006^{ab}	0.85±0.012^{ab}	48.42^{b}
100% TJPW	0.46±0.011^{a}	0.86±0.023^{ab}	53.69^{a}
100% CS	0.31±0.017^{c}	0.62±0.030^{e}	50.00^{ab}
75% OJPW+ 25% CS	0.38±0.004^{bc}	0.76±0.003^{bcd}	49.81^{ab}
50% OJPW+ 50% CS	0.37±0.013^{bc}	0.73±0.019^{cde}	50.63^{ab}
25% OJPW+ 75% CS	0.34±0.006^{c}	0.70±0.007^{cde}	48.83^{ab}
75% TJPW+ 25% CS	0.42±0.012^{ab}	0.82±0.014^{abc}	51.16^{ab}
50% TJPW+ 50% CS	0.38±0.020^{bc}	0.77±0.019^{abcd}	49.73^{ab}
25% TJPW+ 75% CS	0.31±0.028^{c}	0.63±0.044^{de}	49.60^{ab}
50% OJPW+ 50% TJPW	0.46±0.009^{a}	0.90±0.019^{a}	50.79^{ab}

P≤0.05 a, b, c, d, e, the differences between the mean scores of cumulative specific methane and biogas production, represented by different letters in the same column, are significant; P≤0.1 a, b, the differences in the mean scores of the ratio of methane in the biogas indicated by different letters in the same column are significant.

The mixing of CS with OJPW and TJPW significantly reduced the production of methane and biogas in the mixture. In the other word, the increase in the ratio of CS to OJPW and TJPW negatively affected the methane and biogas production of the mixture. At the other side, maximum cumulative specific methane production (0.46 m^3 kg^{-1} ODM, Table 3) existed for %100 TJPW due to its crude

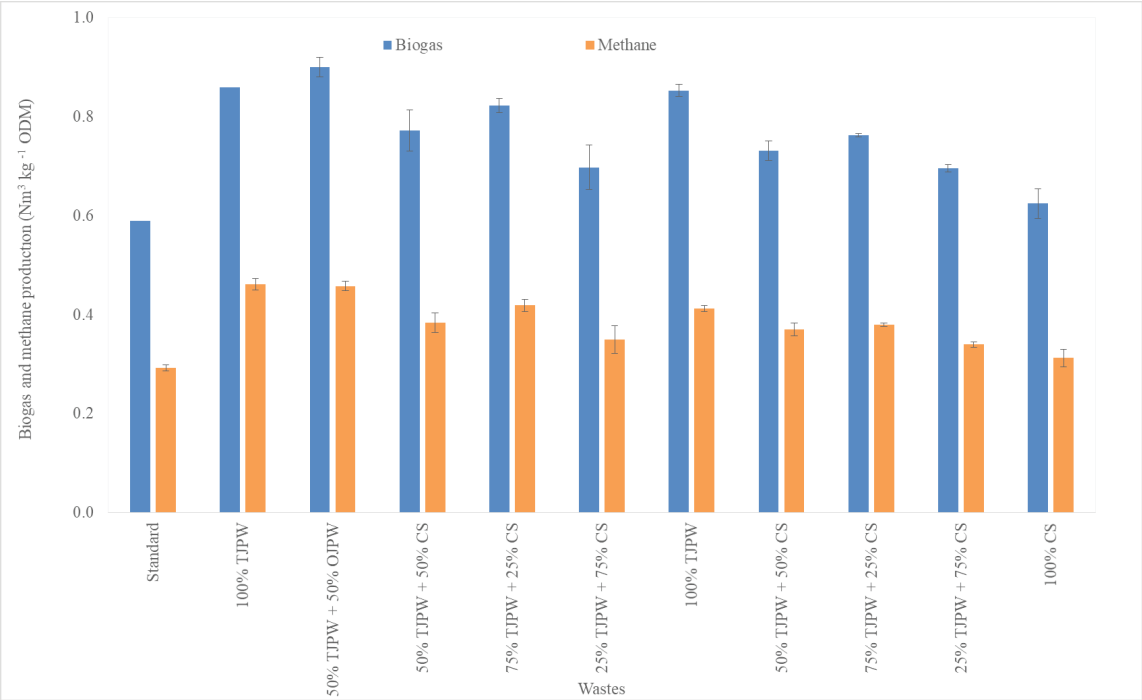

Figure 2- Changes in cumulative specific methane and biogas productions of materials

Table 4- Analysis of variance table of methane, biogas productions and methane ratios of materials

	Source of variance	*SD*	*SS*	*MS*	*F value*	*SEM*	*P value*
Methane	Between groups	9	0.75	0.08	13.236	0.0250	0.000***
	Within groups	20	0.13	0.01		0.0250	
	Total	29	0.88			0.0250	
Biogas	Between groups	9	0.237	0.26	12.653	0.037	0.000***
	Within groups	20	0.042	0.02		0.037	
	Total	29	0.279			0.037	
Methane ratio (%)	Between groups	9	58.174	6.464	2.004	1.466	0.094*
	Within groups	20	64.521	3.226		1.466	
	Total	29	122.696			1.466	

***, the differences between the mean scores of methane and biogas are significant (P≤0.05); *, the differences between the mean scores of methane ratios are important (P≤0.1); *SD*, Standard Deviation; *SS*, Some of Squares; *MS*, Mean Square; *SEM*, Standard Error of the Mean

protein content (10.0%) and crude oil content (5.14%). This findings is supported by the statement of "the amount of methane production increases with the increase in protein and fat by Avcıoğlu (2011).

The cell contents are fast, NDF-ADF (hemicellulose) is slow and ADF (cellulose and lignin) is digested more slowly in the inoculum (Van Soest 1994). Therefore, the digestion of CS occurred later due to high rates of ADF and NDF (Figure 1). In the statistical analysis, both methane and biogas productions of mixes differed significantly (P≤0.05). Methane ratios in the biogas also differed significantly (P≤0.1) (Table 4).

The differences in the methane and biogas interaction of the materials that were examined in the study were also evaluated. There was no interaction for OJPW, TJPW, and CS. Mixtures formed by mixing these samples at certain ratios will also be subjected to co-fermentation, so their interactions will be different. For example, the specific methane production values were 0.38 m^3 kg^{-1} ODM for the mixture of 75% OJPW + 25% CS, 0.41 m^3 kg^{-1} ODM for 100% OJPW and 0.31 m^3 kg^{-1} ODM for 100% CS (Table 3). The methane interaction was 75% x 0.41 + 25% x 0.31 = 0.385 m^3 kg^{-1} ODM for the mixture of 75% OJPW + 25% CS (Table 5). The average specific methane production values were 0.38 m^3 kg^{-1} ODM for the mixture of 75% OJPW+25% CS and 0.385 m^3 kg^{-1} ODM as a result of its interaction. The value of difference was calculated to be -0.005 m^3 kg^{-1} ODM, and the value of decrease was calculated to be -1.30% (Table 5). The values in other materials (50% OJPW+50% CS, 25% OJPW+75% CS, 50% TJPW+50% CS, 25% TJPW+75% CS, 50% OJPW+50% TJPW) in the study were calculated in the same evaluation method which was applied to the other mixtures (50% OJPW+50% CS, 25% OJPW+75% CS, 50% TJPW+50% CS, 25% TJPW+75% CS, 50% OJPW+50% TJPW). The increase in the production of methane and biogas as a result of co-fermentation interactions resulted in a maximum of 5.41% in the mixture of 50% OJPW+50% TJPW (Table 5).

Table 5- Interaction differences of methane and biogas

Waste materials	*Methane interaction difference (%)*	*Biogas interaction difference (%)*
100% OJPW	0.00	0.00
100% TJPW	0.00	0.00
100% CS	0.00	0.00
75% OJPW+25% CS	-1.30	-4.10
50% OJPW+50% CS	2.87	-0.56
25% OJPW+75% CS	1.44	-12.14
75% TJPW+25% CS	-0.87	2,87
50% TJPW+50% CS	-0.33	4.32
25% TJPW+75% CS	-10.79	-6.74
50% OJPW+50% TJPW	5.41	5.27

The methane values of the OJPW and TJPW were reported as 0.45 Nm^3 kg^{-1} ODM and 0.48 Nm^3 kg^{-1} ODM (Gunaseelan 2004), biogas and methane production from CS, 0.70 Nm^3 kg^{-1} ODM and 0.34 Nm^3 kg^{-1} ODM, respectively (Amon et al 2007). The findings in this study were close to the limit values reported by Gunaseelan (2004) and Amon et al (2007). It is thought that this difference, which was observed at a small level in the study, may be due to the chemical structure of the materials (Avcıoğlu 2011).

Results showed that the wastes examined, the highest raw protein and crude oil ratio was in 100% TJPW, the highest dry matter ratio was in 100% CS, the highest organic dry matter ratio was in the mixture of 25% OJPW+75% CS, the highest ADF ratio was in 100% CS, and the highest NDF ratio was in the mixture of 25% OJPW+75% CS.

5. Conclusions

The highest cumulative specific methane and biogas production values were found in 100% TJPW, 100% OJPW and the mixture of 50% OJPW+50% TJPW, while the lowest such values were found in mixtures of OJPW and TJPW containing 75% CS, and 100% CS. A significant difference ($P \leq 0.05$) was found between the methane and biogas productions of the agricultural wastes examined in the statistical analysis. The highest increase in the production of methane and biogas as a result of co-fermentation interaction occurred in the mixture of 50% OJPW+50% TJPW. The co-fermentation of the wastes resulted in differences in the proportions of the cumulative methane, biogas, and methane in biogas. CS mixed at different ratios to OJPW and TJPW reduced methane and biogas production of the mixture.

Recommendations

The biogas production potential of OJPW, TJPW, and CS, and their co-fermentations is high. For this reason, it can be said that they are important materials for biogas plants. New wastes to be generated in case of using the mentioned waste for production of biogas can be used for organic fertilizer in agriculture. Greenhouse gases (methane and carbon dioxide)

that will be released into the atmosphere due to the storage of wastes on uncontrolled conditions will be prevented with the launch of the biogas process. As a result of the elimination of wastes, the odor intensity in the air will decrease, and the environmental problems will be reduced. The level of methane ratios in the cumulated methane, biogas, and biogas in methane that are formed as a result of appropriate co-fermentation interactions can be increased. By providing co-fermentation at an appropriate level, energy production in biogas plants is going to be an able to increase.

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Morphological Changes Caused by *Bacillus megaterium* on Adult Emergence of Fall Webworm's Pupa, *Hyphantria cunea* (Drury) (Lepidoptera: Erebidae)

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ABSTRACT

Insecticidal effects of *Bacillus megaterium* isolates maintained from bacterial oozed pupae were studied on pupae and 2nd instar larvae of *Hyphantria cunea.* The bacterial isolates were identified according to morphological, physiological, biochemical and sequence analyses. This study reported that *Bacillus megaterium* isolates, HMA5 and BM1, caused different abnormal wings shapes and hair loss on thorax and abdomen of *Hyphantria cunea*. In addition, the HMA5 and BM1 treated pupae showed significantly reduced ability of adult emergence rates. The percentages of the adult emergence from pupae were 1% and 2% for HMA5 and BM1, respectively.

Keywords: *Bacillus megaterium*; *Hyphantria cunea*; Morphological changes

1. Introduction

Hyphantria cunea (Drury) is a moth in the family Erebidae, and a widespread pest of ornamental and fruit trees. The pest is highly polyphagous and causes economic damage due to the reduced crop yields and destroys overall productivity (Su et al 2008). The life cycle of the pest consists of four different periods; adult, pupa, larva and egg. Different larval stages of the pest eat large amount of leaves, causing complete defoliation. One of the control methods against the pest is to use pesticides from different groups to prevent larval damage in plants. However, chemical control of this pest can be difficult due to their webbing, reducing the penetration of insecticides to the point that the pest may not come in contact with a lethal dose. In addition, the use of pesticides causes a negative effect on atmosphere and the development of high level pest resistance (Li et al 2013). Since chemical control is harmful to the environment, biological control agents for *Hyphantria cunea* have been used in agricultural lands as a better approach for pest control. *Bacillus thuringiensis*, an entomopathogenic bacterium, has been used in biological control of *Hyphantria cunea*. The bacterium is an effective biocontrol agent of this pest and can be used in preference to insecticides (Zibaee et al 2010). For controlling pests, the use of another species of *Bacillus*, *Bacillus*

megaterium, producing insecticidal metabolites is a new tool for biotechnological research (Aksoy & Ozman-Sullivan 2008). Also the bacteria can be used as biological control agent of nematodes (Huang et al 2010). *Bacillus megaterium* is a rod-like, gram positive, mostly aerobic, spore forming microorganism discovered in many different types of habitat such as dried food, sea and soil (Gu et al 2007). So far there have been many reports on microorganisms as potential microbial biological control agents (Ozsahin et al 2014; Aksoy et al 2015). However, *Bacillus megaterium* against pupae of *Hyphantria cunea* were not used in these studies.

The aim of the study is to isolate bacterial isolates from diseased pupae of *Hyphantria cunea* in hazelnut orchards in Samsun province in Turkey, to evaluate the isolates by molecular techniques**,** to categorise insecticidal activity on 2nd instar larvae of *Hyphantria cunea* and to evaluate efficacy of the bacterial isolates on adult emergence from *Hyphantria cunea* pupae. This is the first study carried out on *Bacillus megaterium* against pupae and larvae of *Hyphantria cunea*.

2. Material and Methods

2.1. Insects

Healthy pupae and 2nd instar larvae of *Hyphantria cunea* were provided by Ondokuz Mayis University, Agricultural Faculty, Plant Protection Department, Entomology Lab. (Samsun, Turkey).

2.2. Bacterial isolation

Diseased pupae of *Hyphantria cunea* were randomly collected from the bark cracks of different hazelnuts orchards or in the soil of fall webworm damaged areas where have not been previously treated with any *Bacillus* biopesticide in Samsun province. The collected samples were kept at about 4 °C in the refrigerator until further use for bacterial isolation. To eliminate external contamination, diseased *Hyphantria cunea* pupae were sterilized in 1% sodium hypochlorite solution for 3 min. The samples were then washed three times in sterile distilled water and transferred aseptically into a sterile mortar and macerated with a sterile pestle in 1.5 mL of sterile distilled water. The suspension was then heated at 75 °C for 10-15 min because non-spore isolates were eliminated. 1×10^{-2} and 1×10^{-4} dilutions were streaked on nutrient agar plates incubated at 30 °C for 48 h (Cavados et al 2001) and candidate isolates were selected based on colony morphology.

2.3. Biochemical characteristics

The morphological, physiological and biochemical characteristics of *Bacillus* isolates were determined according to Logan & Vos (2009).

2.4. DNA extraction

The total genomic DNA was extracted from bacterial suspension (after 12 h incubation in LB) using Qiagen DNA extraction kit. DNA concentration was standardized at about 50 ng μL^{-1} for PCR assay.

2.5. 16S rRNA gene sequencing

16S rRNA gene region of two isolates was amplified by PCR tests for sequence analysis using the universal primers 27F (AGAGTTTGATC(AC) TGGCTCAG); positions 8 to 27 and 1492R (ACGGTTACCTTGTTACGACTT); positions 1508 to 1492, (Weisburg et al 1991). Amplifications of the *16S rRNA* gene were performed in a final volume of 50 µL containing 25 µL of 2x BioMix™ Red (Bioline), 1µL of each primer, and 1 µL of template DNA and 17.5 µL of dH_2O. Reactions were performed in a Bio-Rad T100 Thermal Cycler. The PCR conditions were 15 min at 94 °C, 35 cycles of 30 s at 94 °C, 1 min at 52 °C and 90 s at 72 °C, followed by a final extension at 72 °C for 7 min.

Sequencing for PCR products of the selected isolates was performed in both directions, carrying out by the Medsantek Company, Turkey. The Chromas Pro Software (Technelysium Pty Ltd, Qld, Australia) was used for editing and regenerating the obtained sequences. Resulted partial *16S rRNA* gene sequences of *Bacillus megaterium* were searched in GenBank database (http://www.ncbi.nlm.nih.gov/Genbank) with available sequences using nucleotide-nucleotide Basic Local Alignment Search Tool (BLAST). Moreover, Mega 6 software

was used for alignment analyses and also construct a phylogenetic tree (Tamura et al 2013).

2.6. Bioassay for the pupae

Twenty healthy pupae of *Hyphantria cunea* were placed in 1 L plastic ice-cream cups (sterilized by ethanol) containing filter papers moisturized by sterile distilled water. Twenty healthy pupae of *Hyphantria cunea* were put into each ice-cream cup and sprayed with a 10^8 cfu mL^{-1} suspension of the bacterial isolates grown on nutrient agar at 30±2 °C for 24 h. Harvested cells with a drigalski spatula suspended in sterile distilled water. The turbidity of the each bacterial suspension was adjusted to an optical density at 600 nm (OD_{600}) of 0.7 (10^8 cfu mL^{-1}, colony-forming units mL^{-1}).

The suspension was applied on pupae placed on the cups from a distance of 25-30 cm with a hand spray atomiser of 50-mL capacity until the pupae surface was just wetted with very fine droplets. The negative control cups were sprayed with sterile distilled water and incubated at 24±2 °C with 65±5% relative humidity (RH), 16:8 h light:dark photoperiod for 30 days in a Binder incubator (Model KBWF 240, Germany). Polyethylene sheets were used together with rubber in order to cover the open sides of cups. The pupae were checked daily to collect emerged adult. The causal agents were re-isolated from the pupae according to Cavados et al (2001) and found identical to the originals cultures characterized by sequencing of *16S rRNA* gene.

The treatments consisted of: (i) 20 pupae of *Hyphantria cunea,* bacterization with *Bacillus megaterium* (isolate-HMA5); (ii) 20 pupae of *Hyphantria cunea,* bacterization with *Bacillus megaterium* (isolate-BM1); (iii) 20 pupae of *Hyphantria cunea,* bacterization with *Bacillus thuriginesis* (isolate-27.1a, isolated previously from soil (*16S rRNA* Accession Number: KX683870); (iv) 20 pupae of *Hyphantria cunea* were sprayed with sterile distilled water (Control-C). The bioassays were conducted twice with five replications.

2.7. Bioassay for the 2nd instar larvae

Twenty 2nd instar larvae of *Hyphantria cunea* were put into each 1 L plastic ice-cream cups (sterilized by ethanol) containing filter papers moistured by sterile-distilled water. Fresh acer leaves were sprayed with suspension of the bacterial isolates and allowed to air dry. The control leaves were treated with distilled water. The larvae were then fed on leaves treated and non-treated with suspension of the bacterial isolates. The uneaten leaves were removed every 24 h and the larvae were fed fresh untreated leaves. The larvae were incubated at 25±1 °C and 65±5% relative humidity (RH), 16:8 h light:dark photoperiod for 10 days in a Binder incubator (Model KBWF 240; Germany) and checked every day to collect dead larvae. On the fourth day after starting the experiment mortality of larvae were recorded. The causal agents were re-isolated from dead nymphs according to Cavados et al (2001) and shown to be identical to the organisms characterized by sequencing of *16S rRNA* gene.

The treatments consisted of: (i) 20 2nd instar larvae of *Hyphantria cunea,* bacterization with *Bacillus megaterium* , isolate HMA5; (ii) 20 2nd instar larvae of *Hyphantria cunea,* bacterization with *Bacillus megaterium* , isolate BM1; (iii) 20 2nd instar larvae of *Hyphantria cunea,* bacterization with *B. thurigiensis* strain 27.1a; (iv) 20 2nd instar larvae of *Hyphantria cunea* were sprayed with sterile distilled water (Control-C). The bioassays of larvae were conducted twice with five replications.

2.8. Data analysis

Kolmogorov-Smirnov One Sample Test results showed that all traits could be assumed normally distributed ($P<0.05$). Levene variance homogeneity test results indicated that all traits had homoscedasticity ($P<0.05$). Then, One-Way ANOVA test was applied to the all data, least significant difference (LSD) multiple comparison tests were used to compare the means. All analyses were evaluated using SPSS v21.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Bacterial isolation

Two *Bacillus megaterium* isolates, HMA5 and BM1, with insecticidal activity were selected based on morphological physiological and biochemical characteristics.

3.2. Biochemical characteristics

The isolates, HMA5 and BM1, were motile, rod-shaped and gram-positive. On nutrient agar, the fresh colonies were 1-2 mm in irregular shaped, translucent whitish, diameter, slightly convex with entire edges and smooth after 24-48 h at 30 °C. Both isolates showed positive results for different tests such as catalase, hydrolysis of gelatin and starch, deamination of phenylalanine, acid production from sugars such as d-glucose, l-arabinose and d-mannitol. They were negative result for voges-proskauer test. They were approved to be members of the genus *Bacillus* by means of the ability to form spores, gram staining and colony morphology. On the basis biochemical diagnostic tests, these isolates were identified as *Bacillus megaterium.*

3.3. 16S rRNA gene sequencing

The *16S rRNA* sequences of these strains analyzed in the research were provided from public database (GenBank). The generated nucleotide sequences of HMA5 and BM1 were deposited in the GenBank database with accession numbers are KY231928 and KY231929, respectively.

The *16S rRNA* gene sequences of HMA5 and BM1 shared 100% sequence similarity with representative *Bacillus megaterium* strains and the isolate 27.1a had 99% sequence similarity with *Bacillus thuringiensis* strains in the Genbank database at the nucleotid level.

3.4. Bioassay for the pupae

The total number of adult emerged from pupae were recorded daily for each treatment and compared with untreated control groups. The bioassay results indicated HMA5 and BM1 were highly toxic against adult emergence from the pupae. The percentages of adult emergence were in the range of 1-99.5%, where they were 84.5±1.71%, 2.0±0.77% and 1.0±0.41% for the isolates 27.1a, BM1 and HMA5, respectively. *Bacillus megaterium* isolates, HMA5 and BM1 treated pupae showed significantly reduced ability to adult emergence rates. The mean adult emergence decreased from 99.5±1.23% in the control to 1±0.41% and 2±0.77% for bacterial suspension treated with HMA5 and BM1 isolates, respectively. The mean adult emergence for the treated pupae with 27.1a also decreased, but this decrease was less than for the bacterial suspension treated with HMA5 and BM1. In contrast, the results showed a significant difference between the control and all of the samples (P<0.05) (Figure 1).

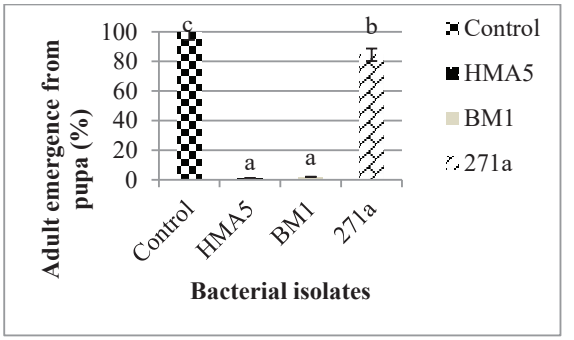

Figure 1- Effect of *Bacillus megaterium* isolates; HMA5, BM1 and *Bacillus thuringiensis* isolate 27.1a on adult emergence from the pupae (Different letters above the bars showed significiancy, P<0.05 by LSD test)

Bacillus megaterium isolates, HMA5 and BM1, induced embryonic mortality and morphological abnormality in the adult emergence of the treated pupae. There was no morphological change in the adult emergence of control group, while HMA5 and BM1 isolates caused different wings defects and hair loss on thorax and abdomen of the adults (Figure 2). In addition, the isolates inhibited almost all adult moths from the pupae. Only few adult moths emerged from the pupae with abnormal morphological deformations (Figure 3).

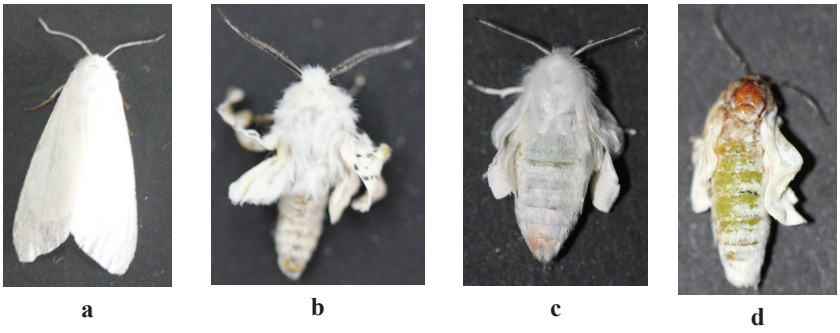

Figure 2- Morphological abnormality in the adult emerged from treated pupae with *Bacillus megaterium* isolate, HMA5. The pupae sprayed with: a, sterile water (control), healthy adult emergence; b, HMA5 isolate, adult emergence with short twisted wings; c, adult emergence with short wings and swelled abdomen; d, adult emergence with short frizzled wings and partial hair loss on thorax and abdomen; e, adult emergence with short wings and completely hair loss on thorax and abdomen; f, adult emergence with cabbage leaf form wings and completely hair loss on thorax and abdomen; g, and adult emergence with fringed wings and completely hair loss on thorax and abdomen

Figure 3- Morphological abnormality in the adult emergence of treated pupae with *Bacillus megaterium* isolate, HMA5. The pupae sprayed with: a, sterile water (control), healthy pupa; b, no adult emergence, diseased pupa; c, partial pupa cracking; d, and semi pupa opening

3.5. Bioassay for the 2nd instar larvae

Bacillus megaterium and *Bacillus thuringiensis* isolates were significantly attack the mortality of 2nd instar larvae of *Hyphantria cunea*. Four days after treatment, the 2nd instar larval mortality was 62.25±1.46%, 59.25±3.28%, 57.0±2.22% and 0.75±0.12% for the isolates HMA5, BM1 and 27.1a and control, respectively. However, the percent mortalities caused by the *Bacillus megaterium* isolates (HMA5 and BM1) did not show statistically significant differences (P<0.05). The percentage of mortality was highest for HMA5 treatment, and lowest for 27.1a treatment (Figure 4).

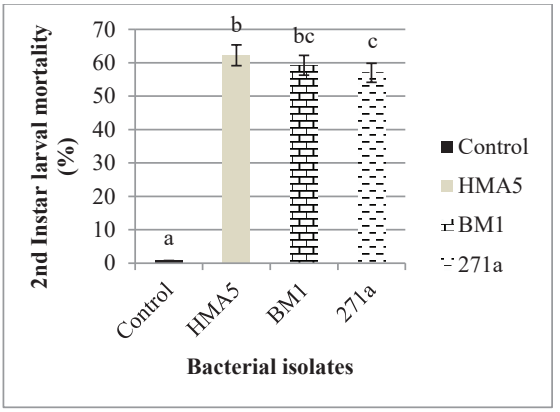

Figure 4- Mortality effect of *Bacillus megaterium* isolates; HMA5, BM1 and *Bacillus thuringiensis* isolate 27.1a on the 2nd instar larvae of *Hyphantria cunea* (Within columns followed by same letters indicated no significiancy among them (LSD test P<0.05)

The isolates, HMA5 and BM1, caused mortality with dry and blacken body of the larvae, while the isolate 27.1a caused mortality with blacken body and softness abdomen of the larvae (Figure 5).

Bacillus species including *Bacillus thuringiensis, Bacillus megaterium* and *Lysinibacillus sphaericus* have been successfully used as biological agents for controlling undesirable organims. These strains exhibit toxicity against caterpillars, beetles, and flies due to their ability to create chemically poison during sporulation (Wagner et al 1996; Berry

a b c d

Figure 5- Mortality symptoms of *Bacillus megaterium* isolates; HMA5, BM1 and *Bacillus thuringiensis* isolate 27.1a on the 2nd instar larvae of *Hyphantria cunea*: a, control; b, HMA5; c, BM and d, 27.1a

2012). Various researches have been studied on the nematicidal and insecticidal effects of *Bacillus megaterium* . Khyami-Horani et al (1999) reported that populations of *Bacillus megaterium* represents one of the best biological agent and highly toxic to the 4th instar larvae of *Culiseta longiareolata*. Aksoy & Ozman-Sullivan (2008) showed that the isolates of *Bacillus megaterium* were successfully used for *Aphis pomi* within five days of the treatments, causing 92% to 100% mortality. For nematodes, *Bacillus megaterium* has also been applied as bioagents. For reducing J2 penetration of sugar beet (*Beta vulgaris* L.), several bacterial isolates were successfully used against the sugar beet cyst nematode, *Heterodera schachtii* (Schmidt) (Neipp & Becker 1999). In addition, to reduce J2 penetration of potatoes, *Bacillus megaterium* has been effectively applied as a biological control agent against the Columbia Root-knot nematode, *Meloidogyne chitwoodi* and the root-lesion nematode, *Pratylenchus penetrans* (Al-Rehiayani et al 1999).

In this study, the two isolates, named HMA5 and BM1 were identified based on biochemical characterization tests following Logan & Vos (2009). HMA5 and BM1 were closely related to *Bacillus megaterium* and *Bacillus thuringiensis*. *Bacillus* species have similarities and some of them

only differ in biochemical test so the determination of the species level is very difficult. However, *Bacillus megaterium* and *Bacillus thuringiensis* can be distinguished by Voges-Proskauer test; acid production from d-glucose; deamination of phenylalanine and hydrolysis of casein. *Bacillus megaterium* can produce acid from d-glucose and hydrolyse casein while not for *Bacillus thuringiensis* (Sneath 1986; Slepecky & Hemphill 2006; Logan & Vos 2009). Analysis of the *16S rRNA* gene confirmed that isolates HMA5 and BM1 were *Bacillus megaterium*, with 100% sequence similarity with the available *Bacillus megaterium* strains in Genbank database.

4. Conclusions

Bacillus species have been used as biological pesticides. These isolated species from diverse regions around the biosphere have been determined as several genotypes (Ben-Dov et al 1997; Xu et al 2006). Although the isolates, HMA5 and BM1 were not fully effective on larvae of *Hyphantria cunea*, the results showed that the *Bacillus megaterium* isolates caused different abnormal wings shapes and loss of hair on thorax and abdomen (Figure 2). In addition, these isolates highly inhibited adult emergence from the pupae. On the other hand, *Bacillus thuringiensis* isolate, 27.1a caused no abnormal morphological deformations on the pupae (Figure 4). The reason may be effective secondary metabolite(s) is/are secreted from the vegetative cells of HMA5 and BM1. Abnormal morphological deformations may occur due to the diffusion effects of these metabolites into the chitin layer. It is hypothesized that these metabolites disrupt the structure of the regulator proteins of the wings and hair on thorax and abdomen. This present study represents the first report of morphological changes on the pupae of *Hyphantria cunea.* Further studies are needed to clarify the active secondary metabolites affecting the healthy adult emergence from pupae to be use in broad spectrum for controlling insects and also to find out the mode of action of these secondary metabolites.

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Polymorphisms of *TLR1*, *TLR4* and *SLC11A1* Genes in Some Cattle Breeds Reared in Turkey

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ABSTRACT

The objective of this study was to examine the allelic and genotypic profiles of four SNPs of bovine Toll-Like receptor 1 (TLR1), Toll-Like Receptor 4 (TLR4) and Solute Linkete Carier 11A1 (SLC11A1) genes in Holstein, two Turkish native breeds and their crossbreds in Turkey. For this purpose, a total of 1023 cattle from Holstein (HL, n= 410), Anatolian Black (AB, n= 106), East Anatolian Red (EAR, n= 84), Anatolian Black Crossbreed (ABC, n= 124) and East Anatolian Red crossbreed (EARC, n= 299) breeds were examined for four SNPs. Samples were genotyped using by the PCR-RFLP method. According to the TLR1 (+1380) SNP, EAR was in Hardy-Weinberg equilibrium (HWE) while the AB, HL and native crossbreeds were deviated from HWE. All cattle breeds were in HWE for the TLR1 (+1596) SNP. In terms of TLR4 (+10) SNP, the CC genotype had the highest frequency in HL and native crossbreeds whereas the AB and EAR breeds were monomorphic and only CC genotype was found these breeds. According to the SLC11A1 (+1066) SNP, the CC genotype had the highest while the GG genotype frequency the lowest in all breeds. In addition EAR breed was in HWE while the AB, HL and native crossbreeds deviated from HWE.

Keywords: Polymorphism; Turkish native cattle; Toll-Like Receptor; *SLC11A1*; Marker-assisted selection

1. Introduction

The immunity is divided into two namely the innate and adaptive immunity, in mammals. Innate immunity is present both in vertebrates and invertebrates, whereas adaptive immunity is only present in vertebrates (Takeda & Akira 2001). Host genetic resistance is mainly sustained by innate immunity, providing protection against pathogens without being vaccinated or exposed to diseases (Prakash et al 2014). Deciphering host genotypes for disease resistance can help us to control livestock diseases and develop strategies for decreased economic losses in farm animal breeding.

TLR1 is associated with immune responses against many bacterial pathogens. It includes a TLR subfamily which creates heterodimers with *TLR2*. The resulting TLR1/TLR2 complex recognises different bacterial cell wall ingredients such as lipoproteins and lipopolysaccharides, thus mediating a natural immune response against Gram-positive and Gram-negative bacteria species (Buwitt-Beckmann et al 2006; Russell et al 2012). In this way,

TLR1/TLR2 heterodimers and also *TLR4* recognise mycobacterium Pathogen-Associated Molecular Patterns (PAMPs), then macrophages and dendritic cells are activated for immune response (Brightbill et al 1999; Chang et al 2006; Hawn et al 2007). It was reported that some mutations in the *TLR1* and *TLR4* genes decrease immune response against lipopeptide and lipopolysaccharide bacterial cell wall components (Hawn et al 2007). *TLR4* plays on important role in immune response against both Gram negative and positive bacteria (Underhill et al 1999). Additionally, the *TLR4* gene was reported to be a strong candidate gene for disease resistancy, such as against mastitis (Ogorevc et al 2009), paratuberculosis (Mucha et al 2009), and brucellosis (Prakash et al 2014).

Solute Linkete Carier 11A1 (*SLC11A1*) is a transmembrane protein and was reported to be one of the best known potential candidate genes that promote innate immunity against different intracellular pathogens (Kumar et al 2011). Therefore, the *SCL11A1* gene variants may be used to nature resistance or susceptibility to some important infections such as tuberculosis in water buffalo (Le Roex et al 2013) and brucellosis in cattle (Kumar et al 2011; Prakash et al 2014).

The present study was aimed at determining the allele and genotype frequency of four SNPs of bovine *TLR1, TLR4* and *SLC11A1* genes in Holstein, Anatolian Black (AB), East Anatolian Red (EAR) cattle breeds and their crossbreeds in Turkey.

2. Material and Methods

A total of 1023 cattle made up of the Holstein (n= 410, from Kayseri, Kahramanmaraş, Balıkesir, Burdur, İzmir and Çanakkale), AB (n= 106, from Kayseri, Sivas, Ankara, Çankırı, Niğde and Yozgat), EAR (n= 84, from Erzurum, Kars and Ardahan), Anatolian Black crossbreed (ABC, n= 124 from Kayseri, Sivas, Çorum, Niğde and Yozgat) and East Anatolian Red crossbreed (EARC, n= 299, from Erzurum, Kars, Ardahan, Kayseri, Sivas, Çorum, Niğde and Yozgat) cattle were examined for four SNPs in the *TLR1* (*+1380, +1596*), *TLR4* (*+10*) and *SLC11A1* (*+1066*) genes. Genomic DNA was isolated from whole blood samples using the phenol-chloroform method (Sambrook et al 1989). Genotyping of *TLR1*, *TLR4*, *TLR9* and *SLC11A1* gene polymorphisms was performed by PCR-RFLP. Detailed information about the primers, amplification product lengths and enzymes are used shown in Table 1.

The PCR for all SNPs was performed in 20 µL reaction mixture, which included 1.5 mM $MgCl_2$, 200 µM dNTPs, 200 µM primer, 1×PCR buffer, 1U Taq polymerase and 50-10 ng genomic DNA. The thermal cycling condition consisted of pre-denaturation (94 °C for 5 min) followed by 35 cycles of 30 s at 94 °C, 30 s at the annealing temperature (Table 1), 30 s at 72 °C, and a final extension of 1 min at 72 °C. For RFLP, each of the PCR products was digested with the appropriate enzyme (Table 1)

Table 1- Primer sequences, amplification conditions, product sizes and restriction enzymes

SNP	*Primer sequence*	*AT (°C)*	*PS (bp)*	*RE*	*RE Heat, inactivation temparatrures and times*	*Reference*
TLR1 (+1380)	F: 5'-TTTAGCAGCCTTTCCATACT-3' R: 5'-TCTACCACGTCACTGGATACT-3'	55	179	*Bs1*I	Activation: 55 °C for 4 hours Inactivation: 80 °C for 20 minutes	Prakash et al 2014
TLR1 (+1596)	F: 5'-TTTAGCAGCCTTTCCATACT-3' R: 5'-CAGATCCAGGTAGATACAGAG-3'	64	354	*Bcl*I	Activation: 55 °C for 4 hours Inactivation: 80 °C for 20 minutes	Sun et al 2012
TLR4 (+10)	5'-CGTAACCCAGCACTGCTTTG-3' R: 5'-GCCTGTTAATGCCCTGTAACC-3	59.2	405	*BstU*I	Activation: 37 °C for 4 hours Inactivation: 65 °C for 20 minutes	Prakash et al 2014
SLC11A1 (+1066)	F: 5'-ATCTCCTTCCTACTGCCCG-3' R: 5'-CACAAACTGTCCCGCGTAG-3'	54	374	*Pst*I	Activation: 37 °C for 15 minutes Inactivation: 80 °C for 20 minutes	Prakash et al 2014

AT, annealing temperature; PS, product size; RE, restriction enzyme

in a reaction including 3 µL of the PCR product, 5 U of the restriction enzyme (MBI Fermentas) and 1 µL of the buffer. The reaction was incubated at the appropriate temperature for each enzyme (Table 1) for 4 h. Genotyping for each SNP was performed by 3% agarose gel electrophoresis.

The genotype and allele frequencies of the examined SNPs in each breed were calculated. The Hardy-Weinberg equilibriums (HWE) of the examined breeds for five SNPs in the *TLR1*, *TLR4* and *SLC11A1* genes were analysed using the Chi-square test. All statistical analyses were made by using FSTAT v.2.9.3.2 software.

3. Results and Discussion

After digestion with the *Bs1*I restriction enzyme digestion, the AA (179 bp), AG (179, 93 and 86 bp) and GG (93 and 86 bp) genotypes were observed for the *TLR1* (*+1380*) SNP (Figure 1). The 93 and 86 bp bands were not separated because of their close proximity to each other. However, genotypes could be detected by observing one or two fragments of 179 and around 100 bp. The AG genotype had the highest frequency while the AA genotype frequency had the lowest in all examined breeds. According to the *TLR1* (*+1380*) SNP, EAR breed was in HWE while the AB, HL and native crossbreeds deviated from HWE (Table 2).

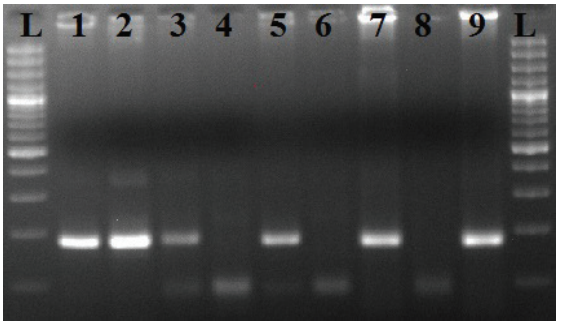

Figure 1- Picture of restriction fragments of *TLR1* (*+1380*) locus after digestion with *Bs1*I restrictiom enzyme Lane L, markers (100 bp); lanes 1, 2, 7 and 9, AA genotypes; lanes 3 and 5, AG genotypes; lanes 4, 6 and 8, GG genotypes

As a result of restriction enzyme digestion, three genotypes for the *TLR1* (*+1596*) polymorphism were detected: 261, 72 and 21 bp for the GG genotype; 333, 261, 72 and 21 bp for the GH genotype; 333 and 21 bp for the HH genotype (Figure 2). The GH genotype had the highest frequency in all breeds followed by HH and GG, respectively, in all examined breeds except for EAR in which HH was found as the most frequent genotype compared to GH. All cattle breeds were in HWE for this SNP (Table 2).

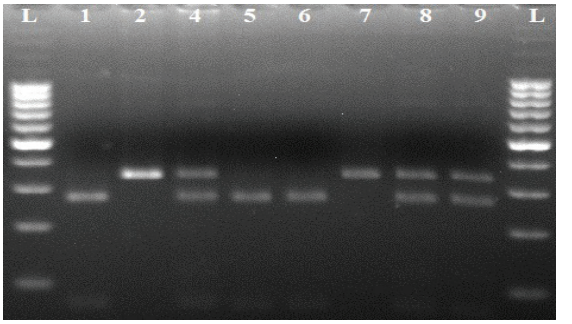

Figure 2- Picture of restriction fragments of *TLR1* (*+1596*) locus after digestion with *Bcl*I restriction enzyme Lane L, markers (100 bp); lanes 1, 5 and 6, GG genotypes; lanes 2 and 7, HH genotypes; lanes 4, 8 and 9, GH genotypes

Following digestion with *BstU*I enzyme for the *TLR4* (*+10*) SNP, in the CC genotype two bands with a length of 246 and 159 bp, and in the CT genotype three bands with a length of 405, 246 and 159 bp were observed (Figure 3). However, the TT genotype was not observed (Figure 3). The genotype CC had the highest frequency in HL and native crossbreeds whereas the monomorphic genotype was found in AB and EAR breeds (Table 2).

The polymorphism in the *SLC11A1* (*+1066*) mutation was identified by digestion of the PCR product with *Pst*I enzyme. In this study, three genotypes were obtained for the *SLC11A1* (*+1066*) SNP (CC, CG and GG) in the examined cattle breeds. After digestion with PstI, one fragment was observed for the CC genotype (348 and 95 bp), two fragments were observed for the GG genotype (293 and 81 bp) and three fragments were found expected

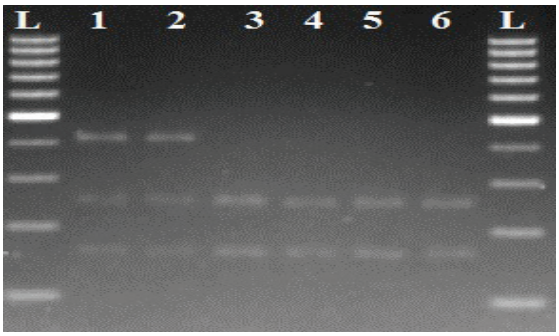

Figure 3- Picture of restriction fragments of *TLR4* (*+10*) locus after digestion with *BstU*I restriction enzyme Lane L, markers (100 bp); lanes 1 and 2, CT genotypes; lanes 3, 4, 5 and 6, CC genotypes

for the AB genotype (374, 293 and 81 bp) (Figure 4). The CC genotype had the highest frequency while the GG genotype frequency was the lowest in all breeds. Turkish native cattle breeds were in HWE while HL and crossbreeds deviated from HWE (Table 2).

The observed heterozygosity varied from 0.284 to 0.568 for *TLR1 +1380*, from 0.401 to 0.502 for *TLR1 +1596*, from 0.000 to 0.018 for *TLR4 +10*, and from to 0.168 to 0.370 for the *SLC11A1 +1066* polymorphism in the five examined cattle populations. The expected heterozygosity, the observed heterozygosity, the allele and genotype

Table 2- Heterozygosity, allele and genotype frequencies of examined loci in cattle breed reared in Turkey

SNP	*Breed*	*n*	*Allele frequency*		*Genotype frequency*			*Heterozygosity*		X^2 *(df= 1)*
			A	*G*	*AA*	*AG*	*GG*	H_E	H_O	
TLR1 (*+1380*)	HL	410	0.270	0.730	0.003	0.539	0.458	0.395	0.537	53.500*
	AB	106	0.190	0.810	0.000	0.387	0.613	0.303	0.371	6.090*
	ABC	124	0.270	0.730	0.008	0.524	0.468	0.395	0.524	13.440*
	EAR	84	0.150	0.850	0.000	0.298	0.702	0.245	0.284	2.570 NS
	EARC	299	0.290	0.710	0.003	0.569	0.428	0.410	0.568	44.880*
			G	H	GG	GH	HH			
TLR1 (*+1596*)	HL	410	0.400	0.600	0.151	0.502	0.347	0.481	0.502	0.730 NS
	AB	106	0.440	0.560	0.217	0.453	0.330	0.493	0.474	0.720 NS
	ABC	124	0.420	0.580	0.210	0.427	0.363	0.490	0.427	1.930 NS
	EAR	84	0.310	0.690	0.095	0.429	0.476	0.429	0.419	0.000 NS
	EARC	299	0.460	0.540	0.258	0.401	0.341	0.497	0.401	10.980*
			C	T	CC	CT	TT			
TLR4 (*+10*)	HL	410	0.998	0.002	0.995	0.005	0.000	0.002	0.004	0.000
	AB	106	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
	ABC	124	0.996	0.002	0.992	0.008	0.000	0.004	0.019	0.000
	EAR	84	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
	EARC	299	0.990	0.010	0.980	0.020	0.000	0.010	0.008	0.030 NS
			C	G	CC	CG	GG			
SLC11A1 (+1066)	HL	410	0.850	0.150	0.763	0.171	0.066	0.254	0.168	45.990*
	AB	106	0.850	0.150	0.755	0.189	0.056	0.262	0.206	7.380*
	ABC	124	0.800	0.200	0.702	0.194	0.104	0.323	0.193	19.720*
	EAR	84	0.760	0.240	0.570	0.370	0.060	0.374	0.370	0.000 NS
	EARC	299	0.780	0.220	0.679	0.201	0.120	0.344	0.200	51.920*

H_E, expected heterozygosity; H_O, observed heterozygosity; X^2, Chi-square; HL, Holstein; AB, Anatolian Black; EAR, East Anatolian Red; ABC, Anatolian Black Crossbreed; EARC, East Anatolian Red Crossbreed; df, freedom degree; *, statistical significance 0.05; NS, Non significant

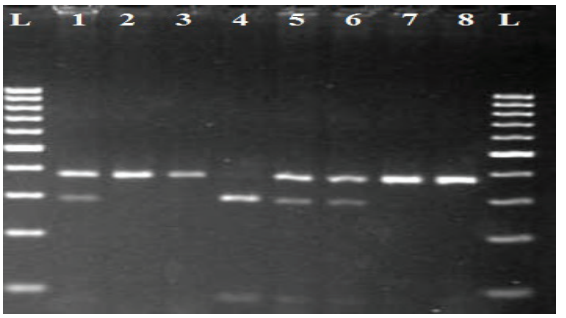

Figure 4- Picture of restriction fragments of *SLC11A1* (+*1066*) locus after digestion with *Pst*I restriction enzyme Lane L, markers (100 bp); lanes 1, 5 and 6, CG genotypes; lanes 2, 3, 7 and 8, CC genotypes; lane 4, GG genotype

frequencies for four polymorphism of the HL, AB, ABC, EAR, EARC populations are shown in Table 2.

Genomic and phenotypic selection for increasing milk yield have resulted in a decrease in the health traits of high yielding cattle breeds such as Holstein (Egger-Danner et al 2015). However, increasing milk production is no longer as important trait as it used to be, compared to health and longevity. In today's dairy cattle breeding, health and longevity are more important for selection than milk production (Egger-Danner et al 2015). The main goal of dairy cattle farming is to increase income by obtaining higher milk and meat yield and to lower the costs caused by lower fertility, high disease and culling rates (Strapáková et al 2016).

It has been shown that TLRs are associated with innate immune response in various livestock species (Le Roex et al 2013; Prakash et al 2014). Therefore, interest in breeding animals which are resistant to major infectious diseases by using TLRs genes has increased tremendously in recent years (Novák 2014).

The *TLR1* (*+1380*) SNP was firstly reported by Prakash et al (2014) in Indian native cattle breeds and their crossbreeds and they observed three genotypes for the *TLR1* (*+1380*) locus. The AG genotype frequency was higher than other genotypes, and AA genotype frequency was found to be lower in Indian native cattle (Prakash et al 2014). In a study conducted in Turkey, five different cattle breeds had been genotyped with this SNP. According to this study, although the genotype GG was the most common genotype in all breeds genotyped, the AA genotype was not found among examined animals. (Çınar et al 2016). Similarly, in this study AA genotype was not found in AB and EAR breeds. The AA genotype was the lowest in Holstein and crossbreeds in our examined population (Table 2). However, in Turkish native cattle breeds (AB and EAR) no AA genotype was observed (Table 2). In the present study the G allele was the most frequently found allele in all the examined breeds and the GG genotype was found to be higher in the investigated Turkish native cattle breeds (Table 2). In Holstein and crossbreeds, the frequencies of the genotype AG were found to be slightly higher compared to the GG genotype (Table 2). The *TLR1* (*+1596*) SNP was firstly reported in the Holstein breed (Sun et al 2012). The authors observed three genotypes (GG, GH and HH) and the frequency of the GH genotype was found to the highest in the Holstein breed (Sun et al 2012). Similarly, we found three genotypes in all examined breeds, and the GH genotype frequency was the highest in Holstein, AB and crossbreeds, whereas the HH genotype was found to be highest in the EAR breed (Table 2). The *TLR4 (+10)* SNP was monomorphic in Turkish native cattle breeds; additionally, this SNP was almost monomorphic in Holsteins and crossbreeds (Table 2). Similarly, Bilgen et al (2016) reported low variation in the AB, EAR and Holstein breeds in terms of *TLR4* SNP with. However, Prakash et al (2014) observed three genotypes (CC, CT and TT) for *TLR4* (*+10*) in Indian native cattle breeds. The *SLC11A1* (*+1066*) SNP was polymorphic in all examined breeds in the present study (Table 2). The frequency of the CC genotype was found to be the highest in the investigated animal populations. Prakash et al (2014) also observed three genotypes; however, in contrast to our study, the CC genotype was the least common in Indian native cattle.

Three of the SNPs genotyped [*TLR1* (*+1380*), *TLR4* (*+10*) and *SLC11A1* (*+1066*)] in this study were taken from a study that investigates *Bos indicus* cattle

derived by Prakash et al (2014). There they reported that samples, they examined were polymorphic and found in HWE. In contrast, in our study, *Bos taurus* origin cattle were used and in terms of these SNPs, deviation from HWE was observed in all breeds except EAR (Table 2). It is thought that this may have been due to the origins of examined cattle breeds (Lin et al 2010). In the light of those findings, it was observed that examined all breeds were in HWEs in terms of *TLR1* (*+1596*) SNP. HWE was not observed just in breed of EARC (Table 2).

This study is the first to investigate the genotype and allele frequencies of *TLR1* (*+1380, +1596*), *TLR4* (*+10*) and *SLC11A1* (*+1066*) SNPs in cattle breeds reared in Turkey. The present study indicated that among the investigated SNPs, heterozygosity was detected as <0.5, which shows low variation for these SNPs (Table 2). Prakash et al (2014) reported an association between the *TLR1* (*+1380*) AA, *TLR4* (*+10*) TT and *SLC11A1* (*+1066*) CC genotypes and resistance to bovine brucellosis. In addition, Sun et al (2012) found an association between the *TLR1* (*+1596*) GG genotype and resistance to bovine tuberculosis. According to the findings of Prakash et al (2014) and Sun et al (2012), we hypothesise that our investigated populations in Turkey had a greater relative risk of incidence to bovine brucellosis and tuberculosis. This is because, the genotypes given by Prakash et al (2014) as indicating susceptibility to bovine brucellosis and tuberculosis were found to have a higher frequency in our examined populations, except for *SLC11A1* (*+1066*).

Prakash et al (2014) reported that the SNPs used in this study might be used in genomic selection against bovine brucellosis and tuberculosis. In conclusion, increasing the frequencies of the *TLR1 (+1380-A*, *+1596-G*) and *TLR4* (*+10-T*) alleles may help to control bovine brucellosis and tuberculosis infections in Turkey. Further studies which study the association between reported SNPs and bovine brucellosis and tuberculosis infections in Turkey are needed.

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