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A GIS Based Land Suitability and Gross Value Evaluation of Mined Lands in Şanlıurfa District

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ABSTRACT
Landmined areas are located along the borders of Şanlıurfa province between Turkey and Syria with 220 km length and 400 m width. Governmental institutions attempted to determine the potential and suitability of mined lands for any agricultural activities before removing active mines. The research was conducted to estimate land suitability and gross value for irrigated agricultural production (GVIAP) of the mined areas using the database for the surrounding land and data obtained by Geographic Information Systems (GIS) techniques. The study was conducted in three steps; data including soil characteristics, digital elevation model (DEM) and orthophoto images of lands located 2 km nearby the mined land along the Turkish border were collected. The data containing DEM and orthophoto images of mined and adjacent lands were integrated in the second step. Finally, a field survey was conducted along the border district and soil samples were collected for each soil boundary nearby the mined lands. The integration of data in GIS allowed to expand the soil boundaries from adjacent lands into the mined lands. Data analysis showed that the total mined land is 6706 hectares of which 90.2% is highly and moderately suitable, 6.9% is less suitable and 2.9% is not suitable for irrigated agriculture. The gross value of irrigated agricultural production was estimated as 2212 US Dollars per hectare. The results revealed that the integration of inaccessible land database with the remote sensing data and GIS can be used to estimate the gross value which is derived from soil characteristics of the mined lands.

Keywords: Gross value added, Adjacent data, Mined lands, Land evaluation, Syria border, Landmined areas

1. Introduction

Conservation and appropriate use of available land resources have gained much attention and became crucial because of global warming and land degradation. The decrease in the agricultural lands due to the pressure of increasing population and degraded soil quality threatens future food production and safety.

The conversion of primary farmlands to urban sprawl and other uses has increasingly become a local, regional, and national concern in Turkey (Kılıç 2011; Bilgili et al. 2018). Turkey is highly vulnerable to drought and desertification due to the various climate, soil characteristics, and topographical structures (Camcı Çetin et al. 2007; OSB 2016). Lands and land uses have a close relationship, therefore, inappropriate and unplanned land management cause irretrievable damage to the lands which eventually turn into useless for agricultural activities (FAO 1977). Land suitability assessment is a prerequisite step of land use planning that is the fitness of a region for different land use types (FAO 1976; FAO 2007), which assists land users in decision-making regarding the level of land suitability (Pramanik 2016; Yalew et al. 2016). Land suitability assessment is often used to manage land resources properly and determine the most appropriate land use type for a particular area (Bodaghabadi et al. 2015). Knowledge on the land suitability is an important tool in planning sustainable land management and integrating the information with crop and soil requirements. Land suitability maps for specific purposes (Gallo et al. 2014) can be obtained by the use of integrating spatial data in GIS (Zhang et al. 2007).

Landmines have been placed between Turkey and Syria borders in 1957 for security and preventing smuggling purposes. Therefore, these lands became inaccessible and closed to all activities. Recently, the need for determination agricultural potential of the mined lands in the Şanlıurfa district has arisen in order for providing data for future planning of agricultural uses. Thus, the objective of this study is to determine the land suitability classes and to estimate gross value of irrigated agricultural production of landmined area by the available data of adjacent lands and mined areas using the GIS techniques.

2. Material and Methods

2.1. Description of the study area

The research was conducted at the mined border belt of Şanlıurfa province located along the 220 km southern border between Turkey and Syria. The width of the mined area is approximately 400 m wide, comprising a total of 6706 ha (Figure 1).

Figure 1- Location of the study area

The dominant climate of the study area is Eastern Mediterranean with a strong continental influence characterized by dry summers. The evaporation reaches 2000 mm, the average temperature is 18.4 °C and the average annual precipitation is approximately 442 mm (DMI 2011). The soil moisture regime is Xeric and the temperature regime is mesic (Soil Survey Staff 2010). The dominant soils within the region are classified as Leptosols, Vertisols, Cambisols, and Calcisols (Çullu et al. 2018) according to IUSS Working Group WRB (2015). The soils are high in pH levels and moderate to high in $CaCO_3$ content. The cation exchange capacity (CEC) is high, organic matter content is low and most soils are clayey in texture (Çullu et al. 2010). Geology of the study area comprises mainly clayey deposits, limestone, sandy marl, shallow marine deposits and the mudflow materials transported during the Pleistocene climatic fluctuation from the surrounding mountains (Kapur et al. 1991).

2.2. Method

2.2.1. Data collection and GIS analysis

The available database was compiled for an area of 2 km wide strip along the border belt, beginning from Turkish territory extending to the mined areas to determine the land suitability classes and to estimate gross value of irrigated agricultural production (GVIAP) of the mined area. The database of mined belt along the borderline, within Turkey, including the former soil map, digital elevation model (DEM) and orthophoto images were integrated into GIS. Soil boundaries, attribute soil data of the adjacent lands of the mined area (Dinç et al. 1988; KHGM 1995) and elevation contours of standard topographic maps were digitized using ArcGIS software (ESRI Inc 2008). A DEM was created by the interpolation of 5 to 50-m interval contour lines digitized from the topographic maps. A slope gradient map was derived from the DEM using standard filtering techniques and classified into five categories. Vector and ancillary data about soil and topography maps were loaded into the GIS by screen digitization and keyboard entry. High resolution-orthophoto image interpretations (1:10.000 scaled) were also used as the reference data for visual interpretation of the soil boundary distributions continuity through the mined land. The study was conducted in three steps;

Database of lands within a 2 km distance of the mined belt within Turkey, including soil polygons and attributes (depth, texture, salinity, organic matter, pH and lime content), slope map and orthophoto images interpretation were firstly generated. A file containing slope map and orthophoto image of mined and adjacent lands were compiled and integrated into GIS in the second step. Finally, a soil survey was carried out by using the analysis of the database along the border to determine the continuity of soil boundary through the mined lands. Soil samples were collected and analysed (texture, salinity, organic matter and pH content averages given in the Table 1) from each soil polygons. The flow chart of the study is shown in Figure 2.

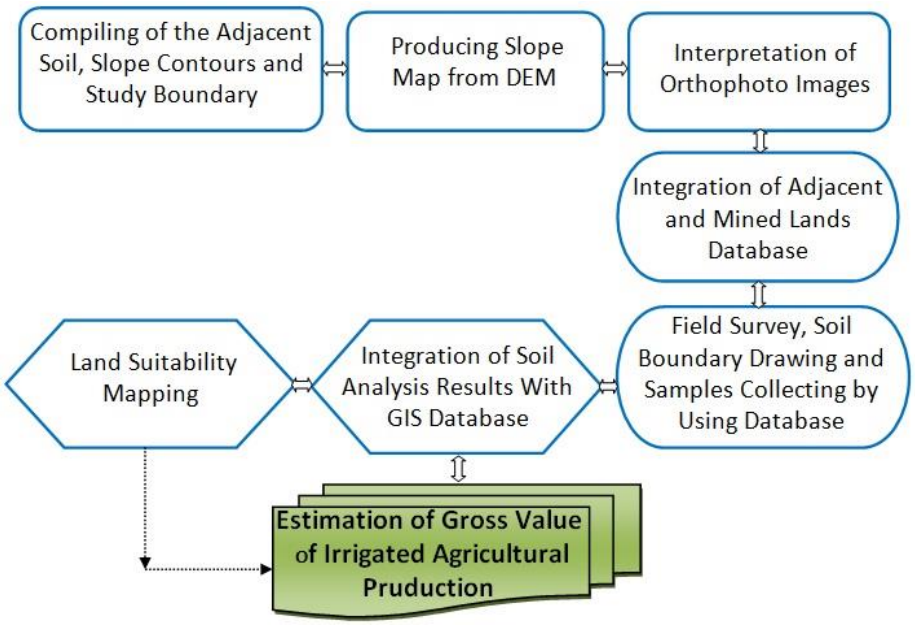

Figure 2- Flow chart used in the study

The soil analysis data, observations in the field survey and the land capability class (LCC) map (1:25.000 scaled) were integrated into the GIS. The LCC map was prepared based on inherent soil characteristics, land features, and environmental factors.

The land suitability map of the study area was prepared in GIS environment using the land database and the results of soil analysis according to the guidelines of FAO (FAO 1976). Soil capability classes, soil depth, slope levels, and soil analysis results of 68 soil samples (the lowest and the highest values) were the main parameters used to determine soil suitability (Table 1).

Table 1- Land suitability classes and their characteristics

Soil Depth (cm)	*Slope Classes (%)*	*OM (%)*	*pH (Sat. Ext.)*	*EC (dS m^{-1})*	*Texture Classes*	*Land Capability Classes*	*Suitability Classes*
> 90	0-3	1.6-2.6	7.94-7.97	0.42-0.6	C-CL	I-II	S1
30-90	3-6	1.3-1.9	7.93-7.97	0.28-0.3	CL	III-IV	S2
10-30	6-25	1.6-3.3	7.96-8.07	0.28-0.4	CL	VI-VII	S3
60-120	0-3	1.5-2.1	7.98-8.03	17.4-22.0	C-CL	V	N1
0-10	> 25	1.1-2.5	7.52-7.66	0.61-0.8	CL	VIII	N2

Land suitability classes are divided into order, class, subclass, and unit classes (FAO 1976). Land suitability order is further divided into suitable (S) and not suitable (N) as *S*1= highly suitable, *S*2= moderately suitable, *S*3= marginally suitable, *N*1= currently not suitable and *N*2= permanently not suitable.

2.2.2. Gross value ırrigated agricultural production (GVIAP)

Gross value of irrigated agricultural production (GVIAP) was estimated by using integrated land suitability map classes into the GIS database. The gross value of irrigated agricultural production of the study area was estimated by using the formula (1) given by ABS (2008):

$$GVIAP = Ai\text{X}\ \frac{\text{Q}}{\text{Ad}/_{Ydiff}+\text{Ai}}\text{XP} \quad (1)$$

Where; *Ai* is the area that will be used under irrigation (kg); *Ad* is the area that is not under irrigation (ha); *Ydiff* is the yield difference factor; i.e. estimated ratio of irrigated to non-irrigated yield, is the total quantity of yield production (t or kg) and *P* is the unit price of production ($ per t or kg)

Estimates of GVIAP values are based on production, commodity price, irrigation data for total area which were obtained from the provincial directorate of agriculture and areal researches (Aydoğdu et al. 2018; Parlakçı Doğan 2019). GVIAP was calculated for commonly produced (kg or tonnes) crops of cotton, second crop maize and wheat in the district.

3. Results and Discussion

3.1. Land suitability mapping

The land suitability map of the mined areas was prepared with the GIS database analysis compiling all the data set given in Table 1. Although, this data set consisted of seven different parameters (Table 1), the most effective ones in the GIS analysis performed for delineating agricultural suitability of the mined lands were soil LCC and slope layers which were presented on the map given in Figure 3. Using the GIS database and land survey along with the mined land provided the opportunity to draw the LCC map that is a base of suitability classification.

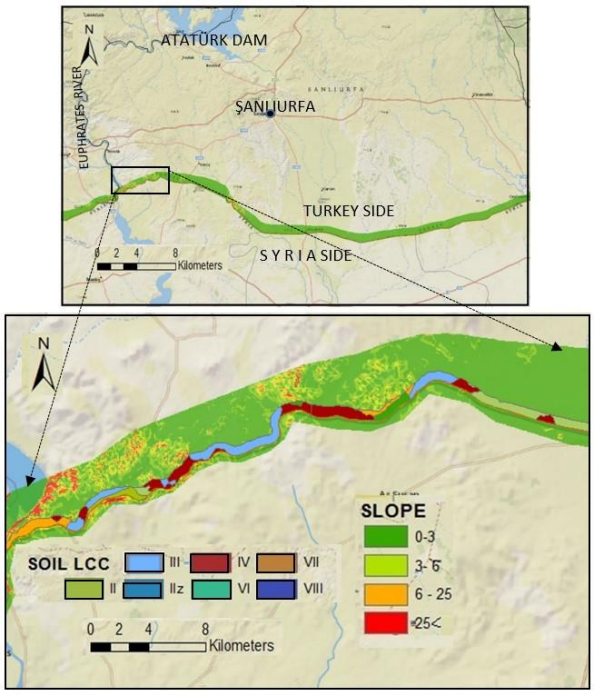

Figure 3- Land capability class and slope map from a selected area

The soil analyses results have been integrated into GIS database for the determination of limiting factors for land suitability classification. The land suitability classes derived from the database along the border are presented in Figure 4.

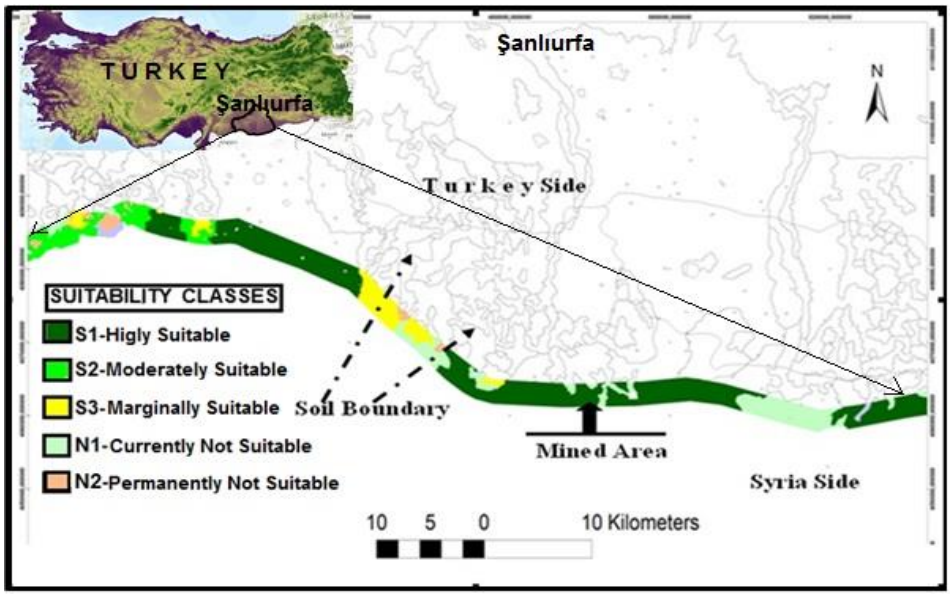

Figure 4- Suitability map of the mined area

The mined lands constituted a total of 6706 ha of which 40.3% is highly suitable (S1), 49.9% is moderately suitable (S2), 3.2% is marginally suitable (S3), 3.7% is currently not suitable (N1) and 2.9% is permanently not suitable (N2) (Table 2).

Limitations such as soil depth, excessive salinity, and high slope constrain the cultivation of several crops. However, the analysis revealed that a considerable part of the land (90.2%) is classified as S1 and S2 classes indicating highly and moderately suitable for agricultural activities. The information on land suitability is an essential prerequisite for land use planning, (Bandyopadhyay et al. 2009) and sustainable development goals related to food, health, water, and climate (Keesstra et al. 2018). The results indicated that a considerable part of lands within the mined areas are suitable for additional agricultural production. Since the lands were not cultivated for a long time, they can be more productive compared to continuously cultivated lands.

3.2. Land evaluation and GVIAP prediction

The mapped areas, which adjacent to water resources and dominated by highly and moderately suitable (S1 and S2) classes were identified as highly suitable to the production of cotton, wheat, barley, corn, soybean, sunflower, and vegetables. While marginally suitable (S3) lands with the shallow depth and high slope gradient were designated as suitable for pistachio, vineyard, olive and, fig orchards. The lands characterized by shallow rooting soil depth formed over limestone parent materials can be suitable for pistachio, vineyard, and olive fruit trees after crushing hard limestones. Crushing the limestone will create an environment to hold water and roots can better use of the available water in the dry summer periods. Therefore, these lands should not only be used for grazing, wildlife and forestry, but also for the other income-generating crops. Salt affected (saline-alkaline) areas, classified as currently not suitable (N1), may be used for salt tolerant-crops such as barley, cotton, and wheat after land reclamation. Other lands (N2), which are located on high slope gradient have shallow soil depth and identified as suitable for settlement, pasture, wildlife, and forestry.

The majority of lands (S1 and S2 suitability classes, 6052 ha) can be taken under irrigated agriculture following the removal of mines and can make a significant contribution to the regional economy.

The gross value of irrigated agricultural production for mined land was estimated by using the GVIAP formula and database of land suitability maps (Table 2).

Table 2- Distribution of the land suitability classes and GVIAPs Values

Land Suitability	*Total Area (ha)*	*Ratio (%)*	*GVIAP (USD)*
S1-Highly Suitable	2 702	40.3	5 976 824
S2-Moderately Suitable	3 350	49.9	7 410 200
S3-Marginally Suitable	209	3.2	462 308
N1-Currently Not Suitable	249	3.7	550 788
N2-Permanently Not Suitable	196	2.9	-
Total	6 706	100.0	14 400 120

The GVIAP results provide a broad range of data about the value of irrigated agricultural commodities. Using the data collected on agricultural production, GVIAP in terms of the gross value of agricultural commodities were simply produced based on irrigation conditions (ABS 2020). We estimated the potential GVIAP values considering common crops of the region that could potentially be produced in the mined lands after removing mine.

Agriculture has a significant impact on the economy of Turkey, thus rational and sustainable management of irrigation facilities are crucial to develop water and soil resources and increase their contribution to the economy. Soils of the region have been irrigated since 1995 and yield was increased up to three to four-fold following irrigation. After the irrigation within the framework of the Southeastern Anatolia Project (GAP) is an integrated regional development project, agricultural production value-added per hectare was determined as $182 (Unver 1997). Income hectare-based values were calculated as 1228.14 USD ha^{-1} for Şanlıurfa in 2018 (Aydoğdu et al. 2020). The GVIAP of the mined lands where located at the southern border of Şanlıurfa was estimated as 2212 USD per hectare and 14,4 million USD for total area/year, in the case of the S1 and S2 lands taken under irrigation.

The mined lands have not been used for agricultural activities since 1957 and are expected to have greater soil quality due to accumulation of soil organic carbon and extractable nitrogen contents (Ozturkmen & Kavdir 2012; Dengiz et al. 2020). Therefore, some parts of these lands can be used for organic agriculture which will increase the value-added compared to conventional agricultural production. Besides, mined lands classified as S3, N1 and N2 groups contributed to the protection of some endemic plants from extinction.

4. Conclusions

The growing population exerts pressure on food-producing lands which are continuously decreasing. Knowledge of the land suitability is the most important tool to develop sustainable land management and estimate regional gross value added.

Determination of land suitability and gross value of irrigated agricultural production considering the conversion of inaccessible lands into agriculture by employing the integrated GIS databases are the major outcomes of the study.

The results indicated the efficiency of GIS analysis in existing and adjacent data integration for mapping and delineating the suitability classes of inaccessible lands. The data derived from the suitability map provides considerable information for the sustainable and economical planning of the mined lands.

Knowing on the potential of lands and soils support the particular uses that have guided to the development of landscapes, accurate land use, industrial investment, and urbanization. Land use potential information supports policy development, planning, and on-ground decision making, and should be consulted when new uses of land are being considered.

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Biochemical Composition and Antioxidant Activity of Different Types of Tomatoes Affected by Ethylene Treatment

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ABSTRACT
The effect of ethylene on biochemical composition and antioxidant activity in beefsteak, heirloom and cluster type of tomatoes were determined. For that purpose, tomato fruit were harvested at breaker maturity stage and divided into two groups one of which was applied with 150 µL L^{-1} ethylene while another remained untreated. Ethylene treated and untreated control fruit were stored at 12 °C and 90±5% relative humidity for 35 days with subsamples removed every 7 days for quality analysis. After each removal time, fruit were kept at 20 °C for additional 3 days to determine shelf life performance. Ethylene treatment enhanced the breakdown of total chlorophyll and accumulation of lycopene and carotenoid contents. At the end of cold storage and shelf life period, the maximum antioxidant activity, carotenoid and flavonoid contents were recorded in ethylene treated heirloom type tomatoes. It can be concluded that ethylene treated heirloom type tomatoes exhibited maximal postharvest quality as compared to beefsteak and cluster type of tomato in term of biochemical composition and antioxidant activity after 35 days of cold storage and shelf life.

Keywords: Ethylene, Antioxidant, Cold storage, Shelf life, Tomato quality

1. Introduction

Tomatoes are vital part of human nutrition around the world. Scientific studies have shown that tomatoes contain high amount of carotenoid, antioxidant, lycopene and are associated with dietary intake that reduces the risk of chronic diseases, cancer, osteoporosis and cardiovascular diseases in humans (Rao et al. 1998; Frusciante et al. 2007; Bhowmik et al. 2012). The presence of carotenoids, especially lycopene, ascorbic acid, vitamin E, phenolic compounds and different antioxidant properties in tomatoes affect the human health (Frusciante et al. 2007; Bhowmik et al. 2012).

Ripening is genetically programmed process which show substantial changes in color, texture, flavor and aroma (Alexander & Grierson 2002). Tomatoes being a climacteric fruit are sensitive to ripening hormone ethylene. Exogenous application of ethylene in climacteric fruit can trigger and enhance the ripening process (Tucker 1993). It is thought that ethylene regulates the formation of carotenoids present in the chloroplast through synthesis of new enzymes and influence mitochondria based organic acid concentrations (Mcglasson 1970). Ethylene does not only affect biochemical composition but also increases respiration rate and aging of fruit and vegetables (Prasanna et al. 2007).

The higher antioxidant activity of tomato could mitigate the effect of ethylene as ripening process involved series of physiological and biochemical changes in which antioxidant properties play fundamental role (Jimenez et al. 2002). The signal transduction by ethylene in important for secondary metabolites synthase. The biosynthesis of flavanol is modulated by ethylene through transcription factor (Lewis et al. 2011). During stress the polyphenol oxidase (PPO) activity is increased through ethylene signalling which supports its involvement in the defence resistance of plant (Bosch et al. 2014). In tea, the phenolic compounds, flavonoids and antioxidant activity were increased by ethylene signalling (Ke et al. 2018).

The nutritional value, color and flavor of tomatoes are mainly dependent on the ratios of lycopene, β-carotene, ascorbic acid and sugars (Nguyen & Schwartz 1999). Epidemiological studies have shown that lycopene and β-carotene serve as an antioxidant and functional food (Tonucci et al. 1995). The assessment of the effects of different tomato varieties on the synthesis of carotenoid and other phenolic compounds are important to enhance the concentration of these antioxidant compounds. The studies conducted previously confirmed that amount of carotenoid and other antioxidant compounds in tomato fruit exhibit differences among genotypes (George et al. 2004). According to Viskelis et al. (2007) the Lithuanian cultivar 'Rutuliai' displayed the highest lycopene content (over 10 mg 100 g^{-1}) which was 1.6-fold more than hybrid 'Admiro' and 2-fold higher than hybrid

'Kassa'. Radzevicius et al. (2013) reported that the different cultivars of tomato have wide variations in term of ascorbic acid contents. The increasing economic importance of tomato throughout the world as a functional food have necessitated to find out the effect of ethylene on the biochemical composition and antioxidant capacity of different types of tomato and therefore this experiment was conducted.

2. Material and Methods

Beefsteak (cv. Tybif), heirloom (cv. Yuksel Koy) and cluster (cv. Merkur) types of tomato were harvested at 'breaker stage'. There was definite break in color from green to tannish yellow, pink or red on not more than 10% of the surface. All fruit were picked from a commercial greenhouse in Antalya, Turkey (36°59'57.3" N and 30°51'20.4" E). During the entire vegetation period, uniform irrigation and fertigation management procedures were applied to the tested tomato types. All fruit were harvested on the same day and immediately brought to the postharvest physiology laboratory at Akdeniz University, Antalya, Turkey. Fruit with any defects i.e. decayed, bruised and non-uniform, were discarded and the remainder were split into two groups. The first group of tomato fruit were applied with 150 µL L^{-1} of ethylene at 20 °C in a 20 m^3 room and the second group were left untreated (control). Both groups of fruit samples were stored at 12 °C and 90±5% relative humidity for 35 days. Fruit samples for different quality analysis were removed from cold room at 7 days intervals and they were also kept at 20 ºC and 60±5% relative humidity for additional 3 days to simulate shelf life performance.

Tomato puree was utilized for analysis of total chlorophyll, lycopene, total phenolic, carotenoid, flavonoid, ascorbic acid contents and antioxidant activity. Homogenization of tomato samples for all quality analysis ultra turrax homogenizer (IKA-Labortechnique Typ T 25 JANKE & KUNKEL GMBH & CO.KG) was used. The samples absorbances for all quality analysis were read through Analytik Jena AG Specord 40 ST spectrophotometer.

The total chlorophyll contents were determined according to the method of Lichtenthaler & Wellburn (1983). Tomato puree of 3 g was homogenized with 80% acetone through ultra turrax homogenizer. The centrifuge of homogenized samples was performed at 8600 x g for 5 min under 4 °C. After centrifuge, sample supernatant was used for determination of chlorophyll content. The supernatant was read through Specord 40 ST spectrophotometer against blank 80% acetone solvent at the wavelengths of 646 and 663 nm. The total chlorophyll contents of tomato fruit were computed through equation (1) given below and given as g kg^{-1} fresh weight (fw).

$$\text{Chlorophyll a} = 12.21 \times A_{663} - 2.81 \times A_{646}$$
$$\text{Chlorophyll b} = 20.13 \times A_{646} - 5.03 \times A_{663}$$
$$\text{Total chlorophyll} = (C_a + C_b) \quad (1)$$

The method explained by Fish et al. (2002) was used for the determination of lycopene contents in different types of tomato. For that purpose, the tomato samples were homogenized through homogenizer and 0.5 g of samples were weighed and put in 50 mL test tubes. 5 mL butylated hydroxytoluene (BHT) prepared with acetone (0.05% w/v), 5 mL ethanol (95%) and hexane at 10 mL concentration were added to sample. Prepared samples were shaken at 4 °C for 5 min at 180 rpm through shaker. After, 3 mL distilled water was added to sample and shaken again for 5 min. Then, the samples were left to separate the phase for 5 min at room temperature to obtain colored layer of hexane at the top surface. The supernatant containing hexane layer was read in spectrophotometer at 563 nm of absorbance. Data obtained from the measurements were calculated by equation (2) below and reported as mg kg^{-1} fw.

$$\text{Lycopene (mg kg}^{-1}) = A_{503} \text{ x } 0.0312/\text{kg, sample} \quad (2)$$

A_{503} = The absorbance value at 503 nm
0.0312 ($\mathcal{E}$) = Extinction coefficient of lycopene.

The extraction of fruit samples was done with 80% methanol for antioxidant activity, total phenolic and total flavonoid contents analysis. For this purpose, tomato puree of 20 g and 80% methanol of 20 mL was homogenized with the help of Ultra-Turrax homogenizer. The samples were centrifuged at 8600 x g for 20 min at 4 °C after homogenization. The antioxidant activity of tomato was determined according to DPPH method described by Benvenuti et al. (2004). For that purpose, 1 m*M* DPPH* radical solution of 600 µL was taken in 4 test tubes and 1, 2, 3 and 4 mL of extracted samples were added into test tubes. After, 80% methanol was used to bring total volume in each tube to 6 mL. The mixture in tubes were vortexed and left to incubate in dark at room temperature for 15 min. Additionally, the control sample was prepared by taking 600 µL of 1 m*M* DPPH* radical solution and 5.4 mL of methanol in a tube and allowed to incubate in a dark place at room temperature for 15 min. After incubation samples absorbance were read at 517 nm wavelength by using spectrophotometer against blank solvent of 80% methanol and control sample. Percent inhibition values proportionate to each sample volume were computed by using equation (3).

$$\% \text{ inhibition} = A_{DPPH} - A_{Extract} / A_{DPPH} \text{ x } 100 \quad (3)$$

A_{DPPH}: DPPH control sample absorbance value; $A_{Extract}$: Test sample absorbance value

The inhibition values and sample volumes were used to obtain a graph. Linear regression analysis was applied to the graph, sample curve and equation explaining the curve was acquired. Equation was used for calculation of EC_{50} (effective concentration) value of the sample. The antioxidant activity by DPPH method is determined through EC_{50} value. The EC_{50} value reflects amount of antioxidant substances present in fruit and vegetables sample that inhibit 50% of DPPH radical. Decrease in EC_{50} value exhibit increase in the antioxidant activity (Cemeroglu 2010). The EC_{50} value was reported in g kg^{-1} fw EC_{50}.

The total carotenoid content analysis was performed by using the method of Witham et al. (1971). For that purpose, tomato puree of 0.25 g was homogenized with 10 mL 80% acetone for 3-4 min through ultra-turrax homogenizer and 80% acetone solvent was used to bring the total volume of sample to 15 mL. After, the samples were centrifuged at 8600 x g for 10 min at 4 °C after homogenization. The carotenoids content was determined by using the supernatant fraction. The samples absorbance used for chlorophyll a, chlorophyll b and carotenoid were 663 nm, 645 nm and 440 nm respectively. The samples absorbance was read through spectrophotometer against a blank solvent of 80% acetone. The total carotenoid content was calculated through equation (4) and expressed as g kg^{-1} fw.

Chlorophyll a (g kg^{-1}) = [12.7 (D663) – 2.69 (D645)] x V/1000 x W Chlorophyll b (g kg^{-1}) = [22.9 (D645) – 4.68 (D663)]×V/1000×W
Carotenoids (g kg^{-1}) = [4.69 (D440) – (chlorophyll a + chlorophyll b)×0.286]×V/1000×W (4)

V = Extract volume
W = Sample quantity

The total flavonoid content of tomatoes was analysed through the procedure explained by Karadeniz et al. (2005). In 50 mL tube, 1 g of tomato puree, distilled water of 5 mL and 5% $NaNO_2$ (Merck) of 0.3 mL were added, respectively. The test tubes were closed and strongly mixed. 5 min later 0.6 mL of 10% $AlCl_3.6H_2O$ (Merck) was added and after 5 min 2 mL of 1 mol L^{-1} NaOH was added. The distilled water was used to bring total volume in tube to 10 mL. Tubes were then vortexed and samples absorbance was measured at wavelength of 510 nm using spectrophotometer against solvent of blank 80% methanol. The standard calibration curve prepared with catechin were used to compute the total flavonoid content of tomatoes and given as mg kg^{-1} fw.

The total phenolic contents analysis was performed according to the Folin-Ciocalteu method explained by Spanos and Wrolstad (1990). Extract of 0.1 mL was blended with distilled water of 0.9 mL and 0.2 mol L^{-1} N Foline-Ciocalteu reagent of 5 mL. After 3 min, aqueous solution of Na_2CO_3 (75 g L^{-1}) at 4 mL concentration was added into blend and samples were kept for 2 h in the dark at room temperature. The samples absorbance was recorded at the wavelength of 765 nm against blank 80% methanol solvent through spectrophotometer. The total phenolic contents calculated were reported as mg of gallic acid equivalent per kg (mg kg^{-1} GAE) fw.

The total ascorbic acid contents analysis was conducted according to Cemeroglu (2010). For that purpose, the tomato samples were extracted with 6% metaphosphoric acid and in 50 mL tube, extract of 5 mL, acetate buffer solution (pH 4.0) of 5 mL, 2,6 dichlorophenolindophenol dye solution of 1 mL, and xylene of 10 mL were added. After, the tubes were stirred for 10 s and centrifugation was performed at 8600 x g for 10 min at 4 °C. The control sample was prepared in a test tube containing 6% metaphosphoric acid of 5 mL, acetate buffer solution (pH 4.0) of 5 mL, 1 mL of 2,6 dichlorophenolindophenol dye solution and 10 mL of xylene. Samples absorbance was recorded at wavelength of 500 nm through spectrophotometer against xylene and control sample. The ascorbic acid calibration curve equation was $y = 0.0123x + 0.0134$ and coefficient of determination was $R^2 = 0.9557$. The equation (5) was used to determine the total ascorbic acid contents and expressed as mg 100 g^{-1} fw.

Ascorbic acid (mg $100g^{-1}$) = $A_2 – A_1/a$ x DF (5)

A_1: Extract sample absorbance value; A_2: Control sample absorbance value; DF: Dilution factor; a: Ascorbic acid standard curve slope

The experiment was designed according to completely randomized design (CRD) with three replications. Each replication contained ten fruit. Duncan's multiple range test was used to determine significant differences among means. Mean values obtained were analysed with SAS program.

3. Results and Discussion

3.1. Total chlorophyll content

Ethylene treated tomatoes had less chlorophyll content and different types of tomatoes exhibited decrease in total chlorophyll content by the end of storage period. After cold storage, the maximum amount of total chlorophyll content (0.0030 g kg^{-1}) was found in untreated heirloom type while the minimum total chlorophyll content (0.0001 g kg^{-1}) was obtained in ethylene treated cluster type of tomatoes (Table 1). There were no significant differences between ethylene treated heirloom and cluster type of

tomatoes by the end of cold storage. After shelf life period, the highest total chlorophyll content (0.0016 g kg^{-1}) was noted in control beefsteak type while lowest total chlorophyll content (0.0004 g kg^{-1}) was obtained in ethylene treated cluster type of tomatoes (Table 2). There were no significant differences between heirloom and cluster type of tomatoes at the end of shelf life period.

Table 1- Effect of ethylene on the total chlorophyll, lycopene, total phenolic contents, antioxidant activity, carotenoid, flavonoid, and ascorbic acid contents of different types of tomatoes under cold storage at 12 °C

		Storage duration (Days)					
Parameters	**Treatments**	**0**	**7**	**14**	**21**	**28**	**35**
Total chlorophyll content (g kg^{-1} fw)	**BS**†	0.050ab*	0.0042ac	0.0024cj	0.0017dj	0.0011gj	0.0010gj
	BS+Ethyl.	0.0038af	0.0030bi	0.0021cj	0.0018di	0.0014fj	0.0007hj
	HL	0.0044ac	0.0039ae	0.0038ae	0.0038af	0.0032bg	0.0030bi
	HL+Ethyl.	0.0040ad	0.0037bf	0.0031bh	0.0029bi	0.0012gj	0.0005j
	CL	0.0061a	0.0042ac	0.0033bg	0.0033bg	0.0030bi	0.0012gj
	CL+Ethyl.	0.0053ab	0.0021cj	0.0016ej	0.0008hj	0.0007ij	0.0001j
	$LSD_{5\%}$: St. Dur.: 0.0008 **St. Dur. × Trt.:** 0.0019 **Trt.:** 0.0008						
Lycopene content (mg kg^{-1} fw)	**BS**	1.93k	6.47ik	9.96gk	17.50eh	26.12de	25.84de
	BS+Ethyl.	5.10ik	6.62ik	12.63fk	18.11eg	22.84df	26.65de
	HL	2.98jk	5.131ik	14.26fj	18.59eg	39.08ac	45.25a
	HL+Ethyl.	4.07ik	5.14ik	10.15gk	31.06bd	42.95a	47.37a
	CL	2.31k	2.46k	6.76hk	25.57de	38.18ac	40.30ac
	CL+Ethyl.	2.98jk	4.87ik	14.81fi	26.59de	30.02cd	41.46ab
	$LSD_{5\%}$: St. Dur.: 3.825 **St. Dur. × Trt.:** 9.3694 **Trt.:** 3.825						
Antioxidant Activity (g kg^{-1} fw EC_{50})	**BS**	1.53a	0.75di	1.09bd	0.56fl	0.66el	0.75di
	BS+Ethyl.	1.40ab	1.14bc	0.72dj	0.72dj	0.85cg	0.89cf
	HL	0.44gl	0.36il	0.25l	0.27kl	0.47gl	0.43hl
	HL+Ethyl.	0.30kl	0.27kl	0.66ek	0.32jl	0.42hl	0.33jl
	CL	1.02ce	0.79ch	0.52fl	0.62el	0.83ch	0.65el
	CL+Ethyl.	1.50a	0.77ci	0.59fl	0.67ek	0.72dj	0.65el
	$LSD_{5\%}$: St. Dur.: 0.1369 **St. Dur. × Trt.:** 0.3353 **Trt.:** 0.1369						
Carotenoid content (g kg^{-1} fw)	**BS**	0.0046jk	0.0062ik	0.0084hk	0.0113gk	0.0201eg	0.0287de
	BS+Ethyl.	0.0042jk	0.0116gk	0.0149fj	0.0155fj	0.0231ef	0.0680b
	HL	0.0048jk	0.0049jk	0.0050jk	0.0112gk	0.0156fj	0.0172fi
	HL+Ethyl.	0.0045jk	0.0058ik	0.0062ik	0.080hk	0.0142fj	0.1182a
	CL	0.0020k	0.0046jk	0.0051jk	0.0068ik	0.0149fj	0.0250df
	CL+Ethyl.	0.0051jk	0.0099gk	0.0148fj	0.0185eh	0.0340d	0.0545c
	$LSD_{5\%}$: St. Dur.: 0.0039 **St. Dur. × Trt.:** 0.0095 **Trt.:** 0.0039						
Flavonoid content (mg kg^{-1} fw)	**BS**	252.7a	709.7cd	57.6cd	56.3cd	25.2d	9.8d
	BS+Ethyl.	237.2ab	123.9ad	65.8cd	61.9cd	37.2cd	22.6d
	HL	113.5bd	72.7cd	40.0cd	34.7cd	29.4d	23.0d
	HL+Ethyl.	106.0bd	77.6cd	59.1cd	47.4cd	45.5cd	42.2cd
	CL	133.2ad	60.8cd	52.0cd	33.0d	30.1d	12.3d
	CL+Ethyl.	183.5ac	101.6bd	55.5cd	41.7cd	32.7d	27.5d
	$LSD_{5\%}$: St. Dur.: 49.4 **St. Dur. × Trt.:** 121 **Trt.:** 49.4						
Total phenolic contents (mg kg^{-1} GAE fw)	**BS**	26.0be	20.6cg	20.0cg	16.3dg	16.2dg	13.7fg
	BS+Ethyl.	24.2bf	22.4bf	21.6cf	19.7cg	17.1dg	12.8fg
	HL	33.4ab	30.1ac	21.1cf	20.0cg	18.7cg	16.2dg
	HL+Ethyl.	37.2a	26.2bd	23.4bf	23.4bf	17.1dg	17.1dg
	CL	21.0cf	19.7cg	19.3cg	14.7dg	13.3fg	9.0g
	CL+Ethyl.	23.1bf	21.8cf	21.2cf	18.9cg	16.8dg	14.4eg
	$LSD_{5\%}$: St. Dur.: 3.9 **St. Dur. × Trt.:** 9.5 **Trt.**: 3.9						
Ascorbic acid (mg $100g^{-1}$ fw)	**BS**	32.17a	22.90dj	23.57cg	19.5.fk	19.52fk	17.18k
	BS+Ethyl.	29.55ab	23.41ch	19.46gk	17.17k	19.56fk	18.92ik
	HL	30.31ab	25.34cd	25.23cd	19.84fk	19.07ik	20.32ek
	HL+Ethyl.	25.43cd	27.42bc	22.33dj	19.91ek	18.65jk	17.69k
	CL	33.23a	23.82cf	23.12di	19.90ek	19.71fk	16.18k
	CL+Ethyl.	30.20ab	23.53ch	24.10ce	19.35gk	19.23hk	16.07k
	$LSD_{5\%}$: St. Dur.: 1.45 **St. Dur. × Trt.:** 3.55 **Trt**.: 1.45						

*: Means with different letters are statistically significant at P≤0.05 according to Duncan's multiple range test; †BS: Beefsteak; BS+Ethyl.: Beefsteak+Ethylene, HL: Heirloom; HL+Ethyl.: Heirloom+Ethylene; CL: Cluster; CL+Ethyl.: Cluster+Ethylene; LSD: Least significant difference; St. Dur.: Storage duration, St. Dur. × Trt.: Storage duration × Treatments; Trt: Treatments

Table 2- Effect of ethylene on the total chlorophyll, lycopene, total phenolic contents, antioxidant activity, carotenoid, flavonoid, and ascorbic acid contents of different types of tomatoes under shelf life conditions at 20 °C

		Storage duration (Days)					
Parameters	**Treatments**	**0**	**7+3**	**14+3**	**21+3**	**28+3**	**35+3**
Total chlorophyll content (g kg^{-1} fw)	**BS**†	0.0050ad	0.0041af	0.0036ag	0.0034ci	0.0018ej	0.0016fj
	BS+Ethyl.	0.0038ag	0.0036ag	0.0025dj	0.0020ej	0.0020ej	0.0015fj
	HL	0.0044ae	0.0044ae	0.0044ae	0.0016fj	0.0007j	0.0005j
	HL+Ethyl.	0.0040af	0.0043ae	0.0036ag	0.0024ej	0.0006j	0.0006j
	CL	0.0061ab	0.0061a	0.0035bh	0.0013il	0.0008ij	0.0006j
	CL+Ethyl.	0.0053ac	0.0015fj	0.0014fj	0.0009hj	0.0007j	0.0004j
	LSD$_{5\%}$: St. Dur.: 0.0009 **St. Dur. × Trt.:** 0.0022 **Trt.:** 0.0009						
Lycopene content (mg kg^{-1} fw)	**BS**	1.93p	5.88np	16.87kn	19.81km	25.16gl	37.18dg
	BS+Ethyl.	5.10op	9.72mp	21.99il	25.33gl	32.40ej	37.31dg
	HL	2.98p	23.93hl	37.05dg	37.72df	38.85df	54.94ab
	HL+Ethyl.	4.07op	21.69jl	28.37fk	42.75ce	42.83ce	56.36a
	CL	2.31p	21.75jl	35.29eh	39.51df	44.11be	48.71ad
	CL+Ethyl.	2.98p	14.98lo	33.83ei	34.50eh	39.90df	53.85ac
	LSD$_{5\%}$: St. Dur.: 4.2219 **St. Dur. × Trt.:** 10.342 **Trt.:** 4.2219						
Antioxidant Activity (g kg^{-1} fw EC$_{50}$)	**BS**	1.53a	0.96bd	0.41jn	0.87be	0.72dh	0.60fl
	BS+Ethyl.	1.39a	0.98bc	0.80bf	0.89be	0.67ej	0.69ei
	HL	0.44in	0.26n	0.34ln	0.36ln	0.38kn	0.47hn
	HL+Ethyl.	0.30mn	0.30mn	0.47hn	0.39kn	0.51gn	0.41jn
	CL	1.02b	0.69ei	0.55fm	0.66ej	0.63ek	0.81bf
	CL+Ethyl.	1.50a	0.56fm	0.46in	0.74cg	0.81bf	0.76cg
	LSD$_{5\%}$: St. Dur.: 0.0888 **St. Dur. × Trt.:** 0.2175 **Trt.:** 0.0888						
Carotenoid content (g kg^{-1} fw)	**BS**	0.0046e	0.0072de	0.0078de	0.0118de	0.0051e	0.0279be
	BS+Ethyl.	0.0042e	0.0048e	0.0055e	0.0149ce	0.0384ad	0.0449ac
	HL	0.0048e	0.0061e	0.0133de	0.0192be	0.0240be	0.0290be
	HL+Ethyl.	0.0045e	0.0094de	0.0128de	0.0122de	0.0230be	0.0660a
	CL	0.0020e	0.0020e	0.0041e	0.0087de	0.0098de	0.0149ce
	CL+Ethyl.	0.0051e	0.0048e	0.0087de	0.0099de	0.0315ad	0.0454ab
	LSD$_{5\%}$: St. Dur.: 0.0105 **St. Dur. × Trt.:** 0.0256 **Trt.:** 0.0105						
Flavonoid content (mg kg^{-1} fw)	**BS**	252.7a	190.8ac	112.7be	87.3ce	30.5e	24.1e
	BS+Ethyl.	237.2ab	55.7ce	31.1e	45.7de	41.6de	8.9e
	HL	113.5be	96.9ce	82.7ce	43.7de	26.1e	19.3e
	HL+Ethyl.	106.0be	51.6ce	50.7ce	49.9ce	47.4ce	44.3de
	CL	133.2ae	128.5ae	66.0ce	41.8de	21.4e	11.4e
	CL+Ethyl.	183.5ad	68.7ce	43.2de	42.0de	22.4e	12.3e
	LSD$_{5\%}$: St. Dur.: 48.2 **St. Dur. × Trt.:** 118.1 **Trt.:** 48.2						
Total phenolic contents (mg kg^{-1} GAE fw)	**BS**	26.0be	23.4cf	25.6be	19.3ej	24.8ce	12.6ij
	BS+Ethyl.	24.2cf	19.0ej	23.2cf	21.0dh	22.5cf	11.6j
	HL	33.4ab	33.4ab	28.2bd	22.7bd	20.1di	12.5ij
	HL+Ethyl.	37.2a	30.0ac	37.2a	22.3cf	16.2fj	12.3ij
	CL	21.0dh	18.5ej	18.5ej	16.1fj	13.5gj	13.3hj
	CL+Ethyl.	23.1cf	21.7cg	17.5ej	23.1cf	13.7gj	12.4ij
	LSD$_{5\%}$: St. Dur.: 2.8 **St. Dur. × Trt.:** 6.9 **Trt.**: 2.8						
Ascorbic acid (mg 100 g^{-1} fw)	**BS**	32.17a	29.16ac	25.31be	19.57fj	18.18hk	13.96kl
	BS+Ethyl.	29.55ab	25.48bd	23.43ch	19.56fj	17.22il	16.19il
	HL	30.31ab	29.71ab	23.83cg	19.84ej	21.25di	17.05il
	HL+Ethyl.	25.43bd	23.96cf	21.17di	20.37di	19.91ej	17.25il
	CL	33.23a	25.56bd	18.76fk	19.86ej	14.67jl	17.35il
	CL+Ethyl.	30.20ab	23.51ch	20.97di	18.49gk	19.35fj	12.54l
	LSD$_{5\%}$: St. Dur.: 1.82 **St. Dur. × Trt.:** 4.45 **Trt.**: 1.82						

*: Means with different letters are statistically significant at P≤0.05 according to Duncan's multiple range test; †BS; Beefsteak, BS+Ethyl.; Beefsteak+Ethylene; HL: Heirloom; HL+Ethyl.: Heirloom+Ethylene; CL: Cluster; CL+Ethyl.: Cluster+Ethylene; LSD: Least significant difference, St. Dur.: Storage duration; St. Dur. × Trt.: Storage duration × Treatments; Trt: Treatments.

During this study, the decline in chlorophyll content may be because of progress in ripening that caused color change in fruit from green to red maturity stage with the transformation of chloroplast into chromoplasts, breakdown of chlorophyll and synthesis of carotenoids occurred as reported by Alexander & Grierson (2002). Watada et al. (1986) expressed that ethylene treatment enhances the degradation of chlorophyll in citrus fruit; turns it to yellow and then to orange color from green that agrees with the finding of less chlorophyll content obtained in this study in ethylene treated tomatoes.

3.2. Lycopene content

Extending storage time had considerably increased the lycopene content in tomatoes. At the end of cold storage, the highest lycopene content (47.37 mg kg^{-1}) was recorded in ethylene treated heirloom type while lowest lycopene content (25.84 mg kg^{-1}) was observed in control beefsteak type of tomatoes (Table 1). There were no significant differences between ethylene treated and control heirloom type of tomatoes at the end of cold storage. At the end of shelf life, the maximum lycopene content (56.36 mg kg^{-1}) was also recorded in ethylene treated heirloom type whereas the minimum lycopene content (37.18 mg kg^{-1}) was noted in control beefsteak type of tomatoes (Table 2). However, there were no significant differences between ethylene treated and control heirloom type of tomatoes at the end of shelf life period.

Increase in lycopene concentration of tomato with extension in storage of tomato was reported by Khairi et al. (2015) which agreed with the findings in this study. Tadesse et al. (2016) reported as the maturity of tomato fruit at green mature stage enhances it converts chloroplast into chromoplast where the lycopene is present in membrane bound crystals. Dhall & Singh (2013) reported higher lycopene content in ethylene treated tomatoes as obtained in this study. The lycopene content determined in the shelf life period were higher than cold storage in our experiment which was in confirmation with Tadesse et al. (2015) who expressed higher lycopene content in tomato kept at 20 and 30 °C than at 4 °C.

3.3. Antioxidant activity

Antioxidant activity showed an increase in beefsteak and cluster types of tomatoes whereas in heirloom type of tomatoes it had decreased. There was no significant difference between control and ethylene treated cluster type of tomatoes on 35th day of cold storage. The minimum EC_{50} value indicate the maximum antioxidant activity. After cold storage, the highest antioxidant activity (0.33 g kg^{-1} EC_{50}) was recorded in ethylene treated heirloom type while the lowest antioxidant activity (0.89 g kg^{-1} EC_{50}) was noticed in ethylene treated beefsteak type of tomatoes (Table 1). After shelf life period, the highest antioxidant activity (0.41 g kg^{-1} EC_{50}) was recorded in ethylene treated heirloom type while the lowest antioxidant activity (0.81 g kg^{-1} EC_{50}) was observed in control cluster type of tomatoes (Table 2).

Tilahun et al. (2017) reported that antioxidant activity in tomatoes was higher at red maturity stage than those of harvested at green maturity stage which can be because of increase in lycopene concentration. This result confirmed our outcomes of ethylene treatment increased lycopene concentration and antioxidant activity.

3.4. Carotenoid content

Extending storage time had increased the carotenoid contents. Ethylene treated tomatoes had more carotenoid content than untreated ones. After cold storage, ethylene treated heirloom type tomatoes had maximum carotenoid content (0.1182 g kg^{-1}) while minimum carotenoid content (0.0172 g kg^{-1}) was noted in control heirloom type of tomatoes (Table 1). At the end of shelf life period, maximum carotenoid content (0.0660 g kg^{-1}) were also noted in ethylene treated heirloom type whereas minimum total carotenoid content (0.0149 g kg^{-1}) was observed in control cluster type of tomatoes (Table 2).

In this study, ethylene treated tomatoes had higher carotenoid content at the end of storage which was in confirmation with the outcomes of Cruz et al. (2018). These researches expressed that ethylene regulates the carotenoid synthesis during ripening of tomatoes. Increase in carotenoid content in this study can be because of advancement in maturity that change color of tomato from green to red with conversion of chloroplast to chromoplast, resulting degradation of chlorophyll and accumulation of carotenoid as explained by Alexander & Greirson (2002).

3.5. Flavonoid content

Flavonoid content decreased during storage. There was a significant interaction ($P \leq 0.05$) between storage duration and treatments. After cold storage, maximum flavonoid content (42.2 mg kg^{-1}) occurred in ethylene treated heirloom fruit with minimum (9.8 mg kg^{-1}) flavonoid content was in control beefsteak tomatoes (Table 1). During shelf life period, the highest flavonoid content (44.3 mg kg^{-1}) was recorded in ethylene treated heirloom type with the lowest flavonoid content (8.9 mg kg^{-1}) was in ethylene treated beefsteak tomatoes (Table 2).

Riadh et al. (2016) stated that different cultivars of tomatoes significantly affected flavonoid content which agreed with significant effect obtained between different types of tomatoes treated with ethylene in our study. Flavonoid content showed decrease with increase in storage duration during our experiment which was supported by outcomes of Howard et al. (2000) who described decrease of flavonoid content during maturation of peppers. The losses in flavonoid content may be because of metabolic transformation to secondary phenolic compounds as reported by Barz & Hoesel (1979).

3.6. Total phenolic contents

Prolonging storage duration decreased the total phenolic contents. At the end of cold storage, the maximum total phenolic content (17.1 mg kg^{-1} GAE) was exhibited by ethylene treated heirloom type whereas the minimum total phenolic contents (9.0 mg kg^{-1} GAE) was found in control cluster type of tomatoes (Table 1). There were no significant differences between ethylene treated and control heirloom type of tomatoes by the end of cold storage. At the end of shelf life period, highest total phenolic content (13.3 mg kg^{-1} GAE) was reported in control cluster type while lowest total phenolic content (11.6 mg kg^{-1} GAE) was recorded in ethylene treated beefsteak type of tomatoes (Table 2).

Extension in storage showed decrease in total phenolic contents of different types of tomatoes in our experiment. According to Day (2001) the higher respiration rate can be the reason of degradation of phenolic compounds**.** Control beefsteak type of tomatoes during cold storage had resulted more total phenolic contents which agreed with Dominguez et al. (2016) who demonstrated reduction in ethylene had increased the total phenolic contents in 'Delizia' tomato cultivar. The higher amount of total phenolic contents in heirloom type of tomatoes in this study during cold storage may be attributed to higher content of lycopene as described by Riadh et al. (2016).

3.7. Ascorbic acid content

Ascorbic acid displayed declining trend with extension in storage duration. At the end of cold storage, highest ascorbic acid content (20.32 mg 100 g^{-1}) was found in control heirloom type whereas lowest ascorbic acid content (16.07 mg 100 g^{-1}) was reported in ethylene treated cluster type of tomatoes (Table 1). There were no significant differences between control beefsteak, ethylene treated heirloom, control cluster and ethylene treated cluster type of tomatoes by the end of cold storage. At the end of shelf life period, untreated cluster type of tomatoes had the maximum ascorbic acid contents (17.35 mg 100 g^{-1}) while the minimum ascorbic acid content (12.54 mg 100 g^{-1}) was displayed by ethylene treated cluster type of tomatoes (Table 2). There were no significant differences between both ethylene treated and untreated heirloom and cluster type of tomatoes.

Declining trend in ascorbic acid content were exhibited by different types of tomatoes with extension in storage duration during this study which agreed with the findings of Tudor-Rado et al. (2016) who stated that decrease in ascorbic acid content of various tomato cultivars and the reason for decline in ascorbic acid may be because of oxidation caused by oxidizing enzymes. Our findings were in contradiction with Dhall & Singh (2013) who reported ethylene treated tomatoes had more ascorbic acid content when comparison was made with control.

4. Conclusions

In conclusion, ethylene treatment resulted in higher lycopene and carotenoid contents with lower total chlorophyll contents in all tested tomato types. After cold storage and shelf life period, the maximum antioxidant activity, carotenoid and flavonoid content were recorded in ethylene treated heirloom type tomatoes. Furthermore, heirloom type tomatoes retained better postharvest quality as compared to beefsteak and cluster type of tomatoes at the end of 35 days of cold storage.

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Palynological Study on Some Grape (*Vitis vinifera* L.) Cultivars Using Scanning Electron Microscopy

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ABSTRACT
Pollen is an important morphological parameter for cultivar identification. This is of great importance in detailed investigations by scanning electron microscope (SEM). In this study, the pollen morphology of selected grape cultivars was examined by SEM. The pollen length, width, P/E ratio of pollen and features of surface were observed. The pollen differed in some microstructural characteristic. Pollen width exhibited significant according to the varieties (10.12-22.44 µm). Similarly, the statistical difference occurred among the thirty *Vitis* cultivars in terms of mean pollen length (16.26-29.65 µm). Areolat pollen was determined in some cultivars. Depending on the cultivars there was significant differences in terms of pores diameter. According to PCA performed in 30 grape cultivars, 3 principal components were revealed and they defined 94.98% of the variance. Cultivars were divided into groups according to pollen features on the cluster. Consequently, the cultivars were categorized under two main groups. The present research is a contribution to a more detailed analysis of grapevine cultivars.

Keywords: Pollen, Morphology, Scanning electron microscope (SEM), Palynology, Classification, Description

1. Introduction

Grapes are most widely grown fruit crop. The grape is a member of the Vitaceae commonly called the grape family, *Vitis* contains around 60 species, plus some natural interspecific hybrids, and they are mostly distributed in temperate areas throughout the world (Mebberely 1987; Lombardi et al. 2007; Najmaddin et al. 2013). The grapes are one of the most important produce from the enological and economical point of view in the Anatolia. Generally, ampelographic studies have focused on the morphology of *Vitis* varieties. Pollen has hereditary properties that determine genotype. Pollen morphology confirms phylogenetic relationships among genera, species and varieties. Thus, it is used in systematic studies regarding to similarity and diversity of pollen. The morphology of pollen can be examined in detail via the scanning electron microscope (Tanaka et al. 2004).

On the basis of surface ornamentation and pollen grain dimensions, different classifications have been made on various plant species, such as grapevines (Wodehouse 1935; Erdtman 1952; Hyde & Adams 1958; Faegri & Iversen 1989). In the description of the pollen, qualitative characters, such as exine microrelief, separate elements, and quantitative characters, such as polar axis, equatorial axis, mesocolpium, apocolpium, and length and width of the colps are used (Roytchev 1995). For example, Uzun & llter (1987) and Kharitonashyili et al. (1989) studied pollen grains in different types of flowers of *Vitis vinifera* L., using scanning electron microscopy (SEM). Ahmedullah (1983) characterized different grape cultivars based on pollen morphology. Martens et al. (1989) studied pollen size variability within genotypes of *Vitis*. Slimane &Askri (1990) characterized 30 grapevine varieties based on pollen size, Roytchev et al. (1994) obtained information on the ultrastructure of exine surface apertures in 27 Bulgarian and repetition seedless grape cultivars. Palynology has presented considerable opportunities for some indigenous grape cultivars identification in grapevines, besides its importance in plant taxonomy (Marasalı et al. 2005). Gallardo et al. (2009) studied 14 Spanish *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi populations. Jovanovic-Cvetkovic et al. (2016) analysed the pollen morphology of indigenous cvs. Žilavka and Blatina to determine their morphological specificities.

The objective of the present study was to classify the thirty grape cultivars according to the shape and microrelief of pollen grains and to establish the possibilities for using the parameters of the different apertures as classification indices using scanning electron microscope (SEM).

2. Material and Methods

Pollen samples. This study was carried with pollen from thirty different of *Vitis vinifera* L. are located at the Department of Horticulture, Agriculture Faculty, Ege University, İzmir, Turkey (Table 1). The inflorescence was isolated. The pollen grains were gathered after these inflorescences were collected at the morning hours at the beginning of the blooming period (Eichhorn & Lorenz 1977). The pollen was sampled by cutting flowers and brushing the anthers and pollen into an Eppendorf tube using a soft brush (Gökbayrak & Engin 2016). The pollen was stored until analysis (Storey 1975).

Table 1- List of the cultivars studied

Cultivars	*Type**	*Cultivars*	*Type**	*Cultivars*	*Type**	*Cultivars*	*Type**	*Cultivars*	*Type**
Vitis vinifera L. "Abiguş"	T	*Vitis vinifera* L. "Alphonse Lavallée""	T	*Vitis vinifera* L. "Alicante Bouschet"	W	*Vitis vinifera* L. "Beyaz Şam"	T	*Vitis vinifera* L. "Buca Razakı"	T
Vitis vinifera L. "Cardinal"	T	*Vitis vinifera* L. "Cinsault"	W	*Vitis vinifera* L. "Çeşme Pembesi"	T	*Vitis vinifera* L. "Foça Karası"	W	*Vitis vinifera* L. " Hafızali"	T
Vitis vinifera L. "İtalia"	T	*Vitis vinifera* L. "Kırmızı Şam	T	*Vitis vinifera* L. "Kozak Gemresi"	T	*Vitis vinifera* L. "Mahrabaşı"	T	*Vitis vinifera* L. "Morsleleh	T
Vitis vinifera L. "Malbec"	W	*Vitis vinifera* L. "Morseyhative"	T	*Vitis vinifera* L. "Müşküle"	T	*Vitis vinifera* L. "Papaz Karası"	W	*Vitis vinifera* L. "Pembe Gemre"	T
Vitis vinifera L. "Pek Üzümü"	T	*Vitis vinifera* L. "Siyah Gemre"	T	*Vitis vinifera* L. "Syrah"	W	*Vitis vinifera* L. "Şika"	T	*Vitis vinifera* L. "Tarsus Pembesi"	T
Vitis vinifera L. "Trakya İlkeren"	T	*Vitis vinifera* L. "Öküzgözü"	W	*Vitis vinifera* L. "Ohannes"	T	*Vitis vinifera* L. "Yuvarlak Çekirdeksiz"	T, SE	*Vitis vinifera* L. "Yuvarlak Razakı"	T

*T: table grape; W: vine grape; SE: seedless grape

Pollen grains were air dried for investigation in SEM. Dry pollen was sputter-coated (Leica model) with 10 µm of gold-palladium. Pollens were measured directly on the screen of the electron microscope. Each of the tested samples were observed with scanning electron microscope (Thermo Scientific Apreo S model) were photographed at 10000 x for whole grain. The pollen length, width, length/width ratio and pore diameter, distance between pores and colpi length were measured at 10 pollen grains for each genotypes. The pollen shape was stated by considering the length/width ratio (Erdtman 1952). The types of aperture found in pollen were described according to Wang et al. (2014). The polar (P) and equatorial (E) axes, P/E relationship were determined according to Van der Pluym & Hideux (1977). The terminology of Erdman (1952) was used in the morphological descriptions of the pollen.

The data were subjected to analysis of variance using SPSS (SSPS Inc. 10.0, USA, 1999) statistical package program. The differences between the means were determined with Fischer's Least Significant Difference (LSD) test. The mean, minimum, maximum" and standard deviation values of the properties were found out. These values were revealed by conducting Pearson's correlation analysis. Further, Principal Component Analysis (PCA) and Clustering Analysis (CA) were also performed and indicated by dendrogram. Differences or similarities of cultivars were evaluated according to their analyzed properties by applying PCA to the findings obtained. Moreover, cluster analysis was utilized to create a dendrogram showing similarities and differences between genotypes.

3. Results and Discussion

Given the characteristics of the thirty grape cultivars, a general description was established for all, according to the values of the various parameters corresponding to the max. and min. records of the cultivars. The statistical difference appeared among the thirty different of *Vitis vinifera* L. genotypes in terms of the pollen grains (pollen length, width and length/width ratio). The variation in the min., max. mean values and standard deviations of grape pollen parameters are shown in Table 2 and Table 3.

Mean pollen width differed statistically significant among the varieties. Thus, the highest mean values for this feature were determined on "Yuvarlak Çekirdeksiz" (19.09 µm) and "Mahrabaşı" (22.44 µm) and the lowest mean values for this pollen width were found in "Alicante Bouschet*"* (10.12 µm), "Öküzgözü" (10.32 µm) and "Syrah" (10.54 µm) varieties respectively. Pollen length ranged from 29.65 µm "Alphonse Lavallée"" to 16.26 µm "Foça Karası". As followed, differences in "Alphonse Lavallée" genotype (28.28 to 32.32 µm) caused to a higher standard deviation (1.50) (Table 2).

Table 2- Morphological characteristic of pollen of grape cultivars (µm)

Cultivars	*Mean Pollen Size*											
	Pollen length (µm)				*Pollen width (µm)*				*Length/width ratio*			
	Min	*Max*	*Mean*	*SD*	*Min*	*Max*	*Mean*	*SD*	*Min*	*Max*	*Mean*	*SD*
1.	15.70	17.26	16.26 m	0.62	14.17	16.89	15.04 fghi	0.94	1.02	1.13	1.08 n	0.04
2.	23.55	29.87	26.86 abcdefgh	2.12	20.82	24.25	22.44 a	1.03	1.08	1.39	1.20 mn	0.13
3.	27.12	31.43	28.34 abcd	1.30	15.07	21.43	16.54 cdef	1.86	1.47	1.81	1,72 hijk	0.10
4.	22.15	26.46	23.97 hijkl	1.40	16.42	19.10	17.39 bc	0.68	1.16	1.61	1.38 lm	0.12
5.	26.47	30.56	28.18 abcde	1.61	15.28	18.89	17.70 bc	1.01	1.42	1.75	1.60 ijkl	0.14
6.	19.86	26.99	25.32 efghij	2.13	12.09	18.58	16.20 cdef	1.87	1.41	1.65	1.57 kl	0.08
7.	28.28	32.32	29.65 a	1.50	14.50	16.76	15.55 efgh	0.70	1.75	2.08	1.91 cdefghi	0.13
8.	19.23	30.05	26.99 abcdefg	3.45	13.11	16.96	14.72 fghij	1.21	1.44	2.15	1.84 efghijk	0.23
9.	24.34	32.86	28.42 abc	2.64	14.06	17.00	15.90 cdef	1.18	1.50	2.01	1.79 fghijk	0.15
10.	17.89	28.57	22.16 l	3.50	11.78	22.08	14.56 fghij	3.01	1.09	2.02	1.56 kl	0.33
11.	21.43	29.02	25.43 defghi	2.69	8.18	13.07	10.12 l	1.49	2.09	3.35	2.55 a	0.41
12.	22.79	29.10	25.98 cdefghi	1.98	10.07	16.48	12.98 jk	2.32	1.66	2.50	2.04 cdefg	0.26
13.	20.44	26.11	22.59 jkl	1.95	9.59	18.56	12.77 jk	2.56	1.41	2.44	1.82 efghijk	0.36
14.	20.82	29.52	25.74 cdefghi	2.99	9.65	17.03	12.94 jk	2.79	1.46	2.95	2.08 cdef	0.54
15.	20.25	31.90	25.04 ghijk	3.46	9.11	12.28	10.54 l	0.97	1.70	3.06	2.40 ab	0.39
16.	17.21	30.71	22.48 kl	4.25	9.20	11.96	10.32 l	0.96	1.59	3.25	2.21 bc	0.54
17.	18.61	32.13	27.19 abcdefg	4.09	12.41	20.23	15.10 fghi	2.57	1.31	2.32	1.84 efghijk	0.37
18.	20.31	30.47	25.13 fghijk	3.77	12.97	19.42	15.67 defgh	2.60	1.19	2.24	1.64 ijkl	0.34
19.	21.83	30.91	25.68 cdefghi	2.69	11.55	15.65	13.74 hijk	1.09	1.68	2.29	1.88defghijk	0.22
20.	19.23	28.47	25.01 ghijk	3.03	11.24	16.36	12.74 jk	1.76	1.62	2.47	1.99 cdefgh	0.31
21.	21.67	29.02	26.53 cdefghi	2.29	11.29	17.73	14.17 ghijk	2.08	1.56	2.29	1.90 cdefghij	0.24
22.	22.00	29.89	25.57 cdefghi	2.36	10.38	16.27	12.69 jk	1.85	1.49	2.38	2.05 cdefg	0.28
23.	20.38	28.01	25.49 defghi	2.19	10.13	14.19	12.26 k	1.65	1.74	2.76	2.11 bcde	0.33
24.	18.96	33.25	28.13 abcde	4.28	14.00	20.74	17.61 bc	2.06	1.19	1.96	1.60 ijkl	0.23
25.	20.84	27.40	23.67 ijkl	2.52	11.01	18.35	13.86 hijk	2.64	1.29	2.13	1,75 ghijk	0.27
26.	26.98	32.27	29.43 ab	1.74	11.43	16.90	13.96 ghijk	1.74	1.78	2.60	2.14 bcde	0.27
27.	26.98	32.27	29.43 ab	1.74	11.43	16.90	14.02 ghijk	1.82	1.71	2.60	2.13 bcde	0.28
28.	24.16	31.30	28.04 abcdef	2.72	10.85	16.74	13.32 ijk	1.94	1.59	2.74	2.16 bcd	0.43
29.	23.19	34.47	26.47 cdefghi	3.37	14.83	23.81	19.09 a	3.15	1.03	1.67	1.42 lm	0.26
30.	22.76	29.02	26.60 bcdefgh	1.97	11.79	21.58	17.18 cdef	2.64	1.26	2.46	1.59 jkl	0.30

* Min: minimum values; Max: maximum values; SD: standard deviations; **1.** *Vitis vinifera* L. "Foça Karası"; **2.** *Vitis vinifera* L. "Mahrabaşı"; **3.** *Vitis vinifera* L. "Yuvarlak Razakı"; **4.** *Vitis vinifera* L. "Beyaz Şam"; **5.** *Vitis vinifera* L. "Müşküle"; **6.** *Vitis vinifera* L. "Trakya İlkeren"; **7.** *Vitis vinifera* L. "Alphonse Lavallée'"; **8.** *Vitis vinifera* L. "Siyah Gemre"; **9.** *Vitis vinifera* L. "Cinsault"; **10.** *Vitis vinifera* L. "Kozak Gemresi"; **11.** *Vitis vinifera* L. "Alicante Bouschet"; **12.** *Vitis vinifera* L. "Buca Razakı"; **13.** *Vitis vinifera* L. "Malbec"; **14.** *Vitis vinifera* L. "İtalia"; **15.** *Vitis vinifera* L. "Syrah", **16.** *Vitis vinifera* L. "Öküzgözü", **17.** *Vitis vinifera* L. "Pembe Gemre", **18.** *Vitis vinifera* L. "Ohannes", **19.** *Vitis vinifera* L. "Papaz Karası", **20.** *Vitis vinifera* L. "Kırmızı Şam", **21.** *Vitis vinifera* L. "Morseyhative", **22.** *Vitis vinifera* L. "Morsleleh", **23.** *Vitis vinifera* L. "Abiguş", **24.** *Vitis vinifera* L. "Hafızali", **25.** *Vitis vinifera* L. "Çeşme Pembesi", **26.** *Vitis vinifera* L. "Cardinal", **27**. *Vitis vinifera* L. "Pek Üzümü", **28.** *Vitis vinifera* L. "Tarsus Pembesi", **29.** *Vitis vinifera* L. "Yuvarlak Çekirdeksiz", **30.** *Vitis vinifera* L.

On the other hand, when the pollen is examined in terms of symmetry and shape, the length/width ratio ranged from 2.55 µm "Alicante Bouschet" to 1.08 "Foça Karası" (Table 2). The terminology of Erdman (1952) was used in the morphological descriptions of the pollen. The pollen grains were prolate-spheroidal "Foça Karası" (8:7-8:8), subprolate "Mahrabaşı" (7:8-6:8), perprolate ("Alicante Bouschet", "Syrah", "Öküzgözü", "Tarsus Pembesi", "Abiguş", "Cardinal",

"Pek Üzümü", "Italia", "Morsleleh", "Buca Razakı" (>8:4)) and prolate ("Kırmızı Şam", "Alphonse Lavallée'", "Morseyhative", "Papaz Karası", "Siyah Gemre", "Pembe Gemre", "Malbec", "Cinsault", "Çeşme Pembesi", "Yuvarlak Razakı", "Müşküle", "Ohannes", "Hafızali", "Şika", "Trakya İlkeren", Kozak Gemresi", "Yuvarlak Çekirdeksiz", "Beyaz Şam" (8:4-8:6)) (Figure 1).

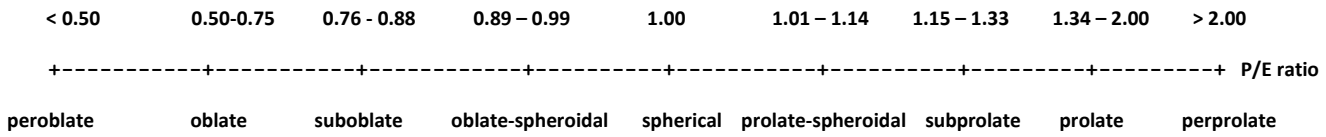

Figure 1- Mean value for P/E ratio (Marasalı et al. 2005; Gökbayrak & Engin 2016)

According to aperture, typically two types were observed. Among the grape varieties examined, it was determined that there was no diaphragm opening in the pollen of a group. Inaperturate pollen grains were observed in some cultivars such as "Foça Karası", "Mahrabaşı", "Trakya İlkeren", "Kozak Gemresi", "Ohannes" and "Çeşme Pembesi", whereas "Yuvarlak Razakı", "Beyaz Şam", "Müşküle", "Alphonse Lavallée'", "Siyah Gemre", "Cinsault", "Alicante Bouschet", "Buca Razakı", "Malbec", "İtalia", "Syrah" "Öküzgözü", "Pembe Gemre", "Papaz Karası", "Kırmızı Şam", "Morseyhative", "Morsleleh", "Abiguş", "Hafızali", "Çeşme Pembesi", "Cardinal", "Pek Üzümü", "Tarsus Pembesi", "Yuvarlak Çekirdeksiz", and "Şika" were tricolporate (Figure 2).

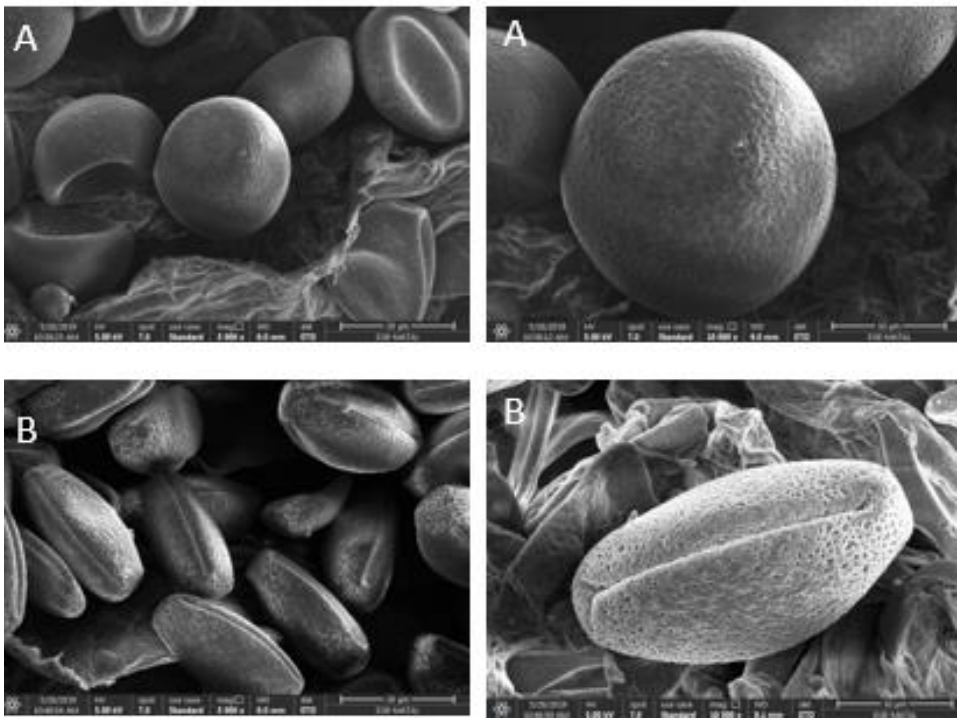

Figure 2- Scanning electron microscope image of pollen A: Inaperturate pollen B: Tricolporate pollen

Circular openings were detected on the pollen grains and such grains are called porate. The pollen had circular apertures on the exine surface, these were not uniformly distributed and the pollen grains were said pantoporate. The pollen grains surface has elongated or furrow-like apertures. These were called colp. Also, the circular apertures on the pollen has circular apertures on the exine surface, they were called pores. The pollen shape and exine patterns of the studied varieties were given in SEM images. There were statistical differences in terms of these properties. For pores length, "Pek Üzümü" (512.29) located at the first group, while the "Alphonse Lavallée'" (112.45) was the last group. Thus, pores width differed statistically significant according to the varieties. For this value, the "Morseyhative" (435.13), "Tarsus Pembesi" (405.98), "Çeşme Pembesi" (405.76) and "Trakya İlkeren" (389.33) varieties were the first group, among the varieties examined, "Syrah" was the smallest diameter of the pores width and, "Syrah" (97.06) located at the last group. In terms of this feature, it was found in different statistical groups in other varieties (Table 3).

Table 3- The length and width values of pores in grape varieties (µm)

Cultivars	*Pores length (µm)*				*Pores width (µm)*			
	Min	***Max***	***Mean***	***SD***	***Min***	***Max***	***Mean***	***SD***
1.	152.91	276.75	204.06 efg	51.24	141.95	260.77	196.85 cdefg	48.74
2.	109.80	332.63	208.54 efg	111.45	149.30	315.66	220.11 bcdef	70.65
3.	103.26	373.89	221.16 defg	120.53	106.60	193.19	136.22 efg	33.33
4.	321.56	482.35	396.57 abcd	66.87	159.84	498.23	328.72 ab	141.31
5.	144.56	280.39	186.67 fg	55.82	134.75	225.46	161.38 cdefg	36.37
6.	265.00	502.46	411.81 abc	91.41	250.00	483.65	389.33 a	93.90
7.	99.46	146.52	112.45 g	20.15	84.91	135.83	102.32 fg	20.13
8.	181.26	398.55	291.52 cdefg	78.13	166.76	289.55	247.52 bcde	47.16
9.	322.00	492.54	397.45 abcd	71.91	124.56	176.55	158.09 defg	22.20
10.	159.94	293.33	208.50 efg	51.79	156.54	267.45	195.59 cdefg	44.84
11.	194.20	280.33	217.18 defg	35.64	167.56	200.18	185.69 cdefg	16.11
12.	167.40	851.10	413.34 abc	276.47	150.80	510.30	280.92 bc	135.39
13.	217.20	349.90	298.82 bcdefg	52.02	180.51	250.54	209.07 cdefg	25.73
14.	159.30	305.50	219.18 defg	63.19	94.86	136.80	118.63 fg	21.30
15.	111.20	288.90	178.48 fg	75.82	86.00	133.50	97.06 g	20.43
16.	99.49	356.50	228.46 defg	108.11	80.68	235.60	121.91 fg	63.99
17.	113.30	372.50	218.54 defg	114.11	100.80	190.16	134.22 efg	33.32
18.	149.90	493.20	330.24 bcdef	146.35	135.23	350.21	196.34 cdefg	90.53
19.	118.80	347.20	213.46 defg	115.16	115.55	135.66	160.39 defg	79.70
20.	176.20	978.20	384.28 abcde	334.34	84.98	267.80	189.01 cdefg	68.75
21.	197.60	778.70	474.54 ab	282.97	150.11	770.80	435.13 a	270.89
22.	214.90	354.70	311.98 bcdef	56.52	180.66	340.91	272.57 bcd	73.78
23.	141.90	217.50	175.20 fg	27.92	120.37	177.80	155.79 defg	24.60
24.	142.90	270.70	198.57 efg	51.63	140.05	166.35	152.57 defg	11.09
25.	419.80	439.20	430.93 abc	7.83	386.51	421.35	405.76 a	16.57
26.	178.39	398.80	265.36 cdefg	91.58	165.72	281.35	245.68 bcde	46.05
27.	498.65	546.65	512.29 a	19.54	191.60	203.63	198.14 cdefg	4.33
28.	291.00	523.66	431.81 abc	113.64	280.00	498.70	405.98 a	98.63
29.	147.30	295.60	214.60 defg	57.80	135.81	228.64	161.62 cdefg	37.84
30.	188.50	333.30	232.32 defg	61.57	170.80	328.74	212.80 cdefg	65.79

Min: minimum values; Max: maximum values; SD: standard deviations ;**1.** *Vitis vinifera* L. "Foça Karası", **2.** *Vitis vinifera* L. "Mahrabaşı", **3.** *Vitis vinifera* L. "Yuvarlak Razakı", **4.** *Vitis vinifera* L. "Beyaz Şam", **5.** *Vitis vinifera* L. "Müşküle", **6.** *Vitis vinifera* L. "Trakya İlkeren", **7.** *Vitis vinifera* L. "Alphonse Lavallée'", **8.** *Vitis vinifera* L. "Siyah Gemre", **9.** *Vitis vinifera* L. "Cinsault", **10.** *Vitis vinifera* L. "Kozak Gemresi", **11.** *Vitis vinifera* L. "Alicante Bouschet", **12**. *Vitis vinifera* L. "Buca Razakı", **13.** *Vitis vinifera* L. "Malbec", **14.** *Vitis vinifera* L. "İtalia", **15.** *Vitis vinifera* L. "Syrah", **16.** *Vitis vinifera* L. "Öküzgözü", **17.** *Vitis vinifera* L. "Pembe Gemre", **18.** *Vitis vinifera* L. "Ohannes", **19.** *Vitis vinifera* L. "Papaz Karası", **20.** *Vitis vinifera* L. "Kırmızı Şam", **21.** *Vitis vinifera* L. "Morseyhative", **22.** *Vitis vinifera* L. "Morsleleh", **23.** *Vitis vinifera* L. "Abiguş", **24.** *Vitis vinifera* L. "Hafızali", **25.** *Vitis vinifera* L. "Çeşme Pembesi", **26.** *Vitis vinifera* L. "Cardinal", **27**. *Vitis vinifera* L. "Pek Üzümü", **28.** *Vitis vinifera* L. "Tarsus Pembesi", **29.** *Vitis vinifera* L. "Yuvarlak Çekirdeksiz", **30.** *Vitis vinifera* L. "Şika"

Scrobiculate pollen was detected in "Alicante Bouschet*",* "Buca Razakı", "Ohannes", "Papaz Karası", "Morseyhative", "Morsleleh", "Hafızali", and "Tarsus Pembesi", on the other hand striate pollen was found among the other varieties (Figure 3). Pollen of some cultivars had not furrows. These pollens were found in "Alicante Bouschet*",* "Buca Razakı", "Syrah", "Ohannes", "Morseyhative", "Hafızali", "Cardinal", "Tarsus Pembesi", and "Şika" cultivars. Areolat was observed in "Foça Karası", "Mahrabaşı", "Yuvarlak Razakı", "Beyaz Şam", "Müşküle", "Trakya İlkeren", "Alphonse Lavallée'", "Siyah Gemre", "Cinsault", "Kozak Gemresi", "Malbec", "Italia", "Öküzgözü", "Pembe Gemre", "Papaz Karası", "Kırmızı Şam", "Morsleleh", "Abiguş", "Çeşme Pembesi", *"*Pek Üzümü", and "Yuvarlak Çekirdeksiz" (Figure 3).

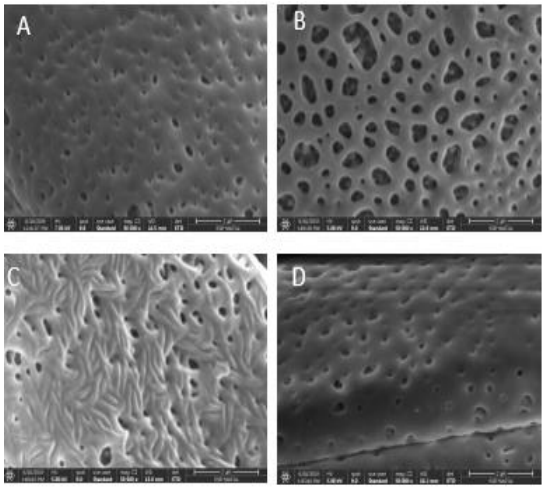

Figure 3- Pollen exine ornamentati on image A: Scrobiculate pollen B: Striate pollen C: Areola pollen D: without furrows pollen

The correlation coefficients of the features are shown in Table 4. Accordingly, the highest positive correlation was determined between pores length and pores width (r= 0.739; P<0.01). From the other side, a negative correlation occurred between the pollen length/width ratio and pollen width value (r= -0.816; P<0.01). Correlation between pollen length/width ratio and pollen width is not meaningful.

Table 4- Pearson correlation coefficient among traits in cultivars

Traits	*Pollen width*	*Pollen length*	*Pollen length/width ratio*	*Pores width*
Pollen length	0.247			
Pollen length/width ratio	**-0.816****	0.315		
Pores width	0.045	-0.073	0.123	
Pores length	0.104	0.076	0.080	**0.739****

Abbreviations: * Significant at P<0.05; ** Significant at P<0.01

Clustering analysis was used to determine the degree of similarity of grape cultivars, is located in Figure 4 as dendograms. Consequently, the cultivars were categorized under two main groups. “Yuvarlak Razakı”, “Pembe Gemre”, “İtalia”, “Öküzgözü”, “Müşküle”, “Abiguş”, “Papaz Karası”, “Yuvarlak Çekirdeksiz”, “ Hafızali”, “Alphonse Lavallée’”, “Syrah” “Foça Karası”, “Kozak Gemresi”, “Alicante Bouschet”, “Mahrabaşı”, “Şika” were included in the first group while “Beyaz Şam”, “Buca Razakı”, “Çeşme Pembesi”, “Tarsus Pembesi”, “Trakya İlkeren”, “Morseyhative”, “Malbec”, “Ohannes”, “Siyah Gemre”, “Cardinal”, “Morsleleh”, “Cinsault”, “Kırmızı Şam”, and “Pek Üzümü”, were collected in the second group. First and second groups divided into different sub-groups. The similarity of pollens of grape cultivars examined with CA showed a correlation with those examined with PCA in terms of examined characteristics.

Table 5 shows the degree of similarity of grape varieties with clustering analysis. Therefore, the relationships between grape genotypes examined with CA. According to PCA performed in 30 grape genotypes, 3 principal components were revealed and they defined 94.98% of the variance (Table 5). In this way, pollen width and the length/width ratio had the highest positive contribution to PC1, constituting 36.68% of the total variance, which is the most important component. The pores length and between pores width contributed to PC2, accounting for 34.81% of the total variance. On the other hand, PC3 constituted pollen length accounting for 34.81% of the total variance (Table 5).

Table 5- Component loading in Principle Component Analysis (PCA)

Traits	*PC1*	*PC2*	*PC3*
Pollen width	**0.960**	-0.029	0.262
Length/width ratio	**-0.944**	-0.026	0.309
Pores length	-0.105	**0.933**	0.086
Pores width	0.101	**0.932**	-0.087
Pollen length	-0.010	0.002	**0.998**
Eigenvalue	1.834	1.741	1.175
Proportion (%)	36.674	34.813	34.813
Cumulative (%)	36.674	71.487	94.977

Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization

Characterization of grape cultivars by pollen grains has been relatively frequent. A number of palynological investigations into cultivated *Vitis* varieties also showed that pollen shape and P/E ratio change from one sample to another (Reille 1966; Cabello et al. 1994; Roytchev 1997). Our results revealed that the thirty cultivars of *V. vinifera* exhibited differences about the pollen morphology. There were difference in the size (pollen width, pollen length), shape of pollen grains, pores on pollen surface and pollen ornamentation. All features reviewed were found to be the most important parameters for characterization.

There were significantly differences in pollen width grains sizes in the cultivars studied. The maximum pollen width size in the "Mahrabaşı" (22.44 µm) and the highest in "Yuvarlak Çekirdeksiz" (19.09 µm). The lenght of pollen grains ranged from 16.25 µm (Foça Karası) to 29.65 µm (Alphonse Lavallée'). Pollen width grains sizes of the "Cardinal" studied has 13.96 µm, and lenght of pollen grains has 29.43 µm. In relation to the results reported by Marasalı et al. (2005) and Gökbayrak & Engin (2016), the "Cardinal" pollens were medium sized, the values obtained in our studies were higher than those of the mentioned studies. The pollen width and length of "Yuvarlak Çekirdeksiz" were 19.09 µm and 26.27 µm, respectively. Roytchey et al. (1994) reported that pollen is same sized (the highest mean values - 24.13 and 24.04 µm) all investigated the seedless grape cultivars. As it is seen, it has been revealed that there are studies on pollen morphology in seedless grape cultivars.

Vitis is characterized by its 3-colporate grains. However, there was difference in pollen shape in our study. Prolate pollen grain was found in the material from "Foça Karası". Subprolate pollen grains was found in "Mahrabaşı". Perprolate pollen grains were on "Alicante Bouschet*",* "Syrah", "Öküzgözü", "Tarsus Pembesi", "Abiguş", "Cardinal", "Pek Üzümü", "Italia", "Morsleleh", "Buca Razakı" and prolate were in "Kırmızı Şam", "Alphonse Lavallée'", "Morseyhative", "Papaz Karası", "Siyah Gemre", "Pembe Gemre", "Malbec", "Cinsault", "Çeşme Pembesi", "Yuvarlak Razakı", "Müşküle", "Ohannes", "Hafızali", "Şika", "Trakya İlkeren", "Kozak Gemresi", "Yuvarlak Çekirdeksiz", "Beyaz Şam". To confirm our findings, pollen shape and P/E ratio differed from the findings of Marasalı et al. (2005) and Gökbayrak & Engin (2016) for grapes. Roytchev (1997) reported in seedless cultivars, this ratio varies from 1.10 (cv. Seedless Red) to 2.08 (cv. Russalka), being < 2 for most of the cultivars. The elliptical oval shape of pollen grains is typical for most of the seedless grapes. Inceoglu et al. (2000) stated that pollens of *Vitis sylvestris* ranged from prolate-spheroidal and subprolate pollen shape.

Erdtman (1952) reported reticulate pollen grains in members of the family *Vitaceae*. Faegri & Iversen 1989, on the other hand, reported that the exine sculpturing of *Vitis* was reticulate, foveolate-perforate and that lumina size increased towards the poles under LM. This study showed that in the thirty grape cultivars, exine sculpturing was obscurely reticulate under SEM, and scrobiculate and striate at the mesocolpia and distinctly reticulate at and around the poles. An increase in lumina size towards the poles, observed by SEM, supports the results for Faegri & Iversen (1989). The findings obtained by electron microscope in our study showed that the pollen morphological characteristics for the *Vinifera* cultivars can be used as a distinctive characteristic.

Ornamentation of the pollen is one of the most significant characteristics that can be used to separate cultivars. This situation reflects the variation between the cultivars. In grape species, the presence or absence furrows can be considered as useful tools for some taxonomic studies. Grape cultivars have also been subjected to palynological investigations (Reille 1966; Cabello et al. 1994; Roytchev 1997). As a result of this study, we found of furrows some of the examined cultivars, such pollens were found in "Alicante Bouschet*",* "Buca Razakı", "Syrah", "Ohannes", "Morseyhative", "Hafızali", "Cardinal", "Tarsus Pembesi", "Şika" cultivars. Areolat is surrounded in exine were determined in cultivars of "Foça Karası", "Mahrabaşı", "Yuvarlak Razakı", "Beyaz Şam", "Müşküle", "Trakya İlkeren", "Alphonse Lavallée", "Siyah Gemre", "Cinsault", "Kozak Gemresi", "Malbec", "Italia", "Öküzgözü", "Pembe Gemre", "Papaz Karası", "Kırmızı Şam", "Morsleleh", "Abiguş", "Çeşme Pembesi", "Pek Üzümü", "Yuvarlak Çekirdeksiz".

Pollen morphological characteristics such as pore structure, the ratio of P/E, and ornamentation at the polar and equatorial view are the most valuable variables for separating the grape species. The results of UPGMA clustering projection for species are quite common. The results from cluster analysis show that the examined members of the thirty grape cultivars that fall into two main groups coincide with pollen morphological features (Figure 4). According to PCA performed in the thirty grape genotypes, 3 principal components were revealed and they defined 94.98% of the variance. Pollen width and the length/width ratio had the highest positive contribution to PC1, constituting 36.68% of the total variance, which is the most important component. While PC1 is related to pollen width, PC2's association with pores length and pores width are a useful value in differentiation between varieties (Table 5).

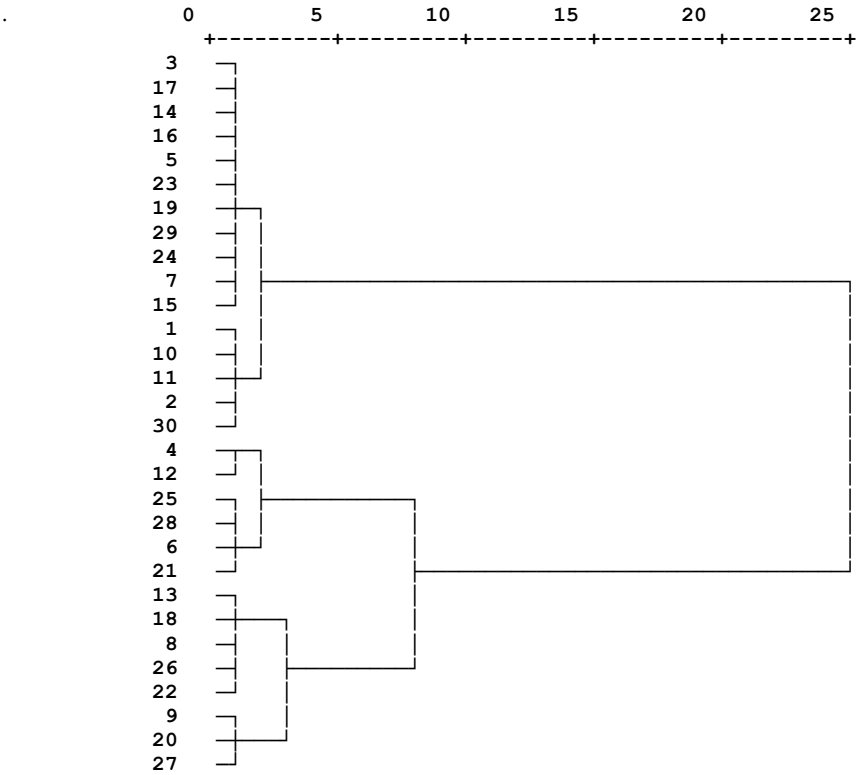

Figure 4- Dendrogram of hierarchical cluster analysis obtained by Ward's clustering method

4. Conclusions

In grape species, pollen morphology, exine characteristics, and the presence or absence of pores and furrows can be considered as useful tools for some taxonomic studies.

Morphological characteristics of pollens showed significant differences among the thirty grape cultivars. These properties are the most influential for classification of cultivars into particular groups. A number of palynological investigations into cultivated *Vitis* varieties also showed that pollen shape and P/E ratio change from one sample to another. The pollen features were found to be the most important parameters for characterization.

The present study confirms the inaperturate and tricolporate pollen grains were observed in cultivars. Some differences in size, polarity and ornamentation were observed among some of the studied cultivars in some cases among the thirty grape cultivar. There were differences in pollen ornamentation in the cultivars studied. In this regard "*Alicante Bouschet",* "Buca Razakı", "Syrah", "Ohannes", "Morseyhative", "Hafızali", "Cardinal", "Tarsus Pembesi", "Şika" cultivars were without furrows. On the other hand, areolat pollen determined in some cultivars were, such as "Foça Karası", "Mahrabaşı", "Yuvarlak Razakı", "Beyaz Şam", "Müşküle", "Trakya İlkeren", "Alphonse Lavallée'", "Siyah Gemre", "Cinsault", "Kozak Gemresi", "Malbec", "Italia", "Öküzgözü", "Pembe Gemre", "Papaz Karası", "Kırmızı Şam", "Morsleleh", "Abiguş", "Çeşme Pembesi", "Pek Üzümü", "Yuvarlak Çekirdeksiz". These morphological properties of pollen can be used for identification of varieties. Wine grapes and table grapes were distributed among groups. Considering the features examined, the fact that the cultivars can be divided into groups by means of a cluster analysis is an indication that they can be used in the identification of varieties. Palynology of *Vitis vinifera* L., is an adequate and complementary observation for identification.

Disclosure statement

No potential conflict of interest was reported by the author.

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Determining Factors Affecting Cooperative Membership of the Beekeepers Using Decision Tree Algorithms

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ABSTRACT

Agricultural cooperatives have important contributions to farmers. Thanks to cooperatives, agricultural products are sold at high prices, while agricultural inputs can be purchased at low prices. Cooperatives provide their partners with technical support in product processing, grading, standardization, storage and quality. On the other hand, cooperatives contribute to the sustainability of agricultural activities by providing credit support to their members. The current research was carried out Milas district of Muğla province, which is the center of pine honey production in Turkey. In the current research, a survey was conducted face to face with 62 farmers engaged in beekeeping, and the decision tree model, which is one of the data mining methods, was used in determining the factors that affect the beekeepers' membership in cooperatives. As a result of the statistical analyses conducted, it was concluded that on the cooperative membership of beekeepers, their status of using credit, education level and status of receiving beekeeping supports have a highly significant influence.

Keywords: Beekeeping, Farmers, Data mining, Machine learning, Milas

1. Introduction

Agricultural cooperatives are considered as one of the most important means of improving the living levels of farmers living in rural areas, increasing the income levels of producers with limited resources and providing agricultural development and are widely used in the world. Agricultural cooperatives are one of the most important tools used in the fight against poverty in rural areas where more than 70% of the world's poor people live (Mojo et al. 2015a).

When a general evaluation is made, it can be said that farmers are members of cooperatives due to economic concerns. On the other hand, cooperatives also have non-economic social and cultural benefits (Mojo et al. 2015b). For example, cooperatives create jobs and provide quality food and services to their members (Debeb & Haile 2016). Cooperatives contribute to the increase in productivity. Cooperatives can also reduce transaction costs (Gutierrez 2014).

Small-scale farmers are often disadvantaged in bargaining, especially in developing countries. This situation prevents farmers from reaching competitive markets (Liu 2018). Therefore, it can be said that cooperatives are more beneficial especially for small scale farmers in developing countries. Thanks to cooperatives, small-scale producers can reach the market more easily, farmers' incomes increase, product efficiency increases and production costs decrease.

Cooperatives reduce the risks that will be encountered in the market by increasing the bargaining power of economically weak individuals. Thanks to cooperatives, new market opportunities are created. On the other hand, cooperatives are effective in increasing individual capacity, improving cooperative members' revenues and leadership skills (Woldu et al. 2013). Cooperatives establish stronger social ties, solidarity, trust and partnership among their members (Gutierrez 2014). Agricultural cooperatives play an important role in the modernization and commercialization of small-scale enterprises (Bernard & Spielman 2009). Agricultural credit cooperatives contribute to production by giving credit to their members. On the other hand, cooperatives provide cheap agricultural inputs to their members and thus help to reduce production costs. In the study conducted by Aksoy et al. (2017), it was determined that honey productivity is high in agricultural organizations owned by beekeepers who are members of a cooperative and are engaged in migratory beekeeping.

Farmers have to make very different decisions during agricultural production. Farmers must decide the products to be obtained (honey, beeswax, bee resin, pollen, etc.) and the technology to be used (hive type). Farmers' decision to become a

member of the cooperative is also an important decision for themselves. When the previous studies on cooperative membership are examined, it is seen that many factors affect the farmers' decision to become a member of the cooperative. In a study conducted by Karlı et al. (2006), it was determined that education level, communication level, gross income and land size affect farmers' cooperative membership. In a study conducted by Dorgi & Gala (2016), it was found that farmers who have information about the cooperative, who use credit and who have leadership characteristics are more likely to become members of the cooperative. Balgah (2019) determined that total cultivated area, the number of households and duration of agricultural experience have an effect on farmers' cooperative membership. In the study conducted by Kızılaslan & Gürler (1997), factors affecting the membership of the producers to a cooperative were found to be education, level of consciousness of cooperative, social status, the level of opening up to the foreign culture and the use of mass communication tools.

It is important to determine the factors that affect the decision of the beekeepers in the district of Milas to be a member of a cooperative in terms of both the development of agricultural cooperatives and the increase in the number of members of cooperatives. For this reason, the current study aims to determine the membership status of the farmers working in apiculture in the Milas district of the city of Muğla and the factors affecting their membership to cooperatives.

2. Material and Methods

2.1. The method employed in the collection of the data

The main material of the current research is made up of the data collected from the survey administered face-to-face to the participating farmers engaged in beekeeping activities in the Milas district. The main population of the study is comprised of 721 farmers engaged in beekeeping activities according to the records of the Milas Agriculture and Forestry Directorate. The survey was conducted with the farmers determined with proportional sampling volume (Genç et al. 2020; Niyaz & Demirbaş 2018). In the study, the confidence interval is taken as 90% and error margin is taken as 10%. A total of nine neighborhoods (villages) in the Milas district were included in the current study.

$$n = \frac{Np(1-p)}{(N-1)\sigma_P^2 + p(1-p)} \quad \text{(Eq. 1)}$$

In Equation 1; n: Total number of the farmers to be surveyed; N: Main population; p: is taken as 0.50 to arrive at the maximum sampling volume; (1-p): 0.5; σ_{px}^2= Variance

As a result of this calculation, the total number of the farmers to be surveyed was found to be 62. After determining the number of farmers to be surveyed, the farmers to be interviewed were randomly determined.

In the decision tree model, 13 variables that were thought to have an effect on the state of beekeepers' being a cooperative member or not were included (Table 1).

Table 1- Variables used in the decision tree model

Acronym	*Variable description*	*Type of measure*	*Data type*
AGE	Beekeeper's age	Age≤40 means "young" 41≤X≤60 means "middle-aged" Age≥61 means "old"	Numeric
AGREXP	Beekeeper's agricultural experience	Experience <21years means "little experienced" Experience 20<X<40 years means "moderately experienced" Experience >39 years means "highly experienced"	Numeric
NONA	Out-of agriculture income	With out-of agriculture income, without out-of agricultural income	Nominal
HIVE	The number of hives	The number of hives <101 means "small number of hives" The number of hives 100<X<201 means "moderate number of hives" The number of hives >200 means "high number of hives"	Numeric
EDUC	Beekeeper's education level	Period of education <6 means, "elementary school graduate" Period of education 5< means "more educated"	Numeric
CRED	Credit use	Uses credit, doesn't use credit	Nominal
STOR	Storage of honey	Stores honey, doesn't store honey	Nominal
INNO	Application of innovations	Applies innovations, doesn't apply innovations	Nominal
SUPP	Receiving beekeeping supports	Receives support, doesn't receive support	Nominal
MARK	Having access to market data	Beekeepers can have access to market data about bee products, they cannot have access to market data about bee products	Nominal
INFO	The state of needing information about beekeeping	Information about beekeeping is needed, information about beekeeping is not needed	Nominal
BEEXP	Experience about beekeeping	If the experience about beekeeping is <26 years then he/she is experienced, if the experience about beekeeping is >25 years, then he/she is very experienced	Numeric
FAM	Household population	If the household population is <4, then it is a small family, if the household population is >3 then it is a big family	Numeric

2.2. The method employed in the analysis of the data

A scale consisting of a total of 16 items was developed to determine beekeepers' attitudes towards agricultural cooperatives. The scale items were designed in the form of a five-point-Likert scale and the response options were designed as "1. Strongly disagree", "2. Disagree", "3. Moderately agree", "4. Agree", "5. Strongly agree" to elicit the participating farmers' attitudes towards the effect size of each item.

The decision tree model was used to determine the factors that affect the beekeepers' membership to the cooperative. In the current study, J48, naive bayes and random forest algorithms was used.

The decision tree is similar to the tree structure that has different nodes, such as root nodes, stem nodes, and leaf nodes. It is a frequently used technique in data mining for classifying a large amount of data and creating a data set with a similar structure (Ramya et al. 2018). One of the most widely used machine learning algorithms is the decision tree. When the literature is examined, it is seen that the decision tree method is widely used in different disciplines ranging from agricultural sciences to health sciences, social sciences to veterinary medicine.

This method systematically analyses data to derive important relationships between dependent variables and independent variables and to display them in a tree structure. The tree consists of nodes, branches and leaves (Rondovic 2019). At the top is the root node. In this node, a number of properties are tested and branches are derived from the root node according to the different results of this test. Each branch is connected to a new decision node and a new set of properties is tested to extract branches from these nodes. At the bottom of the tree structure are there leaf nodes from which no nodes are derived any more (Seyrek & Ata 2010). During the construction of the decision trees, it is of great importance to determine from which property to start branching; that is, division. The branching of the tree can be carried out according to the value that the entropy will take. The entropy is expressed as follows (Özkan 2016):

$$H(T) = -\sum_{i=1}^{n} p_i \log_2(p_i) \quad \text{(Eq. 2)}$$

In the 2nd Equation, T stands for entropy, p_i represents the likelihood of the presence of the *i.* data class found in the data set in the whole class (Bulut 2017).

3. Results and Discussion

3.1. Some socio-economic features of the beekeepers

The ages of the beekeepers are between 27 and 74 and the mean age is 48.1. In the study by Kızılaslan & Adıgüzel (2012), the mean age of the beekeepers was found to be 51.70. The mean year of education of the beekeepers was found to be 5.9 years. Demirbük & Kızılaslan (2020) found that 66.1% of the farmers are elementary school graduates, 23.8% are high school graduates, 6.5% are university graduates and 3.6% do not have any education. The mean number of family members is 3.6 persons. The mean agricultural experience of the beekeepers was found to be 26.3 while their mean beekeeping experience is 19.5 years. Farmers need working capital to supply inputs. For farmers who lack adequate financial resources, credit is an alternative source of finance. Of the participating beekeepers, 64.5% were found to have used credit.

Of the participating beekeepers, 88.7% (55 farmers) are engaged in other agricultural activities apart from beekeeping, 38.1% of the total agricultural income of the participating beekeepers comes from beekeeping activities and 25.8% of them have other income sources different from agriculture.

3.2. Findings related to the beekeepers' cooperative membership

Agricultural cooperatives include agricultural development cooperatives, agricultural credit cooperatives and agricultural sales cooperatives (Kızılaslan & Kızılaslan 2007). Agricultural development cooperatives are cooperatives founded to develop their members' production in many different product areas including plant production, animal production and forestry production, to conduct activities required to meet their members' needs for supplies, processing, marketing and evaluation, to help their economic and social development, to provide different lines of business, to make use of natural resources to increase their economic power and to be engaged in activities to promote the development of hand and home arts and agricultural industry (Can et al. 2017). Agricultural credit cooperatives are agricultural organizations that provide credit to their members under favourable conditions. The most important goal of agricultural sales cooperatives is to assist their members in marketing; that is, to find the best sales offers for the products of farmers in domestic and foreign markets.

One of the farmers' organizations is the producer associations. Producer associations have many different objectives such as to ensure the development of agricultural production, to provide technical and economic guidance to producers for this purpose, to provide producers with all kinds of agricultural inputs under appropriate conditions, to protect the rights of producers, to make necessary inquiries, to perform farmer training and extension services (Kızılaslan & Doğan 2013).

Of the participating beekeepers, 48.4% are members of agricultural cooperatives while 51.6% are not members. When the agricultural cooperatives of which the beekeepers are member are examined, it is seen that 13.3% of them are members of agricultural development cooperatives, 83.4% are member of agricultural credit cooperatives and 3.3% are members of agricultural sales cooperatives. As can be seen, there are significant differences between the beekeepers' rates of membership in agricultural cooperatives. The biggest reason for this difference is thought to be the difference in the need of beekeepers for the services of the cooperatives. Many of the beekeepers are members of agricultural credit cooperatives. This shows that a significant portion of the beekeepers who are involved in the current research need agricultural credit. On the other hand, 82.3% of the beekeepers were found to be member of the Beekeepers' Association. The farmers who are not members of any cooperative were asked "Do you want to be a member to any cooperative in the future?". To this question, 56.2% of the beekeepers said "yes" while 43.8% said "no". Among the reasons for the beekeepers' not being a member to any agricultural cooperative, the most prominent one is that there is no cooperative in the village (25.0%), followed by the lack of trust in agricultural cooperatives (21.9%) and inadequate number of hives (21.9%).

A significant number of farmers think that cooperatives support farmers in terms of competitiveness (4.02), contribute to their bargaining power (3.98) and help farmers in getting credit (3.95).

3.3. Results related to the decision tree

In order to determine the success in classification, the test and training clusters were determined through 3 different processes. In the first process, the data set was divided by using the percentage split. In the percentage split method, 66% of the data set was used for training and 34% for the test. In the second process, the 10 fold cross validation method and in the third process, the 3 fold cross validation method were used. For the success in classification, the accuracy criterion was used. Moreover, kappa statistics, Mean Absolute Error (MAE) and Root Mean Square Error (RMSE) values of the models were investigated.

The percentage split method was found to have yielded a higher rate of accuracy than the cross validation methods. Naive bayes algorithm was found to have yielded the highest rate of accuracy. For example, for the naive bayes algorithm in the percentage split method, the rate of accuracy was found to be 76.1905%.

Kappa statistics (kappa coefficient) is used to measure the compliance between the observations. 1 shows a perfect compliance (Vierra & Garrett 2005). In the current study, the highest kappa statistics value (0.5249) was obtained for the naive bayes algorithm in the percentage split method (Table 2).

MAE is used to determine the absolute error between the measurement values and model predictions. The closer the MAE value to zero, the better the prediction ability of the model is (Eren & Eyüboğlu 2011). The lowest MAE value (0.3505) was obtained for the J48 algorithm in the percentage split method in the current study (Table 2).

RMSE is used as the error ratio between the measurement values and model predictions and a RMSE value converging to zero indicates increasing prediction ability of the model (Eren & Eyüboğlu 2011). The lowest RMSE value (0.4319) was obtained for the random forest algorithm in the percentage split method in the current study (Table 2).

Table 2- Values of Kappa statistics, MAE and RMSE

	Kappa statistics		
	J48	Naive bayes	Random forest
Percentage split	0.4324	0.5249	0.4324
10 folds CV	0.2282	0.3582	0.0921
3 folds CV	0.0996	0.1935	0.1236
	MAE		
	J48	Naive bayes	Random forest
Percentage split	0.3505	0.3592	0.3777
10 folds CV	0.4581	0.4113	0.4127
3 folds CV	0.4587	0.4279	0.4532
	RMSE		
	J48	Naive bayes	Random forest
Percentage split	0.4845	0.4344	0.4319
10 folds CV	0.5729	0.4888	0.4652
3 folds CV	0.5938	0.5051	0.5047

In the current study, in order to construct the decision tree, a total of 13 independent variables that are the beekeeper's age, the beekeeper's agricultural experience, the beekeeper's status of having out of agricultural income, the beekeeper's number of hives, the beekeeper's education level, the beekeeper's state of using credit, the beekeeper's state of storing honey, the state of beekeepers' having access to beekeeping data, their state of needing data about beekeeping, producers' experiences of beekeeping, household population, the beekeeper's state of adopting innovations, the beekeeper's state of receiving beekeeping supports were included in the model. However, as a result of the analysis conducted, it was determined that there are three variables in the decision tree that are the beekeeper's state of using credit, the beekeeper's education level and the beekeeper's state of receiving beekeeping supports (Figure 1).

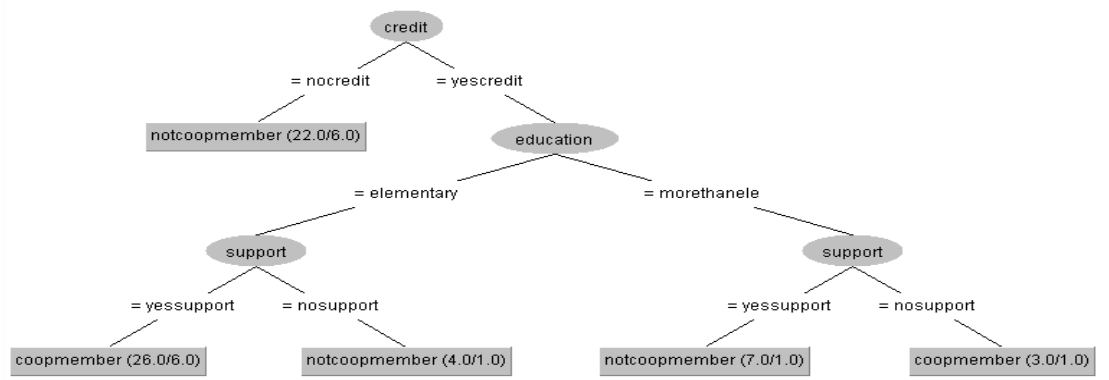

Figure 1- The decision tree for the factors affecting the membership to cooperatives

The decision tree produces rules. The decision tree obtained in the current study was found to have produced five rules. These rules produced by the decision tree are shown below. Weka software was used in the analysis.

Rule 1: If it is =nocredit then
notcoopmember
Rule 2: If it is =yescredit and =elementary and =yessupport then
coopmember
Rule 3: If it is =yescredit and =elementary and =nosupport then
notcoopmember

Rule 4: If it is =yescredit and =morethanele and =yessupport then notcoopmember
Rural 5: If it is =yescredit and =morethanele and =nosupport then coopmember

The important point to be taken into consideration here is not to evaluate the individual variables within a rule separately, but to evaluate the variables within a rule together. For example, when Rule 2 and Rule 4 are examined, it is seen that the status of being a member of a cooperative changes due to the difference in education level.

In the decision tree, first the beekeepers' status of using credit was examined. The beekeepers not using credit are not members to cooperatives. Then, a classification of the beekeepers using credit was made according to their education level. Thus, these beekeepers were divided into two classes as those having elementary education and those having education more than elementary education. If the beekeepers having elementary education are receiving beekeeping support then they are cooperative members. If the beekeepers having elementary education do not receive beekeeping support then they are not cooperative members. If the beekeepers having education more than elementary education receive beekeeping support then they are not cooperative members. If the beekeepers having education more than elementary education do not receive beekeeping support then they are cooperative members.

In the current study conducted to determine the factors affecting the cooperative membership of the farmers working in the Milas district, it was found that education level of the beekeepers is highly influential on their decision to be a cooperative member. Ogunleye et al. (2015), Anigbogu et al. (2017) reported similar results. In these studies, it was also found that the farmers' education level affects their cooperative membership.

In the present study it was also found that the beekeepers' state of receiving beekeeping supports affects their membership to a cooperative. Ertek et al. (2016) found that the amount of the support received by the producers affects their decision to be a member of a cooperative. Similarly, in a study conducted by Debeb & Haile (2016), the amount of the support received was found to be positively affecting the membership to a cooperative.

When the decision tree was structurally examined, it was determined that the producers with primary education and beekeeping support were cooperative members and the producers with higher levels of education and receiving beekeeping support were not cooperative members. These findings clearly show that the factors affecting the beekeepers' cooperative membership are the level of education and beekeeping supports.

Another factor found to be influential on the farmers' membership to a cooperative in the current study is their status of using credit. When the decision tree was structurally examined, it was found that the beekeepers not receiving credit are not cooperative members. This result concurs with the findings of Gashaw & Kibret (2018), Omotesho et al. (2016).

In the current study, it was determined that only three of the 13 variables used in the decision tree model had an effect on the cooperative membership of beekeepers. When the relevant literature was reviewed, it was found that many different variables have an effect on the membership of farmers in a cooperative. In fact, this is an expected result because the personality characteristics of farmers, farmers' perspective on cooperatives, examples of successful/unsuccessful cooperatives, cooperative subsidies, production patterns and agricultural structure can vary greatly from country to country.

The main advantage of decision tree classifiers is their capability to break down a complex structure into a collection of simpler structures, thus providing a solution that is easy to interpret (Cho et al. 2011). Another advantage of decision-tree approach is it visualizes the solution; it is easy to follow any path through the tree (Wang & Lee 2006). When the previous studies on the subject were examined, many studies using decision tree method in beekeeping were found. In a study conducted by Edwards-Murphy et al. (2016), the decision tree method was used in the monitoring of honey bee health. In a study conducted by Aksoy et al. (2018), some data mining algorithms such as MARS and CHAID were used to produce honey production estimates of beekeeping enterprises. In a study conducted by Karadas & Kadırhanogullari (2017), data mining and artificial neural network algorithms were used to determine the amount of honey production. When a general evaluation was made, it was determined that the studies using data mining methods in beekeeping were mostly for estimating the amount of honey production, and the data mining methods were not used sufficiently in the studies to determine beekeepers' perspectives of cooperatives. This current study is seen to be important as it is believed to fill this void in the literature.

4. Conclusions

The beekeepers participating in the current study were found to be middle-aged, elementary school graduates and engaged in beekeeping for a long time in general. In the current study, the beekeepers' education level was found to have a positive influence on their cooperative membership. As it will be difficult to give formal education to these farmers, their education level should be increased through informal education. For this reason, farmer training should be carried out through agricultural extension activities.

In light of the findings of the current study, it can be argued that the cooperative membership ratio of the farmers is not very high. It was also found that the beekeepers do not have much information about cooperatives and they do not much trust in cooperatives. Therefore, a comprehensive extension and training program including information about why cooperatives are established and their economic and social impacts.

High majority of the farmers were found to be members of the Beekeepers' Association. On the other hand, there are three different cooperatives of which beekeepers are members, which shows that there is an awareness of cooperatives in the district. Therefore, through effective agricultural policies and support programs to be implemented, more beekeepers are believed to be convinced for cooperative membership.

In the current study, it was determined that decision tree algorithms can be used to determine the factors affecting the cooperative membership of beekeepers. Therefore, it is thought that the results of this study will provide guidance for other studies to be conducted on the subject. On the other hand, in order to increase the accuracy rate in decision tree models, it is planned to add some variables to the model by increasing the number of questionnaires in future studies.

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Determination of Some Growth and Development Characteristics Between Birth and Twelve Months Age in Yerli Kara Cattle

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ABSTRACT

This study was carried out for the purpose of determination of some growth and development characteristics in the period between birth and twelve months of age in Yerli Kara cattle. The animal material of the study was composed of 104 Yerli Kara calves located in Ankara province. In the study, live weight, withers height, rump height, chest girth, body length, chest depth and front wrist girth values at birth were found as 14.85 kg, 58.00 cm, 60.10 cm, 54.41 cm, 49.36 cm, 24.25 cm and 7.40 cm respectively. The same values at the age of three months were found as 49.37 kg, 73.95 cm, 76.84 cm, 83.45 cm, 79.14 cm, 35.45 cm and 9.46 cm respectively. Values at the age of six months in these calves were detected as 81.22 kg, 87.29 cm, 90.35 cm, 99.36 cm, 92.93 cm, 42.79 cm and 10.58 cm respectively. Values at the age of twelve-month were determined as 97.29 kg, 92.15 cm, 95.55 cm, 106.22 cm, 98.07 cm, 45.26 cm and 10.74 cm in the same order. Average daily gains values were found 0.360 kg in the period between birth and 3 months of age, 0.333 kg from 3 to 6 months, and 0.102 kg from 6 to 12 months. The results showed that the body measurements of animals in this study are generally lower than the values reported in literature. Therefore, a selection program considering this fact will be useful for the development of native Yerli Kara cattle maintained at the farm operations of Ankara province.

Keywords: Average daily gain, Body measurement, Live weight, Yerli Kara cattle

1. Introduction

Turkey has a great potential in terms of cattle presence and it is among the leading countries in the world with 18 million cattle. 9.23% of these cattle are indigenous breeds (TUIK 2020). In this context, Yerli Kara cattle which is the best locally adapted breed to the inefficient conditions is the domestic cattle breed with the highest number and largest living area in Central Anatolia of Turkey (Boztepe et al. 2015; Ünal et al. 2019). They are grown extensively in mountainous regions, and primitive maintenance, feeding and barn conditions.

Growth and development characteristics are of great importance in cattle which is the most important source of meat and milk production. In cattle breeding, as a measure of growth and development, various body measurements are taken as basis. The most important of these parameters is birth weight. Birth weight is the easiest and most reliable measure of prenatal growth and an important factor affecting postnatal growth and development (Akbulut et al. 2001; Karabulut et al. 2012). Özhan et al. (2012) have reported that the calves with an average birth weight of 32 kg growed up faster than the calves with an average of 23 kg in a herd. Therefore, it is also of great importance in economic terms. Other body measurements such as withers height, rump height, chest girth, body length are also characters that are effective on growth and development (Akbulut et al. 2001; Bilgiç and Alıç 2004; Wu et al. 2004; Karabulut et al. 2012). There are several factors that have effect on the birth weight and body measurements of a calf. The effected environmental factors can be listed as maternal age and weight at birth, maternal ability, nutrition, year and season of calving (Souza et al. 1994; Akbulut et al. 1998; Kaygısız et al. 1998).

There are limited number of studies showing about live weight and body measurements for Yerli Kara cattle. It is also important to determine the effect of environmental factors on these characteristics to reveal the growth and development performance up to one year in the conditions of the region where the Yerli Kara cattle is widely grown. This study was carried out to determine some growth and development characteristics in the birth and 3, 6 and 12 months of age of Yerli Kara calves maintained at different farms. In addition, average daily gains of the animals were determined in the specified periods.

2. Material and Methods

The animal material of this study was consisted of Yerli Kara Cattle grown in private farm operations located in Osmansin village, Çamlıdere district of Ankara. This breed has been conserving within the scope of the project of "Conservation of Domestic Genetic Resources and Sustainable Use" conducted by General Directorate of Agriculture Research and Policies (TAGEM). The study was carried out on a total of 104 heads Yerli Kara from 20 different farms. Calves under study was born in January, February, and March of 2018.

In this region, Yerli Kara breeding is mostly carried out as extensive livestock system which is also named as a traditional farming. The animals are kept in the barns during the winter, while they are raised in the pasture in summer. In spring and autumn periods, the animals are kept in the barn of the farms in the village at night, and they are sent to pasture around the village in the daytime. The animals are taken to the uplands of village in early May. In the summer period, the animals are grazed completely in the pasture during the day. They are kept in a corner of the pasture or surrounded barnyards by nights. No additional feed is given to the animals grazed in the pasture. In winter, animals are kept in completely closed village barns under poor care and feeding conditions. In the winter period, animals are fed with only straw and some farms provide very little additional meadow grass and concentrate feed. This situation causes to animals having a skinny and weak appearance after the winter months.

In the study, the data collected from 104 calves at birth and 3 months of age and from 102 and 97 calves at 6 and 12 months of age respectively. The data of birth period were taken within the first 24 hours after calves were born. Weights at 3, 6 and 12 months of age were taken in the mornings with an empty stomach. In addition to live weight (LW), withers height (WH), rump height (RH), chest girth (CG), body length (BL), chest depth (CD) and front wrist girth (FWG) were taken (Anonymous, 2020). Body weights and measurements of the animals were determined with a scale sensitive to 200 g, measuring stick and measuring tape.

The analyses of variance by Minitab 16 were used in the evaluation of data (Minitab 2010). The test of Tukey provided by Minitab were realized for multiple comparisons.-Live weights and body measurements including average daily gains of calves were analyzed by using the following statistical model (General Linear Model procedure):

$$Y_{ijklm}=\mu + a_i + b_j + c_k + d_l + e_{ijklm}$$

Where; Y_{ijklm}: observed data; μ: Overall mean; a_i: i. effect of calf's gender (1:female, 2:male); b_j: j. effect of maternal age (2-3, 4-6, 7 +); c_k: k. effect of birth month (1:January, 2:February, 3:March); d_l: l. effect of farms (1. farm, … 20. farm); e_{ijklm}: random error.

3. Results and Discussion

3.1. Growth and development

The mean and standard errors (SE) for live weight and body measurements at birth, 3, 6 and 12 months of ages are given in Tables 1, 2, 3 and 4, respectively.

Table 1- The least square mean (LSM) and standard error (SE) values of live weight and body measurements in Yerli Kara cattle at the birth period

Character	*n*	*LW (kg)*	*WH (cm)*	*RH (cm)*	*CG (cm)*	*BL (cm)*	*CD (cm)*	*FWG (cm)*
General	104	14.62±0.359	57.68±0.451	59.80±0.468	53.91±0.515	48.84±0.612	23.96±0.277	7.28±0.077
Gender								
Female	50	14.15±0.446	57.30±0.560	59.37±0.581	52.96±0.640^{b}	48.67±0.760	23.84±0.344	7.01±0.095^{b}
Male	54	15.10±0.448	58.06±0.561	60.24±0.583	54.86±0.642^{a}	49.02±0.763	24.08±0.345	7.55±0.095^{a}
P values		*0.079*	*0.254*	*0.213*	*0.015*	*0.696*	*0.555*	*0.001*
Maternal Age								
2-3	18	13.18±0.685^{b}	55.72±0.859^{b}	58.01±0.892^{b}	51.83±0.982^{b}	46.70±1.167^{b}	22.95±0.528^{b}	7.04±0.146^{b}
4-6	52	15.39±0.401^{a}	58.47±0.502^{a}	60.59±0.522^{a}	55.42±0.574^{a}	49.94±0.682^{a}	24.73±0.309^{a}	7.46±0.085^{a}
7 +	34	15.29±0.535^{a}	58.85±0.670^{a}	60.82±0.696^{a}	54.48±0.766^{a}	49.90±0.911^{a}	24.20±0.412^{a}	7.35±0.114^{a}
P values		*0.012*	*0.007*	*0.020*	*0.006*	*0.035*	*0.011*	*0.035*
Birth Months								
January	21	15.43±0.682	57.92±0.855	59.95±0.888	54.96±0.977	48.42±1.161	24.81±0.526^{a}	7.27±0.145
February	46	14.78±0.441	57.40±0.553	59.54±0.575	53.99±0.632	48.03±0.752	24.76±0.340^{a}	7.38±0.094
March	37	13.66±0.506	57.72±0.634	59.92±0.659	52.78±0.725	50.09±0.861	22.31±0.390^{b}	7.19±0.108
P values		*0.074*	*0.811*	*0.843*	*0.167*	*0.132*	*0.001*	*0.296*
Farms								
P values	104	*0.122*	*0.812*	*0.781*	*0.328*	*0.131*	*0.761*	*0.254*

The differences between the averages expressed in different letters in the same column are important (P<0.05)

Table 2- The least square mean (LSM) and standard error (SE) values of live weight and body measurements in Yerli Kara cattle at 3 months of age

Character	*n*	*LW (kg)*	*WH (cm)*	*RH (cm)*	*CG (cm)*	*BL (cm)*	*CD (cm)*	*FWG (cm)*
General	104	47.41±1.117	73.47±0.816	76.32±0.828	82.83±0.759	77.57±0.811	34.96±0.432	9.42±0.078
Gender								
Female	50	45.98±1.386	72.92±1.013	75.69±1.027	82.16±0.942	77.16±1.001	34.43±0.537	9.17±0.097^{b}
Male	54	48.83±1.390	74.02±1.016	76.94±1.031	83.50±0.945	77.98±1.010	35.49±0.538	9.67±0.097^{a}
P values		*0.088*	*0.362*	*0.312*	*0.238*	*0.496*	*0.100*	*0.001*
Maternal Age								
2-3	18	41.98±2.128^{b}	70.94±1.556^{b}	73.67±1.577^{b}	79.61±1.447^{b}	74.98±1.546	34.01±0.824	9.16±0.149^{b}
4-6	52	49.59±1.244^{a}	73.52±0.909^{ab}	76.38±0.922^{ab}	83.76±0.846^{ab}	78.68±0.903	34.78±0.482	9.46±0.087^{ab}
7 +	34	50.66±1.660^{a}	75.95±1.214^{a}	78.90±1.230^{a}	85.13±1.128^{a}	79.06±1.206	36.10±0.643	9.65±0.117^{a}
P values		*0.002*	*0.024*	*0.020*	*0.007*	*0.062*	*0.071*	*0.025*
Birth Months								
January	21	47.57±2.117	73.61±1.548	76.10±1.569	82.13±1.439	76.31±1.538	34.56±0.820	9.38±0.149
February	46	47.36±1.370	73.23±1.002	76.06±1.015	82.79±0.931	77.78±0.995	34.87±0.531	9.41±0.096
March	37	47.29±1.571	73.56±1.148	76.79±1.164	83.58±1.068	78.62±1.141	35.46±0.608	9.47±0.110
P values		*0.994*	*0.957*	*0.864*	*0.690*	*0.466*	*0.611*	*0.848*
Farms								
P values	104	*0.002*	*0.168*	*1.161*	*0.008*	*0.019*	*0.007*	*0.028*

The differences between the averages expressed in different letters in the same column are important (P<0.05)

Table 3- The least square mean (LSM) and standard error (SE) values of live weight and body measurements in Yerli Kara cattle at 6 months of age

Character	*n*	*LW (kg)*	*WH (cm)*	*RH (cm)*	*CG (cm)*	*BL (cm)*	*CD (cm)*	*FWG (cm)*
General	102	78.19±1.705	86.46±0.610	89.67±0.619	98.31±0.789	91.62±0.895	42.11±0.370	10.45±0.081
Gender								
Female	48	75.81±2.151	85.83±0.769	89.24±0.781	98.05±0.996	91.40±1.129	42.13±0.467	10.19±0.102^{b}
Male	54	80.58±2.072	87.09±0.741	90.10±0.752	98.58±0.959	91.85±1.087	42.10±0.449	10.70±0.098^{a}
P values		*0.059*	*0.160*	*0.345*	*0.650*	*0.734*	*0.962*	*0.001*
Maternal Age								
2-3	18	70.26±3.182^{b}	84.10±1.138^{b}	87.04±1.155^{b}	94.59±1.473^{b}	88.47±1.670^{b}	40.84±0.690^{b}	10.13±0.151^{b}
4-6	50	81.72±1.937^{a}	87.45±0.693^{a}	90.67±0.703^{a}	99.98±0.897^{a}	92.61±1.017^{a}	43.14±0.420^{a}	10.50±0.092^{ab}
7 +	34	82.60±2.491^{a}	87.83±0.891^{a}	91.29±0.904^{a}	100.38±1.153^{a}	93.79±1.307^{a}	42.37±0.540^{a}	10.71±0.118^{a}
P values		*0.003*	*0.015*	*0.006*	*0.002*	*0.026*	*0.013*	*0.006*
Birth Months								
January	21	79.62±3.167	87.89±1.133	91.05±1.149	100.74±1.466^{a}	91.97±1.662	42.66±0.687^{a}	10.71±0.151^{a}
February	44	80.16±2.080	86.39±0.744	89.44±0.755	97.98±0.963^{ab}	92.97±1.091	42.58±0.451^{a}	10.52±0.099^{ab}
March	37	74.80±2.363	85.10±0.845	88.51±0.857	96.24±1.094^{b}	89.93±1.240	41.10±0.512^{b}	10.11±0.112^{b}
P values		*0.151*	*0.124*	*0.192*	*0.044*	*0.119*	*0.042*	*0.002*
Farms								
P values	102	*0.001*	*0.016*	*0.021*	*0.001*	*0.003*	*0.024*	*0.001*

The differences between the averages expressed in different letters in the same column are important (P<0.05)

Table 4- The least square mean (LSM) and standard error (SE) values of live weight and body measurements in Yerli Kara cattle at 12 months of age

Character	*n*	*LW (kg)*	*WH (cm)*	*RH (cm)*	*CG (cm)*	*BL (cm)*	*CD (cm)*	*FWG (cm)*
General	97	96.80±1.930	91.47±0.637	94.91±0.638	105.59±0.745	98.16±0.792	45.13±0.373	10.58±0.089
Gender								
Female	47	91.73±2.351^{b}	90.56±0.776	94.13±0.777	104.22±0.907^{b}	97.09±0.965	44.36±0.454^{b}	10.27±0.109^{b}
Male	50	101.86±2.351^{a}	92.38±0.776	95.70±0.777	106.96±0.907^{a}	99.23±0.965	45.90±0.455^{a}	10.88±0.109^{a}
P values		*0.001*	*0.044*	*0.081*	*0.010*	*0.056*	*0.004*	*0.001*
Maternal Age								
2-3	17	91.57±3.535	89.32±1.167^{b}	92.58±1.168^{b}	103.31±1.364	96.15±1.451	44.06±0.683	10.40±0.163
4-6	49	99.44±2.088	92.32±0.690^{a}	95.87±0.690^{a}	106.69±0.806	99.06±0.857	45.77±0.404	10.58±0.096
7 +	31	99.38±2.864	92.76±0.946^{a}	96.30±0.946^{a}	106.76±1.105	99.27±1.176	45.55±0.554	10.75±0.132
P values		*0.111*	*0.035*	*0.020*	*0.063*	*0.146*	*0.078*	*0.182*
Birth Months								
January	19	105.12±3.545^{a}	92.91±1.171	96.24±1.176	108.13±1.368^{a}	100.99±1.455^{a}	46.28±0.685^{a}	10.93±0.164^{a}
February	43	96.79±2.267^{a}	91.35±0.749	94.62±0.749	105.71±0.875^{ab}	99.24±0.931^{a}	45.17±0.438^{a}	10.55±0.105^{ab}
March	35	88.49±2.574^{b}	90.14±0.850	93.89±0.851	102.92±0.994^{b}	94.25±1.057^{b}	43.93±0.498^{b}	10.25±0.119^{b}
P values		*0.001*	*0.132*	*0.228*	*0.005*	*0.001*	*0.014*	*0.003*
Farms								
P values	97	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.004*	*0.001*

The differences between the averages expressed in different letters in the same column are important (P<0.05)

Live weights at birth, 3, 6 and 12 months of ages were found to be 14.85 kg, 49.37 kg, 81.22 kg and 97.29 kg respectively. Live weight from birth to 12 months of age for above mentioned traits were lower than the reported ranges (Demirhan 2008; Kılıçel 2014; Ünal et al. 2019) of 16.97- 21.35 kg, 63.21 - 68.18 kg, 101.04 - 110.33 kg, 152.16 kg- 184.57 kg, respectively. In addition, all body size values reported in the birth period were found to be lower than all values reported in the same literatures. In these periods, only body length value was found to be similar to the literature reports. In the study, the effect of gender was determined to be significant ($P<0.05$) in only the FWG in all measurement periods (Tables 1, 2, 3 and 4). In terms of body measurements, in the birth period the effect of gender on WH, CG, CD and BL values were reported (Demirhan 2008; Kılıçel 2014; Ünal et al. 2019) to be not significant, and FWG values as significant ($P<0.05$) and these findings were consistent with the values in our study. Demirhan (2008) found that the effect of gender was not significant in WH, CG, BL values in the other three periods (3, 6, 12 months). Kılıçel (2014) found that the effect of gender on WH and CG values at 3 months of age CG value BL at 12 months of age was significant while the same values found to be not significant in other periods.

In the studies conducted with our other local breeds in Turkey, Ünalan and Işık (2007) found significant ($P<0.01$) effect of gender on live weight and front wrist girth and not significant for withers height, body length, chest girth and middle rump width in the period of birth for the South Anatolian Red calves. Özlütürk et al. (2007) found that the effect of gender on live weights and body measurements in Eastern Anatolian Red calves were significant ($P<0.01$) in favor of male calves for birth, 3 and 6 months of age and not significant for 9 and 12 months of age.

In the studies conducted with other breeds, the effect of gender on live weight and body measurements of Holstein calves has been found by Hızlı et al. (2017) as significant ($P<0.01$) in birth and weaning (75 days) but not significant at the age of 6 months. Ayaşan et al. (2016) found a significant ($P<0.05$) effect in birth period but not significant for weaning (75 days) and 6 months old Holstein calves. Bayrıl and Yılmaz (2010) have not found a significant effect of gender on birth, weaning (60 days) and 6 months old weight in Holstein calves. Koçak et al. (2008) found a significant ($P<0.001$) effect of gender on the birth period weight in the Holstein, Brown-Swiss and Simental calves grown in Lalahan Livestock Research Institute.

Abera et al. (2012) found that birth weight of the calves significantly ($P<0.05$) influenced by sex, where male calves were heavier than females at birth. However, female calves were superior ($P<0.05$) at weaning and yearling and also had faster growth rate than male calves. Villalba et al. (2000) found a significant ($P<0.05$) effect of sex on body weight in birth, 4, 5 and 6 months of age of Brown Swiss and Pirenaica breeds.

When the maternal age values in Tables 1, 2, 3 and 4 are examined, calves born from 4-6 years old cows and 7 years old cows and above were found to be superior to calves born from 2-3 years old cows in all measurement parameters, for the period of birth. In other periods (3, 6 and 12 months) the 7 years old age and above had higher values, and the 2-3 years old age group was found to be at the lowest values, in all measured parameters. The statistical difference between the groups was found to be significant ($P<0.05$) in all measurement parameters at birth and at 6 months of age. The effect of maternal age at 3-month was found to be significant ($P<0.05$) in LW, WH, RH and CG values. The 12-month period for this factor was found to be also significant ($P<0.05$) in WH and RH values. Demirhan (2008) found BW and WG values in the birth period, all values (LW, WG, CG, BL, WH) at 3 and 6 months of age, LW, WG, CG and BL values at 12 months of age in favor of calves born from first calving cows compared with calves of two or more calving cows. Kılıçel (2014) found the LW, WH, CG and BL values in favor of calves born from two or more calving cows compared with calves from first calving cows, in all measurement periods. Ünal et al. (2019) found the BW, WH, RH, CG, BL, CD and FWG values in the period of birth in favor of calves born from five years old and above calving cows compared with calves from four years and under calving cows.

In the studies conducted with our other local breeds for the effect of the maternal age, Ünalan & Işık (2007) found not significant values for LW, WH, BL, CG, and FWG in the period of birth in the calves of South Anatolian Red calves. Özlütürk et al. (2007) found significant ($P<0.01$) values for live weights in Eastern Anatolian Red calves in favor of male calves in birth, 6 and 12 months of age and not significant at 3 and 9 months of age.

As for the studies conducted with other breeds, the effect of maternal age on live weight; Hızlı et al. (2017) found a significant ($P<0.01$) effect in birth, weaning (75 days) and 6 months weight for Holstein calves. Ayaşan et al. (2016) found significant ($P<0.05$) effect of maternal age on birth, weaning (75 days) and 6 months old weight in Holstein calves. Bayrıl & Yılmaz (2010) did not find any significant effect of maternal age on birth, weaning (60 days) and 6 months old weight of Holstein calves. Koçak et al. (2008) found significant ($P<0.001$) effect of maternal age on the birth weight in the Holstein, Brown-Swiss and Simental calves grown in Lalahan Livestock Research Institute. Villalba et al. (2000) found a significant ($P<0.05$) effect of maternal age on 5 and 6 months of age weight but not significant in birth and 4 months of age in Brown Swiss and Pirenaica breeds calves.

In the current study, live weight and body size values generally were found to be lower than literature reports in all measurement periods. In the study area births are taken place in January, February and March. Therefore, the animals that will give birth in winter are housed in completely closed and stuffy barns, under insufficient care and feeding conditions. These situations prevent of calf development in the period of before and after birth. In addition, calves return from the pasture to the village after 7-8 months of age and housed the winter months on the farmings in the village. The animals cannot get enough nutrients here, which cause slower rate in the growth of the animals. This can explain the reason for the measurement values of

the 12-months calves, which are not significantly higher than the values of the six-months calves. The fact that other study results are better than the findings of the current study may be due to the better care and feeding of the animals in the institute environment.

Although the effect of gender in the growth periods of Yerli Kara cattle except for a few characteristics was statistically not significant, it was determined that the development of male calves was superior in all measurement periods (Tables 1, 2, 3 and 4).

This is the evidence of sex hormones affect growth positively and which is in coincide with the literature (Demirhan 2008; Kılıçel 2014; Ünal et al 2019). When according to the maternal age is examined, calves born from cattle of 4-6 years old age and 7 years old age and above were found to be superior to calves born from cattle of 2-3 years old age in all measurement parameters (Tables 1, 2, 3 and 4). This may be due to the fact that 2-3-year-old cows are not fully able to complete their development, and because they are the first calves in general, their maternity abilities do not fully develop and do not feed the calf adequately. In addition, since the calves are always with their mothers in Yerli Kara cattle, the effect of the maternal age continued in the following periods (6 and 12 months).

3.2. Average daily gains (ADG)

ADG values from birth to the 12th month in Yerli Kara calves presented in Table 5. In the study, ADG values were found to be 0.360 kg in the period between birth to 3 months, 0.333 kg in the period between 3 and 6 months and 0.102 kg in the period between 6 and 12 months. It can be said that the ADG of the animals are at the desired levels since values are above the targeted 300 g increase in both the periods between birth and 3 months and 3 and 6 months. The calf's genetic capacity, environmental factors and especially the milk provided by the mother to the calf determine the sucking period growth of the calves, and in this period, environmental factors have more effect than genetic factors (Alpan & Aksoy 2012).

Table 5- The least square mean (LSM) and standard error (SE) of average daily gains in Yerli Kara cattle between birth and 12 months of age

Period	*n*	*Birth - 3 Months*	*n*	*3 - 6 Months*	*n*	*6 - 12 Months*
General	104	0.360±0.010	102	0.333±0.012	97	0.102±0.006
Gender						
Female	50	0.350±0.013	48	0.320±0.016	47	0.087±0.006^{b}
Male	54	0.371±0.013	54	0.346±0.015	50	0.116±0.006^{a}
P values		*0.184*		*0.146*		*0.001*
Maternal Age						
2-3	18	0.316±0.020^{b}	18	0.305±0.023	17	0.125±0.011^{a}
4-6	52	0.376±0.012^{ab}	50	0.348±0.014	49	0.095±0.007^{ab}
7 +	34	0.389±0.016^{a}	34	0.346±0.018	31	0.086±0.009^{b}
P values		*0.010*		*0.228*		*0.016*
Birth Months						
January	21	0.353±0.020	21	0.347±0.023^{a}	19	0.142±0.011^{a}
February	46	0.358±0.013	44	0.355±0.015^{a}	43	0.096±0.007^{b}
March	37	0.370±0.015	37	0.297±0.017^{b}	35	0.068±0.008^{c}
P values		*0.747*		*0.017*		*0.001*
Farms						
P values		*0.001*		*0.011*		*0.001*

The differences between the averages expressed in different letters in the same column are important (P<0.05)

The values found in the current study comply with the information stated by Alpan & Aksoy (2012) who stated the ability of animals to utilize nutrients at the highest level therefore the highest level for the ADG. Also, it complies with the information that Estermann et al. (2003) reported that calves born at the end of the winter season are relatively higher ADG than other times. In addition, in accordance with the findings in the study, Casasus et al. (2002) reported that calves kept in pasture together with their mothers reached a lower live weight for those born in autumn compared to those born in spring in terms of 6-month live weight.

However, in the study, ADG slowed down significantly in period between 6-12 months. This may be due to the fact that this period coincides with the autumn and winter months and the animals is housed under insufficient care and feeding conditions of winter. Roth et al. (2008) reported that ADG slowed down in cases where weaning performed quickly in calves with weak body resistance and due to of the calves not being fed enough during this period. This information is accordance with the findings obtained in the current study.

4. Conclusions

The results determined in the research show that the live weights and body sizes of the calves are not at the desired level in the Yerli Kara cattle breed. In general, live weight and some body measurements were lower than the literature reports in all measurement periods. It was concluded that this might have been due to the maintenance and feeding conditions of farmer where the study was conducted. In addition, the animal material of this research was selected from different farms and also different breeder conditions. LW and body measurements of animals were taken in pasture conditions. Like these, it is thought that some uncontrollable environmental factors have negative effects. The fact that there is a limited number of resources related to the growth and development of Yerli Kara cattle is another negative factor in terms of comparison of the findings obtained. To prevent this, farmers need to make improvements in environmental factors. It is necessary to provide feed supplements at least to meet the nutrients needed by animals and to improve barn conditions. In addition, the application of adding outbred bulls to prevent inbreeding without disturbing the purity of the populations may prevent the problems that will occur genetically.

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Abbreviations and Symbols	
TAGEM	General Directorate of Agriculture Research and Policies
LW	live weight
WH	withers height
RH	rump height
BL	body length
CD	chest depth
FWG	front wrist girth
SE	standard error
n	number of materials
P	significance value
ADG	average daily gains

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Effects of Normal and Nano-Capsulated Thyme and Peppermint Essential Oils on Intestinal Morphology and Microbial Population of Broilers Fed on Standard and Low Crude Protein Diets

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ABSTRACT
This study was conducted to investigate the effects of normal and nano-capsulated forms of thyme and peppermint essential oils on intestinal morphology and microbial population of broilers fed on normal and low crude protein diets. The study was carried out with a total of 320 Ross-308 broiler chickens as a 2×2×2 (2 medicinal plant essential oils, 2 forms of oils and 2 levels of diet crude protein) factorial arrangements with 32 groups (10 chicks per replicate) according to a completely random design. This experiment was done in three periods included: Starter (1-10 days), grower (11-24 days) and finisher (25-42 days). The experimental diets had significant effects on intestinal morphology ($P<0.05$), so that the use of the usual form of essential oils while reducing the depth of the crypt, increased the ratio of the length of the villa to the depth of the crypt. In use of peppermint essential oil with standard protein, crypt depth increased, and the ratio of villi height to crypt depth was minimal ($P<0.05$). A decrease in dietary crude protein level reduced the population of lactobacilli in the intestine ($P<0.05$). However, the level of crude protein in the diet and the form of use of the essential oil, as well as the type of essential oil and its form, did not have significant effects on the intestinal microbial population ($P>0.05$). Based on the results of this experiment, it can be stated that in broilers, the use of the encapsulated form of thyme essential oil in diets with standard crude protein levels, improved the intestinal morphology and intestinal non-pathogenic microbial population of broilers. Ten percent reduction in dietary crude protein level had adverse effects on measured traits and is not recommended, and in case of reduction of dietary protein, it is necessary to use peppermint essential oils supplements.

Keywords: Crude protein levels, Essential oil, Intestinal morphology, Microbial population, Nano-capsulation

1. Introduction

Recently, the use of medicinal plants in poultry diets has been on the rise (Darabighane et al. 2017; Gadde et al. 2017). Medicinal plants are added to poultry diets for a variety of reasons. These include antimicrobial, antioxidant, growth enhancement, reducing drug use and chemical additives, improving the taste and marketability of poultry products, and improving blood parameters and immunity (Habibi & Ghahtan 2019; Moryani et al. 2020). Among the high-quality and widely used plants in this field, we can mention the medicinal plants of thyme and mint (Hassan 2019; Nouri 2019). Different types of thyme and mint essential oil are special importance due to their abundant contains of active ingredients such as menthol, carvone, limonene, β-pinene, menthone, α- pinene, geraniol, and effective drug combinations (Rabiei et al. 2011; Morsy 2017; Tariq et al. 2019; Aydın & Barbas 2020). In order to save on essential oil consumption and make it more effective, the use of encapsulated forms of various medicinal plants, including thyme, has become common, and it show promising results in the field of working with medicinal plants (Hosseini & Banabazi 2020; Maty & Hassan 2020).

Since protein is an expensive part of the diet and its excretion into the environment causes many environmental problems, in recent years special attention has been paid to reducing the crude protein content of animal rations by responsible institutions and breeders. Since reducing the level of crude protein in poultry rations has adverse effects such as reduces growth, reduces production, weakens the immune system, so this reduction should be in accordance with the criteria and the use of instructions that while achieving the above, animal performance should not be reduced (Shazali et al. 2019; UlAbiden et al. 2019).

Nanotechnology is a general term that refers to all advanced technologies in the field of nanoscale work. Nanocapsules, are capsules that have a nanometer diameter and can be inserted and encapsulated (Ji et al. 2019; Elbaz et al. 2020). Nano-capsuling of aromatic substances can improves the healing properties and facilitates their access. Because of their small size, these substances increase the mechanism of cell uptake and increase their efficiency (Bayramzadeh et al. 2019). Present

experiment carried out to evaluate the effects of using normal and nano-capsulated thyme and peppermint essential oils on intestinal morphology and microbial population of broiler with standard and low crude protein diets.

2. Material and Methods

A total of 320 male Ross-308 broilers (one-day-old) were sexed and weighed before starting the trial, then were divided into 2×2×2 factorial experiments consisting of thyme essential oils (0, 0.2 mL normal and capsulated), peppermint essential oils (0, 0.2 mL normal and capsulated) and crude protein (standard and 10% lower) with 32 units. For copulating, the loading capacity of essential oil at a concentration of 0.5–2% sodium alginate was first investigated, then was encapsulated with biocompatible calcium alginate hydrogel (Dima et al. 2013). Experiment was conducted in a completely randomized design with three growth periods including starter (1 to 10 days), growing (11 to 24 days) and finisher (25 to 42 days). Diets were formulated using the user-friendly feed formulation (UFFDA) program according to the Ross-308 broiler nutrition specification guidelines.

Table 1- Calculated nutrient contents of diets used in broilers

Feed ingredients	*Feeding periods*					
	Starter (1 to 10 days)		*Grower (11 to 24 days)*		*Finisher (25 to 42 days)*	
	Standard CP	*10% Lower*	*Standard CP*	*10% Lower*	*Standard CP*	*10% Lower*
Corn	58.0	63.3	61.7	60.6	65.5	69.0
Soybean meal	37.1	31.8	32.8	28.0	28.0	24.0
Canola oil	1.4	1.4	2.1	2.5	3.0	3.5
Dicalcium phosphate	2.3	2.3	2.3	2.3	2.3	2.3
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin suppliment[1]	0.25	0.25	0.2	0.25	0.25	0.25
Mineral supplement[2]	0.25	0.25	0.2	0.25	0.25	0.25
L-Lysine hydrochloride	0.1	0.1	0.1	0.1	0.1	0.1
DL- Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Calculated nutrients (%)						
Metabolizable energy (Kilocalorie per Kg)	3000	3000	3100	3100	3200	3200
Crude protein (%)	23.2	20.7	21.5	19.35	19.5	17.55
Calcium (%)	0.81	0.74	0.45	0.55	0.56	0.54
Available phosphor(%)	0.5	0.5	0.54	0.54	0.53	0.63
Sodium (%)	0.19	0.19	0.18	0.17	0.18	0.16
Available Lysine(%)	1.17	1.24	1.05	1.05	0.93	0.93
AvailableMethionine + Cystine(%)	0.63	0.63	0.59	0.59	0.53	0.49

[1]Composition of the supplement of used vitamins per Kg including: Vitamin A (IU) 22500, Vitamin D_3 (IU) 5000, Vitamin E (IU)45, Vitamin K (mg) 5, Vitamin B1 (mg) 4.3, Vitamin B_2 (mg) 16.5, Vitamin B12 (mg) 0.04, Acid Pentatonic (mg) 24.5, Acid Folic (mg) 2.5, Niacin (mg) 74, Pyridoxine (mg) 7.3, Biotin (mg) 0.04. [2]Composition of the supplement of used minerals per Kg including: Manganese sulfate (mg) 248, ferrous sulfate (mg) 125, zinc oxide (mg) 211, copper sulfate (mg) 25, iodate Calcium (mg) 25, Selenium (mg) 0.5, Colin (mg) 625, anti-oxidation (mg) 2.5

At the end of the experiment (42 days old), after 6 hours of starvation, 2 birds (10 birds in each replicate) were selected from each cage that close to the mean weight of the cage and after slaughter, intestinal morphology and microbial population were evaluated.

Data were subjected to statistical analysis according to a completely randomized design as a factorial arrangement of 2×2×2 using the general linear model procedure of SAS (9.2). Data were log-transformed before analysing in case of unequal variances (Hosseinpour et al., 2018). Means were compared using Tukey's tests at 5% probability, according to the following model:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + \varepsilon_{ijkl}$$

Where; Y_{ijkl}: dependent variable; μ: overall mean; A_i: the effect of thyme essential oils; B_j: the effect of peppermint essential oils; C_k: the effect of crude protein levels; AB_{ij}: Interaction of Factors A×B; AC_{ik}: Interaction of Factors A×C; BC_{jk}: Interactivity of Factors B×C; ABC_{ijk}: Interaction of Factors A×B×C and ε_{ijkl} = the random error.

3. Results and Discussion

3.1. Intestinal morphology

The effects of experimental treatments on intestinal morphology of broilers are presented in Table 2. Experimental treatments had no significant effects on villi and goblet cells of chickens, but resulted in changing the crypt and villi/crypt parameters, and the difference between means was statistically significant ($P<0.05$). Capsulation in contrast to non-capsulation, significantly increased the depth of crypts and reduced the ratio of villi to crypt ($P>0.05$). In interaction between diet crude protein and

medicinal plant, using standard protein with thyme had the best result about crypt depth and villi height. About interaction between diet protein level and capsulation, the best result about crypt depth and villi height was observed with normal protein level and no capsulated form of oils. In interaction between medicinal plant essential oil and form of use, the lowest crypt depth was resulted in group with normal crude protein level and non-capsulated. Thyme with non-capsulated form of oil had the lowest crypt depth (P>0.05). In interaction between diet crude protein level, medicinal plant essential oil and form of application, the best result was obtained with normal level of crude protein, thyme essential oil and non-capsulated forms of them (P.0.05). Among the crypt and villi/crypt parameters related to interactions (protein levels and essential oil type), the use of peppermint essential oil with normal protein, crypt depth increased and the ratio of villi height to crypt depth was minimal (P<0.05).

In an experiment was conducted by using thyme and oregano extracts on intestinal morphology, observed that the mixture of these herbal extracts effectively increased the heights of villi cells and reduced the numbers of goblets (Manafi et al. 2018). The antimicrobial properties of thyme were reported by many researchers.

Table 2- Effect of experimental treatments on intestinal morphology (µm)

Effects	*Villi*	*Crypt*	*Villi/Crypt*	*Goblet Cells*
Protein				
Abnormal	1117.8	173.2	6.4	42.1
Normal	1124.1	176.2	6.7	45.8
SEM	26.39	2.65	0.15	2.10
P-value	0.867	0.425	0.395	0.223
Medicinal plant				
Peppermint	1144.8	183.8	6.6	46.1
Thyme	1097.2	165.7	6.6	41.7
SEM	26.39	2.64	0.15	2.08
P-value	0.215	<0.0001	0.969	0.156
Capsulation				
Capsulated	1103.0	181.6^{a}	6.1^{b}	44.9
non-capsulated	1138.9	167.8^{b}	6.9^{a}	42.9
SEM	26.39	2.64	0.15	2.08
P-value	0.345	0.001	0.0009	0.517
Protein× Medicinalplant				
Abnormal×Peppermint	1128.1	184.2^{a}	6.2^{b}	44.6
Abnormal×Thyme	1107.5	162.2^{c}	6.7^{ab}	39.5
Normal×Peppermint	1161.4	183.3^{ab}	6.9^{a}	47.5
Normal×Thyme	1086.8	169.1^{bc}	6.4^{ab}	44.0
SEM	37.32	3.74	0.22	2.94
P-value	0.477	0.031	0.016	0.785
Protein×Capsulation				
Abnormal×Capsulated	1092.6	179.2^{ab}	6.0^{b}	44.4
Abnormal×non-capsulated	1143.0	167. 2^{b}	6.9^{a}	39.7
Normal× Capsulated	1113.3	184.0^{a}	6.2^{ab}	45.4
Normal×non-capsulated	1134.9	168.5^{b}	7.0^{a}	46.1
SEM	37.32	3.74	0.22	2.95
P-value	0.703	0.050	0.043	0.371
Medicinal plant×Capsulation				
Peppermint×Capsulated	1111.2	186.8^{a}	6.1	44.1
Peppermint×non-capsulated	1178.2	180.7^{a}	6.9	48.0
Thyme× Capsulated	1094.7	176.4^{a}	6.1	45.6
Thyme×non-capsulated	1099.6	154.9^{b}	6.9	37.9
SEM	37.32	3.74	0.22	2.95
P-value	0.414	0.050	0.948	0.060
Protein×Medicinal plant×Capsulation				
Abnormal×Peppermint×Capsulated	1046.0	182.5^{ab}	5.8	44.5
Abnormal×Peppermint×non-capsulated	1210.2	185.8^{ab}	6.6	44.7
Abnormal×Thyme×Capsulated	1139.2	175.9^{ab}	6.3	44.2
Abnormal×Thyme×non-capsulated	1075.7	148.5^{c}	7.2	34.7
Normal×Peppermint×Capsulated	1176.5	191.0^{a}	6.5	43.7
Normal×Peppermint×non-capsulated	1146.2	175.6^{ab}	7.3	51.2
Normal×Thyme×Capsulated	1050.2	176.9^{ab}	5.9	47.0
Normal×Thyme×non-capsulated	1123.5	161.3^{bc}	6.7	41.0
SEM	52.78	5.26	0.31	4.17
P-value	0.362	0.048	0.828	0.753

a, b, c: Values within a row with different superscripts differ significantly at P<0.05

Since the villi of the gastrointestinal tract are the first place to communicate with nutrients, they play a very important role in digestion and absorption of the small intestine (Sohel et al. 2019; Reynolds et al. 2020). An increase in villi height means an increase in intestinal absorption, and a decrease in crypt depth indicates a decrease in replacement of enterocytes, and a decrease in tissue changes. In other words, increasing the height of the villi and the depth of the crypt are directly related to the increase in renewal in epithelial cells (Rubin & Levin 2016; Martin et al. 2017; Khan et al. 2020).

3.2. Microbial characteristics of the intestine

The effects of experimental treatments on intestinal microbiology are presented in Table 3. In relation to the effects of experimental diets on the beneficial microbial population of small intestine in chickens, a decrease in the level of crude protein in diets reduced the population of lactobacilli in the intestine ($P<0.05$). The type and form of essential oils did not have significant effects on the microbial population of small intestine ($P>0.05$). Regarding the interactional effects of experimental materials on the microbial population of small intestine of chickens, the use of normal protein levels along with thyme essential oil increased the population of lactobacilli and the overall form in the intestines of chickens ($P<0.05$). However, the level of crude protein in the diet and the form of use of the essential oil, as well as the type of essential oil and its form did not have significant effects on the microbial population of the intestine ($P<0.05$). In the study of the three-way effects between the level of crude protein, the type and form of essential oil, the use of normal protein level with the encapsulated form of thyme essential oil, the total microbial population of form and lactobacilli was at its maximum ($P<0.05$).

Table 3- Effect of experimental treatments on intestinal microbiology (CFU/gr)

Effects	*Lactobacylus*	*Choliform*
Protein		
Abnormal	3.78^{b}	5.35
Normal	4.95^{a}	5.53
SEM	0.311	0.365
P-value	0.013	0.713
Medicinal plant		
Peppermint	4.22	5.32
Thyme	4.51	5.56
SEM	0.311	0.365
P-value	0.514	0.654
Capsulation		
Capsulated	4.00	5.64
non-capsulated	4.72	5.24
SEM	0.311	0.365
P-value	0.112	0.437
Protein × Medicinal plant		
Abnormal × Peppermint	4.00^{ab}	5.83^{b}
Abnormal × Thyme	3.55^{b}	4.85^{b}
Normal × Peppermint	4.43^{ab}	4.81^{b}
Normal × Thyme	5.46^{a}	6.26^{a}
SEM	0.439	0.516
P-value	0.032	0.027
Protein × Capsulation		
Abnormal × Capsulated	3.25	5.86
Abnormal × non-capsulated	4.30	4.83
Normal × Capsulated	4.75	5.43
Normal × non-capsulated	5.14	5.64
SEM	0.439	0.516
P-value	0.459	0.240
Medicinal plant × Capsulation		
Peppermint × Capsulated	3.75	5.51
Peppermint × non-capsulated	4.69	5.13
Thyme × Capsulated	4.25	5.77
Thyme × non-capsulated	4.762	5.34
SEM	0.439	0.516
P-value	0.629	0.965
Protein × Medicinal plant × Capsulation		
Abnormal × Peppermint × Capsulated	3.71^{ab}	6.99^{a}
Abnormal × Peppermint × non-capsulated	4.29^{ab}	4.67^{ab}
Abnormal × Thyme × Capsulated	2.78^{b}	4.72^{ab}
Abnormal × Thyme × non-capsulated	4.32^{ab}	4.99^{ab}
Normal × Peppermint × Capsulated	3.78^{ab}	4.03^{b}
Normal × Peppermint × non-capsulated	5.08^{ab}	5.59^{ab}
Normal × Thyme × Capsulated	5.72^{a}	6.82^{a}
Normal × Thyme × non-capsulated	5.21^{ab}	5.69^{ab}
SEM	0.621	0.729
P-value	0.013	0.017

a, b, c: Values within a row with different superscripts differ significantly at P<0.05

Numerous studies have shown the antimicrobial properties of plant extracts that can improve the flora and intestinal health of the bird's digestive tract by reducing the number of pathogenic bacteria (Ebrahimi et al. 2016; Yadav et al. 2016; Jin et al. 2020). Disadvantages include the presence of harmful microbes in the gastrointestinal tract, increased protein and amino acid degradation due to the secretion of substances such as urease enzymes by microbes (Sharma et al. 2019; Thapa et al. 2019). On the base of Hajipour et al. (2015) study, using thyme powder and essential oils in broilers diets significantly increased the lactobacilli population and reduced the numbers of E coli, also there have beneficial effect on intestinal cells morphology, So the highest villi and the lowest depth of crypt cells blogged to thyme treatments. Using capsulated form of oregano essential oil in contrast to normal form can improve the intestine microbial population and health status of broilers (Ghasemloo et al. 2017). Recently on the base of Mahdavi & Nobakht (2018) study, the mixture of thyme and ziziphora extracts and increase the population of lactobacillus and reduced of E coli numbers in broiler digestive tract. As a result, the use of medicinal plants

essential oils was reduced the gram-negative microbial population of the gastrointestinal tract, so the rate of proteins and amino acids degradation were decreased and more of them are absorbed and stored in the body.

4. Conclusions

Based on the results of this experiment, it can be stated that in broilers, the capsulated form of dietary thyme essential oil with standard crude protein level improved intestinal morphology and changed microbial population on the behalf of Lactobacillus. Also, a 10 percent reduction in the level of crude protein in the diet had adverse effects on measured traits and should be not recommended.

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Soil Temperature Prediction via Self-Training: Izmir Case

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ABSTRACT
This paper proposes a new model, called *Soil Temperature prediction via Self-Training* (STST), which successfully estimates the soil temperature at various soil depths by using machine learning methods. The previous studies on soil temperature prediction only use labeled data which is composed of a variable set *X* and the corresponding target value *Y*. Unlike the previous studies, our proposed STST method aims to raise the sample size with unlabeled data when the amount of pre-labeled data is scarce to form a model for prediction. In this study, the hourly soil-related data collected by IoT devices (Arduino Mega, Arduino Shield) and some sensors (DS18B20 soil temperature sensor and soil moisture sensor) and meteorological data collected for nearly nine months were taken into consideration for soil temperature estimation for future samples. According to the experimental results, the proposed STST model accurately predicted the values of soil temperature for test cases at the depths of 10, 20 30, 40, and 50 cm. The data was collected for a single soil type under different environmental conditions so that it contains different air temperature, humidity, dew point, pressure, wind speed, wind direction, and ultraviolet index values. Especially, the XGBoost method combined with self-training (ST-XGBoost) obtained the best results at all soil depths (R^2 0.905-0.986, MSE 0.385-2.888, and MAPE 3.109%-8.740%). With this study, by detecting how the soil temperature will change in the future, necessary precautions for plant development can be taken earlier and agricultural returns can be obtained beforehand.

Keywords: Soil temperature prediction, Self-training, Regression, Machine learning, Agriculture, Artificial Intelligence, STST

1. Introduction

As technology advances, new solutions are emerging to help simplify agriculture and improving productivity and consumer satisfaction with the increased interest in precision agriculture. The applications of modern information technologies, machine learning, and artificial intelligence offer favorable computational as well as analytical solutions by employing data from multiple sources for decision making in the management of crop production (Friedl 2018). Precision agriculture, soil temperature, and climatic parameters have complex inter-relationships and this complex problem can be efficiently solved using machine learning techniques. The estimation of greenhouse gas emission from agricultural soils (Hamrani et al. 2020), evaluation of farm efficiency (Nandy & Singh 2020), weed classification (Dadashzadeh et al. 2020), plant disease detection (Giraddi et al. 2020), and the determination of the concentration of chemical matters in a grain (Niedbała et al. 2020) are some of the agricultural issues where machine learning is frequently implemented, nowadays.

Soil is of great importance in the terrestrial ecosystem by affecting the physical, biological, and chemical processes. Many studies in agriculture are canalized into this field, especially in terms of the effects of soil moisture and soil temperature on crop yields and plant growth and due to their impact on organic and chemical substances found in soil (Hillel 2005; Yang et al. 2019). Soil temperature plays an important role in agriculture since it is closely related to the myriad events occurring in the soil. It is a very important ecological factor that affects plant life at all stages from seed germination to seedling growth and development. It has a great effect on germination speed and duration. Although other conditions are suitable, if the temperature is too low or too high, there will be little or no germination. If the soil temperature is suitable, biological and chemical activities in the soil continue. These activities stop when the temperature drops and the soil freezes. On the other side, soil resistance to the physical events such as erosion and subsidence can drop dramatically at high soil temperatures. For this reason, factors affecting soil warming and control of soil temperature are extremely important. If we predict further changes in soil temperature, we can develop new strategies in different application areas such as setting up automatic cooling / heating system or irrigation system and determining the planting dates of temperature-sensitive crops etc.

Because of the importance of the subject, various different regression and statistical analysis techniques were proposed considering machine learning such as support vector machines (SVM) (Xing et al. 2018), to estimate soil temperature, and decision tree regression (Pekel 2020) and the least-squares support vector machine (Ren et al. 2019) to predict soil moisture and

collaborative data mining using the algorithms of local polynomial regression, neural networks, k-nearest neighbor, support vector machine (Anton et al. 2019) to estimate both soil temperature and soil moisture.

The soils of Turkey are divided into three major groups: zonal, intrazonal, and azonal. Zonal soils that are formed by the dissolution of rocks under the influence of climatic conditions and vegetation consist of brown forest soils, podsolic forest soils, Terra-Rossa's, chernozems, brown steppe soils, chestnut steppe soils. Vertisols, rendzinas, and volcanic sandy soils are in the category of intrazonal soils that reflect the characteristics of the bedrock. Alluvial/colluvial soils and loesses are examples of azonal soils that are not dependent on natural factors such as climate and vegetation but are formed due to accumulation by the effect of external factors such as streams and wind (Gönençgil et al. 2016; Akengin & Dölek 2019).

The soil temperature of Turkey at the depth of 0.5 cm increases from the Black Sea coast to the Aegean and Mediterranean coasts and decreases continuously from the coastal belt to the mountains and Eastern Anatolia. The lowest underground temperature varies between -3 °C and -6 °C in the higher parts of Eastern Anatolia. The temperature varying between 1-3 °C in Central Anatolia rises to 4-6 °C in the Black Sea, Marmara, and North Aegean coastal belt, and to 9-10 °C in the Southern Aegean and Mediterranean coastal belt. It is between 3-6 °C on the low plains of Southeastern Anatolia. Soil temperature varies between 2-14 °C at a depth of 100 cm in winter. The temperature, which is 2-3 °C in the higher parts of Eastern Anatolia, increases to 8-9 °C in the Black Sea coastal zone and to 11-13 °C in the Aegean and Mediterranean coasts. The temperature, which is between 4-6 °C in Central Anatolia, rises above 10 °C in Southeastern Anatolia. The soil temperature at 0.5 cm depth in July, which characterizes the summer period, varies between 20-25 °C in Eastern Anatolia and 25-30 °C in Central Anatolia. The temperature, which is around 25 °C in the Black Sea coastal zone, reaches 30-35 °C in the Aegean coasts and 35-38 °C in Southeastern Anatolia. The temperature changes between 13-27 °C in 100 cm deep soil in July. The lowest decrease in the soil temperature towards the bottom in July occurs in the Eastern Anatolia and the Black Sea coastal zone, the highest decrease in the Mediterranean and Southeastern Anatolia. As a matter of fact, the temperature decrease at the depth of 0.5 cm to 100 cm in July reaches 5 to 7 °C on the Black Sea coast, 7 to 10 °C in Central Anatolia and 10 °C in Southeastern Anatolia (Gönençgil et al. 2016).

Table 1 displays the recent machine learning studies taking soil temperature prediction in Turkey as the main subject. In addition to the past values of soil temperature, meteorological factors such as air temperature, relative humidity, and solar radiation, etc. were generally used as input for the applied models for estimation. The experimental studies were made from 5 cm to 100 cm depth in general. The performed method was mostly artificial neural networks (ANN) among them.

Table 1- Recent studies for soil temperature prediction in Turkey*

Ref / Study Area	*Aim*	*Data*	*Methods*	*Performance Measure*
Alizamir et al. 2020b / The city of Mersin	Monthly ST prediction at depths of 5, 10, 50, and 100 cm	25-year (1986–2010) monthly values of AT, SR, RH, WS, and ST	ELM, ANN, CART, and GMDH	RMSE, NS, R^2
Kisi et al. 2017 / The cities of Adana and Mersin	Monthly ST prediction at the depths of 10, 50, and 100 cm	25-year (1986–2010) monthly values of AT, SR, RH, WS, and ST	ANN, ANFIS, and GP	RMSE, MARE, NS, R^2
Yener et al. 2017 / All of the 81 provinces in Turkey	Monthly ST prediction for shallow geothermal applications at depths of 5, 10, 20, 50, and 100 cm	Monthly values of AT and ST between 1960 and 2015	TIR, ANN, PDV, and soil heat calculator program	Maximum average percentage error
Citakoglu 2017 / 261 stations all over Turkey	Monthly ST prediction at depths of 5, 10, 20, 50, and 100 cm	AT, P, and ST values between 20 and 45 years of data (over the period from 1974 through 2010)	ANN, ANFIS, and MLR	MAE, RMSE, R^2
Kisi et al. 2015 / The city of Mersin	Monthly ST prediction at depths of 5, 10, 50, and 100 cm	25-year (1986–2010) monthly values of AT, SR, RH, WS, and ST	MLP, RBNN, and GRNN	RMSE, MAE, R^2
Bilgili M et al. 2013 / The 7 meteorological stations, namely, Afyonkarahisar, Aydın, Denizli, Kütahya, Uşak Manisa, and Muğla as the neighboring stations, and İzmir as the target station	Monthly ST prediction of a target station only using the data of neighboring stations at depths of 5, 10, 20, 50, and 100 cm	Monthly ST data between 2000 and 2006	SR analysis and ANN	MAPE, R
Bilgili M 2012 / Kütahya, Manisa, Usak, Afyonkarahisar, Izmir, Aydın, Denizli, and Mugla	Monthly ST prediction at depths of 5, 10, 20, 50 and 100 cm	Monthly AT and ST data between 2000 and 2006	ANN	MAE, R
Bilgili M 2011 / The city of Adana	Monthly ST prediction at depths of 5, 10, 20, 50, and 100 cm	Monthly values of AT, AP, RH, WS, R, and ST between 2000 and 2007	ANN	MAPE, R
Ozturk et al. 2011 / 66 Turkish state meteorological service locations	Monthly ST prediction at depths of 5, 10, 20, 50 and 100 cm	Altitude, latitude, longitude, monthly values of AT, SD, SR, AT between 2006-2008	ANN	RMSE, R
Bilgili M 2010 / The city of Adana	Monthly ST prediction at depths of 5, 10, 20, 50 and 100 cm	Monthly values of ST, AT, AP, WS, RH, R, SR, SD between 2000 and 2007	LR, NLR, and ANN	MAPE, R

*ELM, Extreme learning machine; ANN, Artificial neural networks; CART, Classification and regression trees; GMDH, Group method of data handling; ANFIS, Adaptive neuro-fuzzy inference system; GP, Genetic programming; TIR, Thermal infrared technique; PDV, Philip and de Vries model; SR, Stepwise regression; LR, Linear regression; NLR, Nonlinear regression; RMSE, Root mean square error; NS, Nash-Sutcliffe coefficient; R^2, Coefficient of determination; MARE, Mean absolute relative errors; MAPE, Mean absolute percentage error; R, Correlation coefficient; MAE, Mean absolute error; AT, Air temperature; SR, Solar radiation; RH, Relative humidity; WS, Wind speed; P, Precipitation; AP, Atmospheric pressure; R, Rainfall; SD, Sunshine duration.

Differently from the mentioned studies, this is the first study that is performed by one of the semi-supervised learning techniques known as "self-training" in the subject of soil temperature prediction. *Semi-supervised learning* is a machine learning approach in which there are a small number of samples whose output is known and a large number of samples with unknown labels to develop a classification/regression model during training (Belkin et al. 2006). One of the semi-supervised learning methods is *self-training* (Zhu & Goldberg 2009).

A novel model, called *Soil Temperature prediction via Self-Training* (STST) is proposed. The STST facilitates the capability of temperature estimation for new samples in case there are few numbers of labeled samples to discover the hidden patterns.

This is the first time that the analysis is being reported in detail to determine which regression method provides the most accurate predictions under the self-training framework and the variation in the performances. For this purpose, it compares the self-training versions of machine learning algorithms, including Random Forest (RF), Support Vector Regression (SVR), K-nearest Neighbors Regression (KNNReg), Extremely Randomized Trees (ETReg), Decision Tree Regression (DTReg), and Extreme Gradient Boosting (XGBoost).

This study is also original in that it investigates the performances of the semi-supervised machine learning algorithms on soil temperature prediction with different ratios of labeled data varying from 5% to 85% with an increment of 5. It should be highlighted that this paper is the first to propose a multi-depth self-training learning framework that considers estimating the soil temperatures at five different soil depths (10, 20, 30, 40, and 50 cm). It presents a new application of semi-supervised machine learning to provide a smart way of soil temperature prediction. The purpose is to estimate the soil temperature in Izmir, Turkey by investigating the dynamics of the past soil temperature & soil moisture data and the meteorological data.

2. Material and Methods

In this section, the materials used to collect data, the proposed "*Soil Temperature prediction via Self-Training*" (STST) model, and the machine learning methods used in the experiments are presented.

2.1. Data collection

The location of the experimental area is Izmir, Turkey during the dates of 01.09.2019 and 22.05.2020. Izmir is located in the Aegean region of Turkey between the latitude of 38° 24′ 46″ and the longitude of 27° 8′ 18″. It is located in the Mediterranean climate zone and it has hot and dry summers and warm and rainy winters. In the middle latitude zone, it is open to marine effects and has a climate affected by the tectonic characteristics of the coastal Aegean strip and the bay having inland sea character. Depending on the sunshine duration and sufficient amount of rainfall, the soil structure has an agriculturally suitable climate (Turkish State Meteorological Service, 2021). While the yearly mean value of rainfall is 700.2 mm, the yearly mean values of air temperature and soil temperature are 17.6 °C and 19.8 °C, respectively (Republic of Turkey Ministry of Agriculture and Forestry 2021). Its soil moisture and temperature regimes are xeric and thermal (Bolca et al. 2011; Kapur et al. 2018). Figure 1 shows the study area in the location map.

Figure 1- The location map of the study area

The distribution of the soil types are as follows: red-brown Mediterranean soils and limeless brown forest soils with the ratio of 16%, alluvial and colluvial soils with the ratio of 12%, brown forest soils with the ratio of 4%, red Mediterranean soils, and rendzinas with the ratio of 3%, chestnut soils with the ratio of 0.4% and regosols% with the ratio of 0.1. 22.5% of the soils in İzmir are deep, or very deep, 4% medium-deep, 38.5% shallow, and 35% very shallow (Dizdar 2003).

The properties of the collected soils for all depths are as follows: pH (KCL):7.2 pH, organic matter: 7.3%, total nitrogen: 975.0 kg/da, total phosphorus: 300.00 kg/da, total potassium 43.99 kg/da, clay: 9.1%, cation exchange capacity: 347.1 mmol+/kg. The soil texture is sandy loam.

A part of the dataset used in the experiments was collected using IoT devices (Arduino Mega, Arduino Shield) and various sensors (DS18B20 soil temperature (ST) sensor and soil moisture (SM) sensor for measuring hourly data at the soil depths of 10, 20, 30, 40, and 50 cm, and light-dependent resistor (LDR) sensor for hourly light intensity (LI)). The meteorological part of

the dataset was obtained from the web page of weather.com that provides air temperature (AT), humidity (H), dew point (DP), air pressure (AP), wind speed (WS), wind direction (WD), and ultraviolet index (UV). Hourly values of the aforementioned features were taken into consideration. After data collection, the records with missing values were removed. Finally, 4500 instances were left for data analysis.

A sample fragment of the dataset at the depth of 50 cm between 01:00 and 04:00 p.m. on September 19, 2019, is given as hourly in Table 2. SM and LI sensors have raw analogue reading values of 0 to 1023 as shown in Table 2. If the value of SM is close to 1023, it means soil moisture is high, otherwise low. In the same manner, if the value of LI is high, the light intensity is a lot. For the prediction of ST of each depth, SM values from all depths are taken into consideration.

Table 2- A part of the dataset used in the experiments*

AT (°C)	*H (%)*	*DP (°C)*	*AP (mb)*	*UV*	*WS (km/s)*	*WD*	*LI*	*SM_{50}*	*SM_{40}*	*SM_{30}*	*SM_{20}*	*SM_{10}*	*ST_{50} (°C)*
28	40	13	1015.2	7/10	3	Northwest	900	322	335	268	270	282	22.56
29	32	11	1013.9	7/10	5	Northwest	874	316	337	267	267	279	23.00
29	34	11	1014.2	5/10	18	West	866	320	339	267	266	276	23.38
28	28	13	1014.2	4/10	23	West	843	317	339	275	264	272	23.82

*AT, Air temperature; H, Humidity; DP, Dew point; AP, Air pressure; WS, Wind speed; WD, Wind direction; UV, Ultraviolet index; LI, Light intensity; SM, Soil moisture; ST, Soil temperature.

2.2. Proposed method: Soil temperature prediction via self-training (STST)

The rationale behind self-training is to increase the training set size with unlabeled data when there is a very small number of pre-labeled data compared to unlabeled ones so that a more optimized classifier model can be constructed using the updated labeled training set. Considering the problem of soil temperature prediction, we may not always be able to access all past data whose results are known to estimate new values. Because of the high cost of manual labeling, it is hard to obtain sufficient, reliable, and up-to-date labeled data for effective soil temperature prediction. In such cases, by applying the proposed *Soil temperature prediction via self-training* (STST) model, we first estimate the values for the unlabeled historical samples and then use all past records to estimate the new values for the future records.

The proposed approach (STST) has a number of advantages that can be summarized as follows:

- The traditional soil temperature prediction is limited to using only labeled data to build a regression model. Differently from the previous studies, the proposed STST approach overcomes this limitation and deals with the design of prediction models in the presence of both labeled and unlabeled data. In addition to labeled data, the STST approach also exploits unlabeled data to help improve soil temperature prediction performance. Due to the STST approach, the unlabeled data samples provide additional knowledge that is relevant for prediction, and they can successfully be used to improve the generalization ability of the learning system.

- An important advantage of the STST approach is that it can be used with the combination of any supervised base learner such as SVR, KNNReg, and ETReg. The STST approach is entirely unaware of the regression method, in fact, it simply learns from the labeled and pseudo-labeled samples as if they were regular labeled samples.

- Another advantage is that the STST approach can be applied to any soil data without any prior information about the given dataset. It does not make any specific knowledge and specific assumptions for the given data.

- Soil temperature prediction at different depths is useful in agricultural management (Abyaneh et al. 2016; Huang et al. 2020). However, the measured soil temperature data at various depths are rarely available for many locations. In many real-world agricultural applications, a huge amount of unlabeled data is available. The proposed STST approach addresses this inherent bottleneck by automatically allowing the model to integrate the available unlabeled data at various soil depths. Since the proposed STST approach covers multiple soil depths, it enables enormous agricultural applications, and so it expands the application of machine learning algorithms in the field of agriculture.

Figure 2 expresses the main course of this study as a series of processes. The first part is the collection of soil data at different depths, meteorological data, and light intensity by using IoT devices and sensors or by pulling data from web pages. After data collection, if needed, missing data imputation can also be performed, otherwise, the machine learning process including self-training takes part. In the self-training phase, a machine learning model is firstly built with the initial labeled data for the purpose of classifying unlabeled instances, and then, it is re-trained by adding its own estimations to the labeled data. After that, future

samples are assigned their predicted temperature values as an output. The final part is the reporting and presentation facility in order to adjust the findings in an interpretable format.

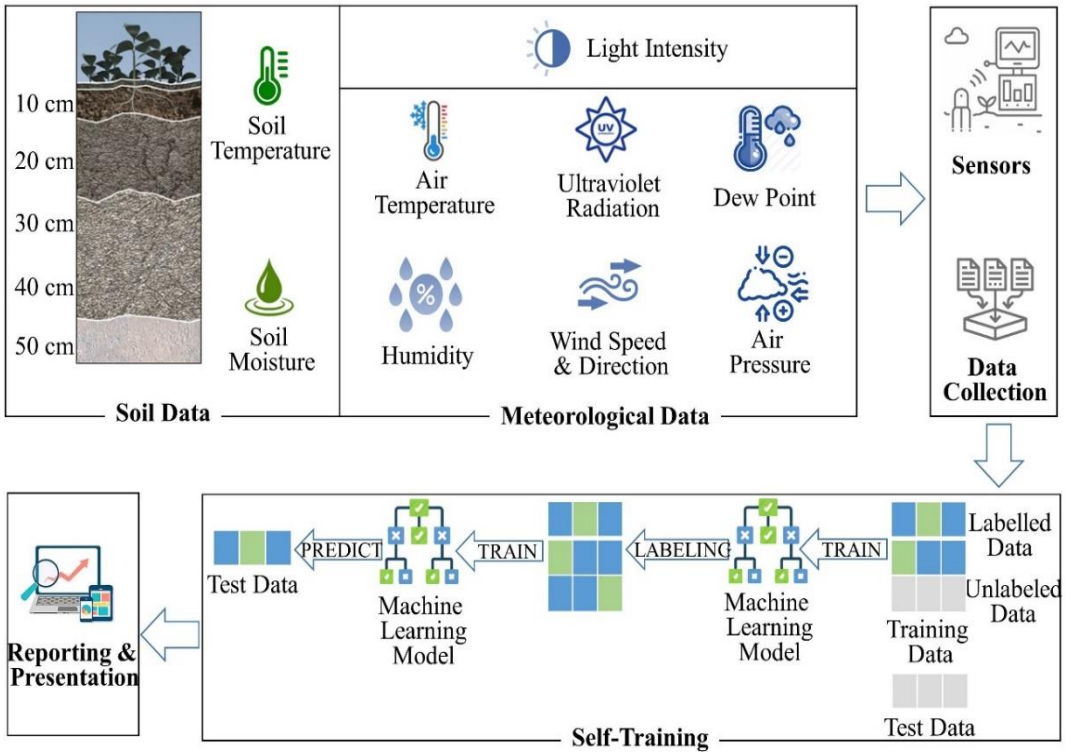

Figure 2- The general framework of the proposed "Soil temperature prediction via self-training (STST)" model

Algorithm 1 given below theoretically displays the pseudo-code of the training part of the proposed model. The aim is to obtain the soil temperature values (Y^*) of new instances in D_{Test} at all depths. In the first part, there are two sets of instances: the labeled dataset (D_L) and the unlabeled dataset (D_U). The hidden patterns affecting the soil temperature can be detected by analyzing the labeled instances. Therefore, using the instances in D_{L_d}, a classifier C_d is trained and a model that facilitates labeling other instances is obtained at the depth d. In this way, the pseudo-label (y_i) of each instance x_i in D_{U_d} is discovered. All the pseudo-labeled instances are then gathered together in D_d. Now, a new labeled training set D_{ALL} is ready by expanding the initially given D_{L_d} with the pseudo-labeled set D_d. The final step is to predict the temperature values (Y_d) of new instances at the depth d by using the new classifier model C_d trained with D_{ALL}. The resulting output is the predicted soil temperature values of all depths as the collection of each Y_d in the set Y^*.

Algorithm 1. Soil temperature prediction via self-training (STST)

Inputs:
D_L: the labeled dataset $D_L = \{(x_i, y_i)\}_{i=1}^{l}$ with l intances
D_U: the unlabeled dataset $D_U = \{x_j\}_{j=l+1}^{l+u}$ with u intances
D_{Test}: test instances
Outputs:
$\boldsymbol{Y^*}$**: predicted values**

foreach depth d **do**
  $\boldsymbol{C_d = Train\ (D_{L_d})}$
  foreach x_i **in** D_{U_d}
    $y = C_d\ (x_i)$
    D_d.Add(x_i,y)
  end foreach
  $D_{ALL} = D_{L_d} \cup D_d$
  $C_d = Train\ (D_{ALL})$
  foreach x_i **in** D_{Test}
    $y = C_d\ (x_i)$
    $Y_d = Y_d \cup y$
  end foreach
  $Y^* = Y^* \cup Y_d$
end foreach

End Algorithm

2.3. Machine learning methods

The machine learning methods performed in the experiments are SVR, RF, KNNReg, ETReg, DTReg, and XGBoost. The parameters of all the applied methods were left as their default values in the sklearn library of Python.

2.3.1. Support vector regression

The main aim is to minimize the error by maximizing the margin around the separating hyperplane. The general formula can be written as an optimization problem as in Equation 1 where w is the normal vector to the bounding planes, C is the penalty associated with the instances which are either misclassified or violate the maximal margin, $\phi(X_i)$ is a function which maps data point X_i into a higher dimensional space, b shows the positions of bounding planes relative to the origin and ξ is a slack variable for soft margins defined for linearly non-separable cases.

$$\min \frac{1}{2}\|w\|^2 + C\sum_{i=1}^{n}\xi_i \quad \text{subject to } Y_i(w^T\phi(X_i) + b) \geq 1 - \xi_i,\ \xi_i \geq 0 \tag{1}$$

In non-linear problems, the kernel functions, $K(X_i, X_j) = \phi(X_i)^T\phi(X_j)$, are used to transform the data into a higher dimensional feature space to make it possible to perform the linear separation. Two kernel functions are generally used for these cases as polynomial kernel (*poly kernel*) or Gaussian radial basis function (*RBF kernel*). For two samples X_i and X_j, the poly kernel function and RBF kernel function can be written as in Equation 2 and Equation 3, respectively.

$$K(X_i, X_j) = (aX_i^T X_j + b)^d \tag{2}$$

$$K(X_i, X_j) = \exp\left(-\frac{\|X_i - X_j\|^2}{2\sigma^2}\right) \tag{3}$$

2.3.2. K-nearest neighbors regression

It is one of the instance-based lazy learners where the method memorizes the training dataset instead of learning a discriminative function for predicting future samples. It compares a given test instance with training instances that are similar to it. The parameter k refers to the number of samples to be considered in the determination of the numeric outcome for a new sample. The

nearest neighbors are calculated using one of the distance metrics such as *Manhattan*, *Euclidean*, *Chebychev*, *Cosine*, etc. In the experimental studies, Euclidean distance given in Equation 4, where two points are described as $X_i = (x_{i1}, x_{i2}, \ldots, x_{im})$ for m features is used. The outcome of the k nearest samples to a specific instance are averaged.

$$\text{Dist}(X_1, X_2) = \sqrt{\sum_{i=1}^{m} (x_{1i} - x_{2i})^2} \tag{4}$$

2.3.3. Decision tree regression

It is one of the supervised learning methods where the regression scenario is represented as a tree-based system in which each branch points out a possible outcome. The depth-first strategy in a top-down recursive and divide-and-conquer manner is applied to predict unknown target values for test cases. Each node of the tree refers to a specific attribute as the branches show their values. The leaves carry the results (the value of the class label).

The construction of a decision tree is started with a root node. The determination of the initial node is based on the mutual information which gives the highest benefit for learning. For this purpose, *information gain* given in Equation 5, which is calculated for each attribute, is used that S is the instances of the parent node, A is an attribute to perform the split, S_{Left} and S_{Right} are the samples found in the left and right child nodes, respectively, and I is the impurity measure. Information gain evaluates the gain of each feature in the context of a target variable. It is performed by taking the mutual information (i.e. the determination of the statistical dependence) between two random variables. I is the mean squared error (MSE) of the children nodes, which is given in Equation 8.

$$\text{InfoGain}(S, A) = I(S) - \left(\frac{|S_{Left}|}{|S|} I(S_{Left}) + \frac{|S_{Right}|}{|S|} I(S_{Right}) \right) \tag{5}$$

2.3.4. Random forest

It is a meta estimator based on decision trees applied to many bootstrapped subsamples of a dataset. First of all, a specified number of decision tree regressors are built. A subset of features is randomly selected to be used as candidates at each split so that the constructed decision trees do not rely on the same set of features and high correlation among trees can be prevented. The bootstrapped instances also prevent the individual trees from overfitting. The numeric predictions of each estimator are averaged and assigned to the test sample as the final output. Equation 6 is used to make a prediction for a new sample x, where B is the number of bootstrapping, T_i is the bootstrapped tree constructed by a set of samples of size n, which is the total number of instances in the training data, and selecting m variables from all features at random for iteration i. The best split point among m variables is determined using the mean squared error as in Equation 8.

$$\hat{f}_B(x) = \frac{1}{B} \sum_{i=1}^{B} T_i(x) \tag{6}$$

2.3.5. Extremely randomized trees

It is an ensemble of decision trees where cut-points are randomly determined while splitting nodes, on the other hand, the whole samples are used as given at the beginning instead of performing bootstrapping. The final output is assigned by averaging the results of ensemble iterations. Two important parameters are the number of randomly selected features and the minimum sample size for splitting a node.

2.3.6. Extreme gradient boosting

XGBoost is also one of the ensemble models of decision trees. It is based on gradient boosting in which errors are minimized by the gradient descent algorithm. By adding models on top of each other iteratively, the errors of the previous model are corrected by the next predictor, until the training data is accurately predicted or reproduced by the model. Instead of assigning different weights to the classifiers after every iteration, gradient boosting fits the new model to new residuals of the previous prediction and then minimizes the loss when adding the latest prediction. XGBoost uses this algorithm with an additional custom regularization term in the objective function. XGBoost uses a loss function to build trees by minimizing the value in Equation 7, where the first part represents the loss function calculating the pseudo residuals of the predicted ($\hat{y}_i$) and the real value (y_i) of the i^{th} instance in each leaf, the second part includes T as the number of leaves, γ as the penalty parameter used for pruning, λ as a regularization term, and w as the leaf weights.

$$\mathcal{L}(\phi) = \sum_i \ell(\hat{y}_i, y_i) + \sum_k \Omega(f_k)$$
$$\text{where } \Omega(f_k) = \gamma T + \frac{1}{2}\lambda \|w\|^2 \tag{7}$$

3. Results and Discussion

From here onwards, the abbreviation of the self-training (ST) method followed by the abbreviation of the base classifier technique is used to refer to the related approach. For example, ST-SVR refers to the self-training method with the SVR base classifier.

Three performance metrics (mean squared error (MSE) given in Equation 8, coefficient of determination (R^2) given in Equation 9, and mean absolute percentage error (MAPE) given in Equation 10, where *ST* is the measured value of soil temperature, $\widetilde{ST}$ is the predicted value of soil temperature, $\overline{ST}$ is the mean of the observed data, and *n* is the number of samples) were calculated to evaluate the usability of the proposed methodology and to select the best one in terms of the given criteria. The results show the outputs of ten-fold cross-validation. By considering the ratio of initially given training data as *p*%, the experimental results were obtained for all varying values of *p* as 5% to 85% with an increment of 5.

$$\text{MSE} = \frac{1}{n}\sum_{i=1}^{n}\left(ST_i - \widetilde{ST_i}\right)^2 \tag{8}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(ST_i - \widetilde{ST_i})^2}{\sum_{i=1}^{n}(ST_i - \overline{ST_i})^2} \tag{9}$$

$$\text{MAPE} = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{ST_i - \widetilde{ST_i}}{ST_i}\right| * 100 \tag{10}$$

Table 3 displays the MSE values of the methods under the self-training framework at the depth of 50 cm. Even though there are very few known cases of soil temperature, in the beginning, the constructed models achieved a remarkable performance by predicting too close to the real values for test cases. The self-training model led to increasing the amount of labeled data by pseudo-labeling the past training data by applying one of the regression methods described in Section 2.3. As a result, the constructed regression model had the advantage of discovering more patterns hidden in data due to more sets of instances with known outputs.

Table 3- The comparison of the applied methods under self-training framework in terms of MSE values

	Methods					
%	*ST-RF*	*ST-SVR*	*ST-KNNReg*	*ST-ETReg*	*ST-XGBoost*	*ST-DTReg*
5	2.293	18.594	6.374	5.128	**2.189**	4.573
10	1.466	16.584	4.020	3.444	**1.279**	3.041
15	1.256	15.459	3.134	3.070	**1.007**	2.448
20	1.197	14.537	2.779	2.607	**0.934**	2.234
25	1.035	14.065	2.411	2.457	**0.772**	2.052
30	0.891	13.553	2.331	2.189	**0.736**	1.702
35	0.816	13.195	2.068	2.140	**0.638**	1.425
40	0.747	12.920	1.991	2.047	**0.582**	1.402
45	0.728	12.554	1.921	1.868	**0.552**	1.530
50	0.670	12.259	1.728	1.687	**0.522**	1.219
55	0.611	12.004	1.732	1.626	**0.506**	1.203
60	0.567	11.730	1.672	1.593	**0.482**	1.197
65	0.539	11.557	1.617	1.452	**0.481**	1.118
70	0.490	11.310	1.559	1.255	**0.419**	0.960
75	0.508	11.113	1.546	1.226	**0.432**	1.008
80	0.467	10.889	1.489	1.160	**0.386**	1.019
85	0.443	10.721	1.457	1.268	**0.385**	1.005

Furthermore, the most noticeable thing is the reduction in error values as the percentage (*p*) value increases. It is because as the number of training instances real labels of which are known rises, the pseudo-labeled training data is less required to predict the outcome of new cases. More accurate estimations can be obtained as a result.

The best results for all the ratio values were achieved when ST-XGBoost was performed. XGBoost is one of the ensemble learning methods which boosts high performance compared to single learners so it was expected that its results were good. ST-RF is another ensemble learning method that followed ST-XGBoost in terms of low error values. The highest errors were obtained when ST-SVR was the learner. It can be inferred that ST-SVR required more labeled training data in order to find the

optimal hyperplane to make more accurate predictions. It could not manage to perform well when the value of *p* is small. The performance of ST-SVR may be improved by changing the kernel type or updating the parameters instead of using default values.

Table 4 shows the MAPE values of the applied methods at the depth of 50 cm. As in Table 3, there is a tendency to decrease in the error values when the percentage of the initially given labeled training data is increased. ST-XGBoost performs the best (7.436% - 3.456%) especially for the p values between 5% and 60%. In addition to ST-XGBoost, ST-RF also achieves the predictions with the least errors (3.434% - 3.059%) for the p values of 65% to 85%. ST-DTReg is the leading one among the single learners with error rates of 9.706% - 4.001%.

Table 4- The comparison of the applied methods under self-training framework in terms of MAPE values (%)

	Methods					
%	*ST-RF*	*ST-SVR*	*ST-KNNReg*	*ST-ETReg*	*ST-XGBoost*	*ST-DTReg*
5	7.544	26.706	13.921	10.899	**7.436**	9.706
10	6.157	24.932	11.410	8.933	**5.939**	8.115
15	5.522	24.107	9.821	8.110	**5.334**	7.120
20	5.125	23.025	9.247	7.549	**4.819**	6.712
25	4.746	22.592	8.440	7.226	**4.395**	6.134
30	4.493	22.141	8.328	6.827	**4.223**	5.948
35	4.267	21.688	7.621	6.555	**4.082**	5.445
40	4.191	21.405	7.456	6.309	**3.914**	5.437
45	3.990	20.906	7.156	5.973	**3.722**	5.177
50	3.828	20.641	6.843	5.827	**3.572**	4.812
55	3.717	20.229	6.820	5.741	**3.581**	4.843
60	3.547	20.053	6.720	5.432	**3.456**	4.622
65	**3.434**	19.842	6.468	5.188	3.486	4.352
70	3.288	19.554	4.338	4.974	**3.275**	4.275
75	**3.258**	19.295	6.266	4.759	3.271	4.243
80	**3.126**	19.095	6.194	4.657	3.156	4.129
85	**3.059**	18.890	6.077	4.665	3.109	4.001

The comparisons of the methods according to R^2 values are indicated in Figure 3 at the depth of 50 cm. R^2 expresses the proportion of the variance for a dependent variable that is explained by the model's inputs. In this study, it is the relationship between soil temperature and other independent variables such as soil moisture, air temperature, ultraviolet radiation, etc. as in the work of Shamshirband et al. (2020) and Tabari et al. (2011). According to the results, the general impression is that there is a steady increase as the value of *p* increases. The same condition as in MSE is valid for R^2 that ST-SVR has the lowest coefficient of determination (0.331 to 0.614) while the best results are found in ST-XGBoost (0.921 to 0.987). Similarly, at the more sensitive depth of 10 cm, the best prediction accuracy was achieved by the ST-XGBoost algorithm. The best learner, ST-XGBoost, has the R^2 values in the interval of 0.927 to 0.986, while the values of the ST-SVR method is in the interval of 0.321 to 0.606.

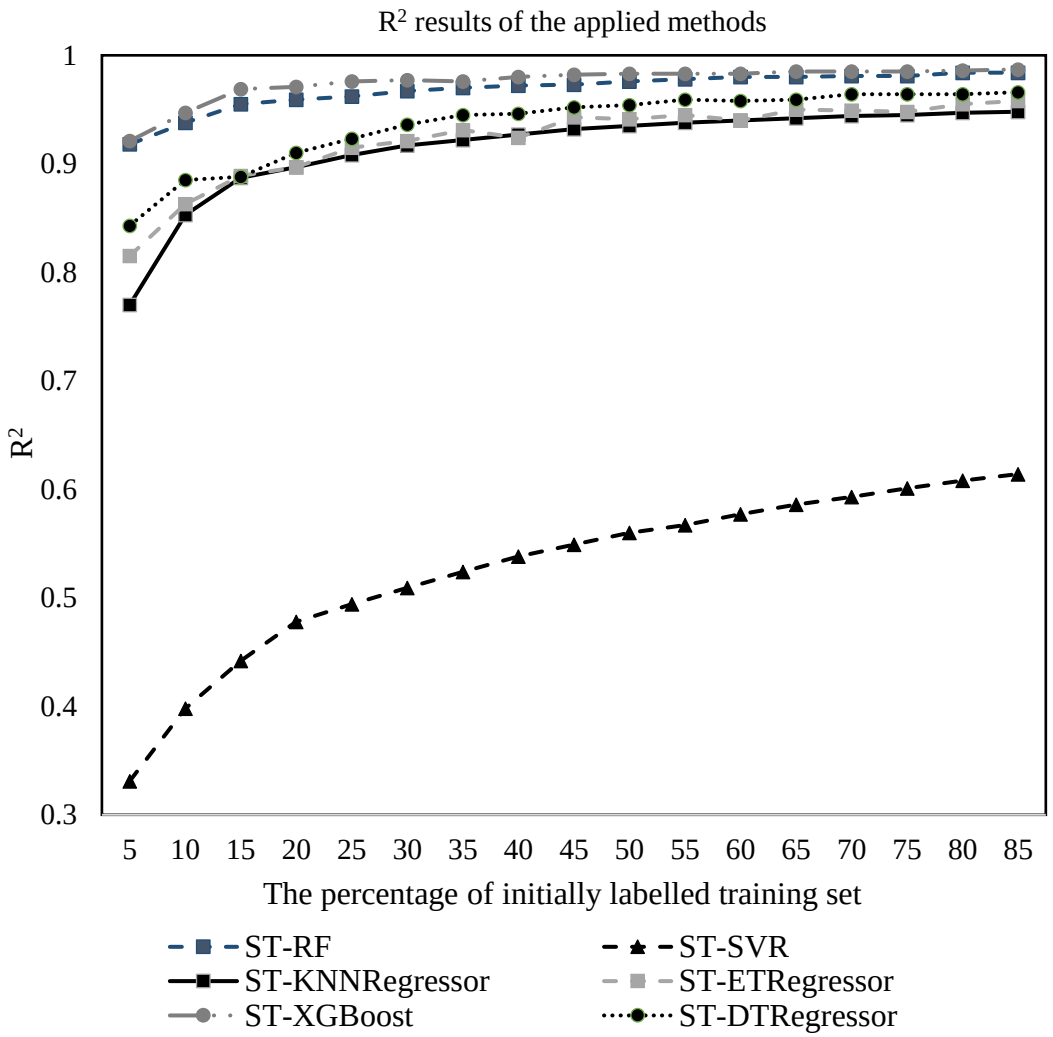

Figure 3- The comparison of the applied methods under self-training framework in terms of R^2 values (ST-RF: the RF method combined with self-training, ST-SVR: the SVR method combined with self-training, ST-KNNRegressor: the KNNRegressor method combined with self-training, ST-ETRegressor: the ETRegressor method combined with self-training, ST-XGBoost: the XGBoost method combined with self-training, ST-DTRegressor: the DTRegressor method combined with self-training)

Figure 4 displays the best-performed model, ST-XGBoost, in terms of the values of MSE at all depths. It is apparent that the model predicts soil temperature well at the depth of 50 cm while the values at the depth of 30 cm are generally estimated worse compared to others. Besides, as in Table 3, MSE values at depths of 10, 20, 30, and 40 cm are decreased when the size of the labeled training set is increased.

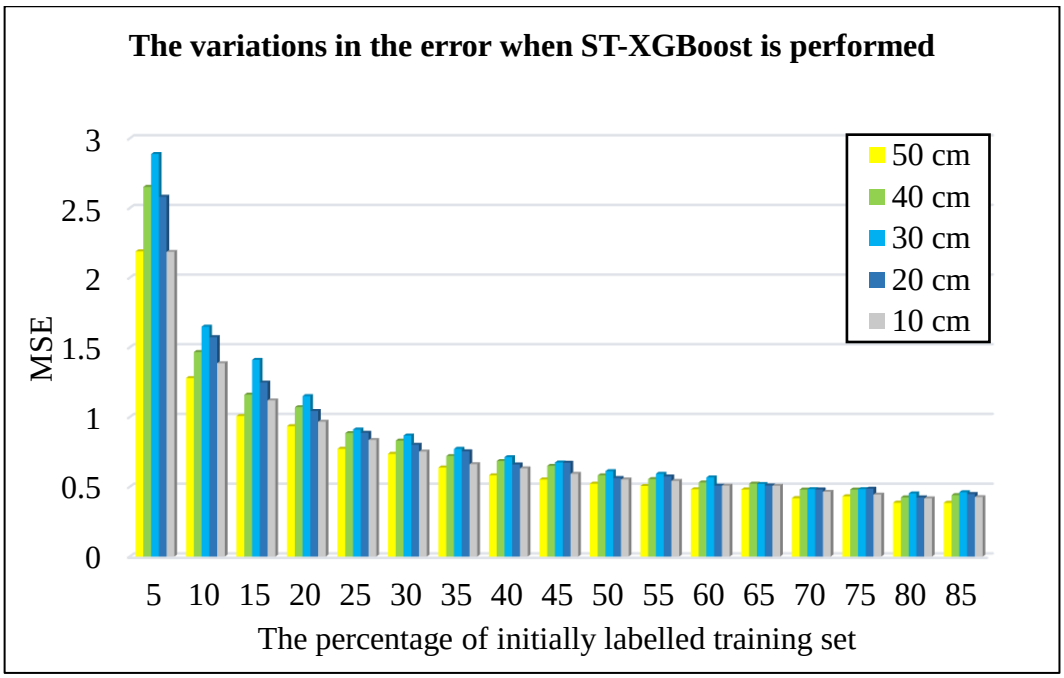

Figure 4- The results of MSE when the best predictor (ST-XGBoost) was performed at all soil depths

Figure 5 demonstrates the predicted and the measured values of test samples of 10-fold cross-validation steps separately when ST-XGBoost is performed at the depth of 50 cm and p is 85% of the whole data. In each iteration, whole data including 4500 samples are divided into training and test (90% of data as the training set and 10% of data as the test set) and this procedure is repeated ten times. In that way, the selection of the samples with different characteristics are increased instead of experimenting only with predefined test cases. The advantage of this is that, perhaps, the samples that are easy to predict in one iteration are collected in the test set, while in another iteration, the opposite (difficult samples for prediction) can be observed. Since the common result of all of them is obtained, a better inference is made than depending on a result of a single sample set. In this direction, Figure 5 shows clearly that the ST-XGBoost model obtains soil temperature values that are very close to the real measurements in each fold.

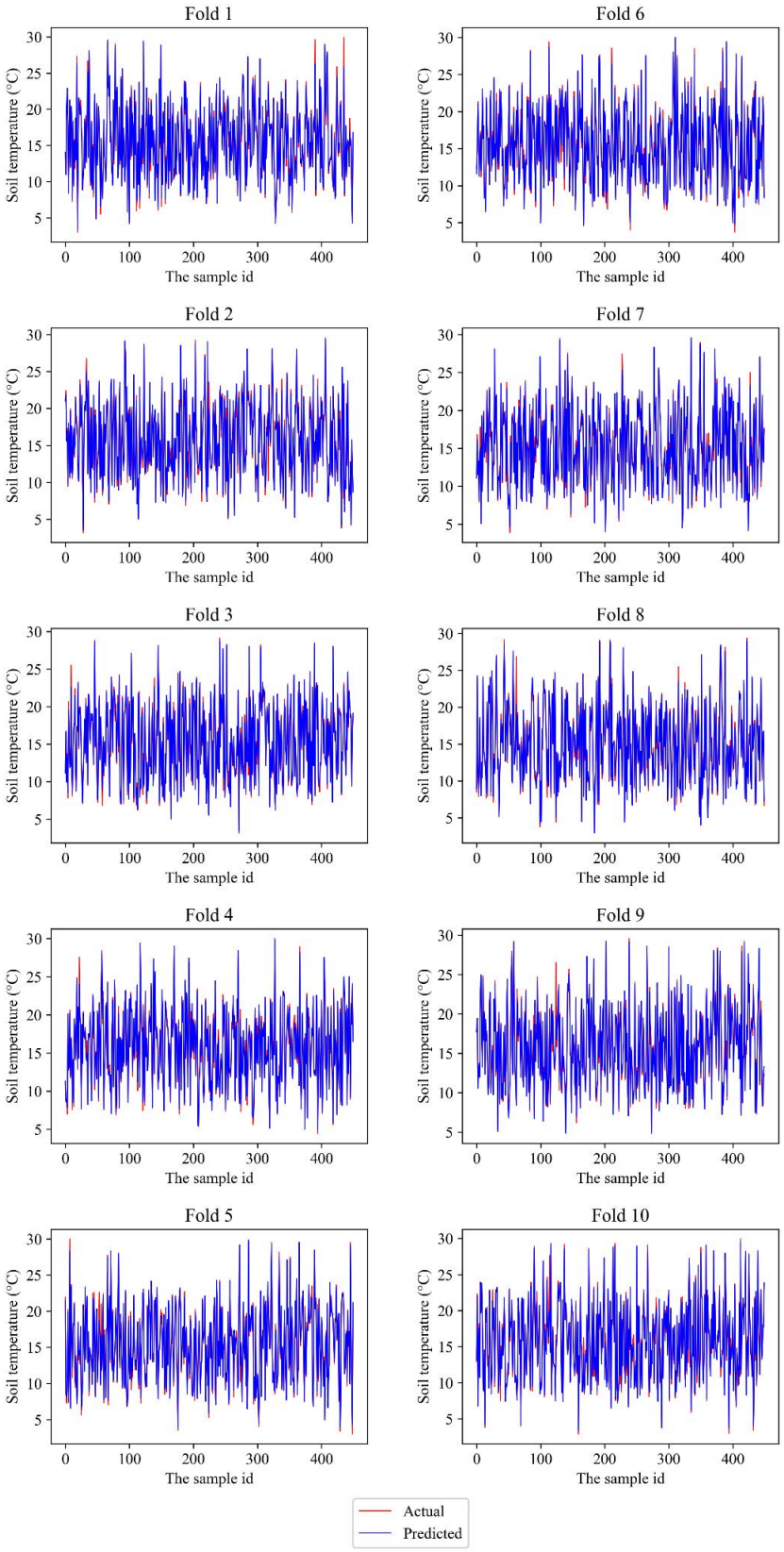

Figure 5- The predicted and the real values of the soil temperature when the ST-XGBoost model was applied at the depth of 50 cm and *p* is 85%

In order to show the trend in the results as the soil depth changes, Table 5 demonstrates the MSE values of each applied method by taking their average for all *p* values from 5 to 85. Especially for ensemble learning methods, there is a general pattern that the error first increases for the soil depth of 10 to 30 and then a decreasing trend follows as the depth increases from 40 to 50 cm. Their common characteristic is that they can better estimate the deepest soil temperature. On the contrary, there is a decreasing trend in most of the cases for single learners from shallow to deeper parts. ST-SVR and ST-DTReg manage to predict the best at the depth of 50 cm while ST-KNNReg performs well at the depth of 40 cm. The main inference for the proposed

model is that the more accuracy is generally obtained the deeper the soil. It is clear that the effect of the meteorological parameters on the soil temperature is greater in the regions close to the soil surface. While the soil in the shallow regions is affected more by external factors such as rainfall and wind, as the soil depth increases, a more stable environment is found in terms of the soil temperature modelling, and regression models, therefore, create better predictive models with less error.

Table 5- The comparison of the mean values of the applied methods in terms of MSE values under self-training framework at different soil depths

Methods	*Depth (cm)*				
	50	*40*	*30*	*20*	*10*
ST-RF	0.866	0.927	0.958	0.941	0.931
ST-ETReg	2.130	2.339	2.492	2.502	2.321
ST-SVR	13.120	13.986	14.412	14.311	14.384
ST-XGBoost	0.724	0.831	0.894	0.836	0.764
ST-KNNReg	2.343	2.286	2.288	2.328	2.637
ST-DTReg	1.714	1.848	1.957	1.941	1.920

Table 6 shows the R^2 results at different soil depths to compare our study with the recent studies in the literature. The values are the best R^2 values obtained with the best parameter combinations in the mentioned studies and our results obtained on our dataset are the optimal R^2 values of the best method, ST-XGBoost, at the specified depths. It is apparent that our proposed method generally outperforms the other models and it manages to estimate soil temperature accurately.

Table 6- The accuracy of the results (R^2) obtained in this study and the similar results obtained in the literature

Ref	*Algorithm*	*Depth (cm)*	*R^2* *Existing Method*	*Proposed Method (STST)*
Alizamir et al. 2020a	• Deep Echo State Network (Deep ESN)	20	0.970	**0.985**
	• Multilayer Perceptron Neural Network (MLPNN)	10	0.890	**0.986**
	• M5Prime Tree	10	0.870	**0.986**
	• Random Forest	10	0.900	**0.986**
Li et al. 2020	• Integrated Bidirectional Long Short-Term Memory Network (BiLSTM)	50	0.920	**0.986**
	• Long Short-Term Memory (LSTM)		0.880	
	• Bidirectional Long Short-Term Memory Network (BiLSTM)		0.860	
	• Deep Neural Network (DNN)		0.870	
	• Random Forest (RF)		0.860	
	• Support Vector Regression (SVR)		0.790	
	• Linear Regression (LR)		0.420	
Penghui et al. 2020	• Adaptive Neuro-Fuzzy Inference System with Grasshopper Optimization Algorithm (ANFIS-mSG)	10	0.977	**0.986**
Guan et al. 2020	• The Hybrid of Multilayer Perceptron by Invasive Weed Optimization (MLP-IWO)	20	0.962	**0.985**
Alizamir et al. 2020b	• Extreme Learning Machine (ELM)	10	**0.986**	**0.986**
	• Artificial Neural Networks (ANN)	50	0.984	**0.986**
	• Classification and Regression Trees (CART)	10	0.984	**0.986**
	• Group Method of Data Handling (GMDH)	10	**0.988**	0.986
	• Multi-Linear Regression (MLR)	50	**0.988**	0.986
Huang et al. 2020	• Multivariate Linear Regression	10	0.915	**0.986**
		20	0.889	**0.985**
		40	0.799	**0.986**
Behmanesh & Mehdizadeh 2017	• Gene Expression Programming (GEP)	10	0.974	**0.986**
	• Artificial neural networks (ANN)		0.980	
	• Multiple linear regression (MLR)		0.971	
Abyaneh et al. 2016	• Artificial Neural Networks (ANN)	10	0.968	**0.986**
		20	0.926	**0.985**
		30	0.893	**0.985**
		50	0.872	**0.986**
Ozturk et al. 2011	• Artificial Neural Networks (ANN)	10	0.960	**0.986**
		20	0.981	**0.985**
		50	0.966	**0.986**

The main findings of the study can be concluded as follows: *1)* It was observed that "self-training" smartly provides many advantages for predicting soil temperature, including reducing cost and providing additional information present in unlabeled data. *2)* The proposed STST approach has the potential to expand the application of machine learning in the agriculture sector, thanks to its advantages. *3)* The ST-XGBoost method outperformed the other methods (ST-RF, ST-ETReg, ST-SVR, ST-KNNReg, and ST-DTReg) in terms of prediction accuracy. *4)* The prediction error changes according to the soil depth. *5)* The accuracy of soil temperature prediction increased as the number of labeled data samples increased.

4. Conclusions

In this study, soil temperature at various soil depths was predicted using the proposed model, *Soil Temperature prediction via Self-Training* (STST). The past soil temperature & soil moisture data and meteorological data of Izmir, Turkey were considered in the interval of 01.09.2019 and 22.05.2020. The experimental results showed that self-training empowered the regression methods by presenting a more labeled pool of data for training a model for prediction. In this way, test samples were estimated more accurately using the information hidden in the expanded labeled instances instead of using few samples with known past values. Especially ensemble learning methods (ST-XGBoost and ST-RF) managed to capture the dynamics better behind the soil temperature prediction compared to other ones under the self-training framework. The best model, ST-XGBoost respectively obtained the results in the range of 0.385-2.888, 3.109%-8.740%, and 0.905-0.986 at depths of 5, 10, 20, 30, 40, and 50 cm for the performance metrics MSE, MAPE, and R^2. In addition, the best predictions were generally made at the depth of 50 cm with the mean MSE values of 0.866, 2.130, 13.120, 1.724, and 1.714 for ST-RF, ST-ETReg, ST-SVR, ST-XGBoost, and ST-DTReg, respectively.

This study contributes to the agricultural field in a way that plant growth can be handled more efficiently by taking the predicted soil temperature values into account. An automated irrigation system or cooling/heating system can be set up according to the variation in the temperature of the predicted time intervals. In the same manner, as future work, the proposed model may be customized and updated in order to estimate the soil moisture, which is another important parameter in plant production.

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The Effects of Natural Boron Mineral on the Essential Oil Ratio and Components of the Spearmint (*Mentha spicata* L.)

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ABSTRACT
This study was carried out to determine the effects of boron mineral on the essential oil ratio and quality in spearmint (*Mentha spicata* L.) species. Different boron doses (0, pure and 1/2 dose, 1/8 dose (8 L decare^{-1})) were applied in this study, but no measurement could be take due to plant death in the pure dose and 1/2 dose. The essential oils of spearmint harvested at two different time were obtained by the method of hydro-distillation. In the analyses by GC_MS/FID (Gas Chromatography/Mass Spectrometry - Flame Ionization Detector), the essential oil ratio based on boron doses was measured as 1.25% for the boron-free condition and 2.22% for the 1/8 dose, the main essential oil components were a total of 35 components in the leaves (without boron), and 29 components for the 1/8 dose. The main essential oil components obtained from the leaves (without boron) were Carvone by 55.12%, Limonene by 9.99%, 1,8-cineole by 8.81% while those for the 1/8 dose were Carvone by 56.02%, Limonene by 14.22%, 1.8-cineole by 6.79% in the first harvest. The essential oil components were found to be rich in terms of terpenes. In this study, which is the first study in which boron mineral was applied to spearmint with the application of boron mineral in solution form, the recommended dose for spearmint was found to be 1/8 boron dose.

Keywords: Spearmint, Liquid boron application, GC/MS-FID, Essential Components

1. Introduction

The mint plant is a member of the Lamiaceae (Labiatae) family. It is a perennial medicinal and aromatic plant which has a prevalent distribution around the world and a very high economic significance due to its valuable essential oil. Mint, which is used prevalently in important industrial fields such as food, perfumery, cosmetics and especially medicine because of its tannin, pulegone, isomenthon, methyl acetate, menthone and especially menthol compounds that it contains, is also a significant spice plant with its leaves (Herro & Jacob 2010; Baydar 2013). As Mentha species have a highly polymorphic structure and a tendency for hybridization, they show high diversity. Highly variable numbers of mint species have been reported between 20 and 90 around the world. 42 species are registered in the Plant List, and there are 2.524 Latin names including synonyms and subspecies for these species. 15 taxa belonging to 10 species are distributed in Turkey. Among mint species, especially species such as *M. piperita* (peppermint), *M. spicata* (spearmint), *M. arvensis* (wild mint) and *M. pulegium* (pennyroyal) are significant species (Neset 2018). *M. spicata* is also named as *M. viridis*. Carvone and D-limonene, 1,8-Cineole and Myrcene are significant components of the essential oil obtained from the plant *M. spicata*. Carvone is prominently used as a scent and flavor addition for food items such as liqueur and chewing gum and to improve the flavor of soaps, perfumes and toothpaste due to its pleasant scent, as potato germination inhibitor, building block and biochemical environment indicator. Carvone has also found an area of usage in the drug industry as it has different biological effects such as antibacterial and antifungal activities (De Carvalho & Da Fonseca 2006). D-limonene is used due to its pleasant citrusy scent, clinically to dissolve cholesterol-containing gallbladder stones and to alleviate heartburn as it has an effect that neutralizes stomach acid and repairs peristalsis. It is also known that D-limonene has a chemo-preventive effect against several types of cancer (Sun 2007). It was reported that mint contains terpenes such as N-menthol, neomenthol, isomenthol, d-menthone, isomenthon, menthofuran, methyl acetate, carvomenthone, cineol, limonene, piperidone, O-pinene, carvacrol, N-pinene, and dipentene, but these compounds vary based on the season, climate and the structure of the plant. In addition to this, it was also stated that mint contains flavonoids such as quercetin, mentosit, isoroifolin, vitamin K, thymol and eugenol. It was reported that all these components have antioxidative and free radical preventive effects and these flavonoids increase the antioxidant enzyme potential (Jagetia et al. 2002). While pure boron minerals have been utilized from the past to the present, with the industrialization and advanced technology in recent years, the demand and need for boron minerals have been increasing day by day. In the agriculture sector, boron minerals are used in areas such as biological improvement and control chemicals, fertilizers, insecticides-herbicides, weed control. As boron is highly compatible with having bonds with oxygen, it

forms several different oxygen compounds. Due to this characteristic of boron, it has 230 different minerals that have been determined so far. The commercial value of seven of these minerals is high. High-value minerals are water-soluble boron salts such as tincal and kernite, and non-water-soluble minerals such as colemanite, ulexite, pandermite, boracite, and sassolite. Boron minerals that have high-grade contents are more valuable and demanded more (Yenmez 2009). The deficiency and toxicity range of boron is remarkably narrow. Fertilization may be the solution of the deficiency problem, while a set of procedures can be utilized to ameliorate soil boron toxicity. However, these approaches are costly and time-consuming, and they do not have permanent effects most of the time. Plant species and also the genotypes within the species are highly different in terms of their boron requirements. So, a sort of soil boron which is accepted deficient for one crop may exhibit toxic effects on another (Brdar-Jokanović 2020).

The region (Kutahya) where this study was carried out is a region that is rich in boron minerals in Turkey. Boron can be very useful and cheap fertilizer for crops in the region. With this study aimed to determine to suitable dose of boron for essential oil yield and quality of spearmint.

2. Material and Methods

2.1. Plant material

Spearmint (*Mentha spicata* L.) was used as plant material in the trial. The plant materials (spearmint seedlings) were obtained from the Kutahya Municipality Hekim Sinan Botanical Garden. *Mentha spicata* L. kind is a perennial and herbaceous species with shorter, bright green color, pale blue flowers, flowers at the ends of the branches and spike-shaped and similar to *M. piperita*. Soil analysis of the trial area in Kutahya is given in Table 1. The Gediz district of the province of Kutahya has a microclimate that is hot and dry in summers, has precipitation in winters. The warmest months in Gediz are July and August, while the coldest ones are January and February. The lowest measured temperature is 5.5 °C. The amount of annual precipitation is 483.08 mm. The months with the most precipitation are May and June, while the one with the least precipitation is September (Anonymous 2017). These temperatures are appropriate for the plant to grow. Boron is found in nature not as a single element but as compounds in combination with multiple elements. Its more frequently encountered compounds include Na, Ca and Mg. Those with Na origins are known as tincal (borax), calcium-rich ones are known as colemanite, sodium and calcium-rich ones are known as ulexite (Yenmez 2009). The type of boron that was used in this study was colemanite. Chemical analysis of the natural boron mineral to be used in the field trials was carried out (Table 2

Table 1- Pre-sowing chemical analysis of experimental soil

Soil variables	*Values*	*Status*
Potassium (K_2O; kg ha^{-1})	20.01	Medium
Phosphorus (P_2O_5; kg ha^{-1})	6.23	Medium
Lime (%)	4.03	Limy
Organic matter (%)	0.78	Very little
Total salts (%)	0.003	Salt-free
pH	7.14	Neutral
Saturation (%)	53.3	Clay and loam

Table 2- Chemical analysis of pure boron mineral

Nutrient/ element	*Calcium*	*Potassium*	*Magnesium*	*Sodium*	*Iron*	*Manganese*	*Zinc*	*Copper*	*Nickel*	*Cadmium*	*Chromium*	*Cobalt*
Units	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}
Values	108.9	19.66	33.22	58.68	0.680	0.042	0.10	<10	<10	<10	0.034	<10

2.2. Field trial

The field trial was carried out in 2016–2017 in the medicinal and aromatic plants trial field of the Gediz Vocational School at Dumlupınar University. It located in the Gediz district of Kutahya, Turkey - 39°0'27.65" N, 29°24'14.66" E; 802 m above sea level. The seedlings started to be planted into the field beginning with April 2016. In both years, experiment was conducted with a randomized complete block design in triplicates.

The dimensions of each planting area were 40 cm × 30 cm. A total of 72 plants were planted by 24 plants per plot. There were 3 rows of plants in each plot, and each plot had an area of 1.6 m × 3 m. Observations and measurements were made on leaf samples obtained from 9 plants labeled in 72 healthy plants in each plot. Since the first year was the plantation year and the plant growth was too low, no and measurement could be taken and no doses were applied. In the trial, 4 different boron mineral doses (0, pure and 1/2 dose, 1/8 dose [8 L $decare^{-1}$]) were applied on the spearmint planted plots. Boron mineral started

to be given when the plant reached 10 cm of height in all plots in the second year. The mineral was given as 100 ml of fluid per plant and re-applied as 50 ml of fluid one month later. Boron minerals that were given without processing led the plants to dry.

Experiment was irrigated considering rainfall, air temperature, and humidity in the soil to save plants from moisture stress. No other additives or fertilizer were applied to the plant neither before nor during the planting session except for boron mineral. The weeds were observed and they were cleared manually. In the second year, two harvests (24.05.2017 and 10.09.2017) were made, and the beginning of flowering was preferred as the harvest time.

2.3. Extract preparation

The extract that was used in our study was prepared from natural boron minerals. The boron mineral obtained from the Emet region was powdered, and afterward, the powdered boron mineral was weighed as 20 g and shaken in 100 ml of distilled water and homogenized for 5 minutes. The boron in the homogenate form was centrifuged at 3500 rpm for five minutes. Its supernatant part was taken and kept in a fridge. This extract to be used was applied as pure or by dilution with distilled water in ratios of 1/2 and 1/8 (Karayel 2006).

2.4. Essential oil isolation and determination of essential oil composition by GC-MS

Samples were diluted 1: 100 with hexane for analysis. At the beginning of the trial essential oil analysis, 20 g of dry material was weighed and taken in a 500 ml flask. Samples were diluted with 1% hexane and injected into gas Chromatography in 1 µl with 40:1 split ratios. Agilent 7890A Capillary columns (HP InnowaxCapillary; 60.0 m x 0.25 mm x 0.25 µm) were used to separate the components. The column was split into two fractions at a rate of 1:1 using a splitter in the FID and mass spectrometry detector (Agilent 5975C). Helium was used as carrier gas at a flow rate of 0.8 mL/min. The injector temperature was maintained at 250 °C, the column temperature program was 10 minutes at 60 °C, raised at 4 °C/minute (40 minutes) at 60 °C and 220 °C and 10 minutes at 220 °C. The detector was set for 60 minutes. The scan range (m/z) for the mass detector was 35-450 atomic mass units and the electron bombardment ionization energy was 70 eV. The data of Wiley and Oil Adams libraries were taken as basis in the diagnosis of the components of the essential oil. The data from the FID detector were used for the volatile oil component ratios (Tabanca et al. 2006).

3. Results and Discussion

3.1. Essential oil ratio (%)

The first year (2016) measurement was not taken to determine the essential oil ratio and components. The reason for this may be explained as that the spearmint plant's adaptation to the soil and the ecological environment could not be completely achieved, and the development of the plant was low. It arrived at the harvest period by completing 50% flowering till the end of the 2017 May. Two harvests for aerial parts of plants at flowering periods were made on 24.05.2017 and 10.09.2017 in the second year. Considering the flowering dates of the plant based on the doses, it was determined that the 1/8 boron dose reached the first and complete flowering dates earlier than the other doses. The plants under the treatment of the 1/8 boron dose started flowering earlier, while those under the boron-free started later. In this context, in our study, the 1/8 boron dose provided more than 1.5% essential oil contents. The lowest essential oil ratio was 1.25% in the boron-free, while the highest one was 2.22% in the 1/8 boron dose. Figure 1 shows the comparison of the amounts of essential oil ratio for the boron-free dose and the 1/8 boron dose (%).

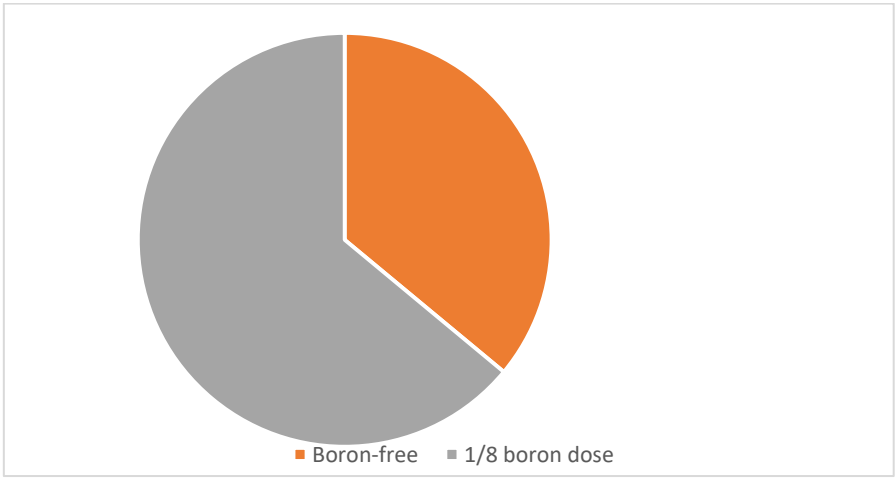

Figure 1- Comparison of the essential oil ratios of the spearmint based on the boron-free dose and the 1/8 dose of boron

Considering previous studies on the essential oil ratio, the ratios of essential oils obtained by hydro-distillation of the spearmint plant dried with different methods were found between 0.95 and 3.16% (Kripanand et al. 2015). In Pozantı location of Adana, the essential oil yields of spearmint varied in the ranges of 0.25 – 3.11 L da^{-1} in the 1st year and 0.37 – 3.66 L da^{-1} in the second year (Ozguven & Kırıcı 1999). There were significant differences among the essential oil ratios of spearmint dried in different drying conditions (Shade, stove 35 °C and 50 °C). The highest essential oil ratio (1.7%) was obtained from the samples dried in shade. In the spearmint dried in a stove, the essential oil ratio was 1.3% in those dried at 35 °C and by 0.6% in those dried at 50 °C (Karakapan 2017). In parts of the mint plant (*Mentha piperita* L.), the highest amount of oil was obtained as 3.18% by drying the tip in shade, while the lowest was obtained as 2.40% by drying the same part in sunlight. In the middle part, the highest amount of oil was obtained as 2.07% by drying in a stove, the lowest was obtained as 1.58% by drying in sunlight, while in the bottom part, the highest was obtained as 1.11% by drying in the stove, and the lowest was obtained as 0.92% by drying in sunlight (Ozer 2012). Essential oil is obtained from the flowering fresh plant by steam distillation with an average yield of 0.6%. Its major components are; 1-carvone (40-70%), limonene (20%), carveols, carvone isomers, carvyl acetate, as well as menthol, borneol, linalool, menthol, jasmone, perilalcol, monoterpenes and sesquiterpenes (Akgul 1993). The essential oil amounts of the *Mentha spicata* L. (spearmint) species determined based on different fertilizer doses (0 da^{-1} N, 5 da^{-1} N and 10 kg da^{-1} N) and drying methods varied between 1.4% and 2%. It was reported that fertilizing with nitrogen increased the essential oil content (Buyukbayraktar 2014). In Mentha arvensis, boron supply between 0.5 and 1.0 g (B) m^3 was optimal for the maximum shoot and root growth and essential oil yield per plant. There was no significant effect of B supply on the relative percentage of menthol, menthone and methyl acetate in the oil. The incorporation of (U-14C) saccharose into the essential oil increased significantly with increasing B supply. The impacts of foliar boron fertilization on the yield of flower heads as well as the chemical composition, content, and yield of essential oils (EO) of *Arnica montana* L. and *A. chamissonis* were studied. The content of EO was from 0.174% to 0.200% and from 0.158% to 0.188%, respectively. The highest EO content in the inflorescences of the two plants was noted at the B rate of 200 g ha^{-1}. Similarly, this dose caused a ca. 35% and 43% increase in the EO yield in *A. montana* and *A. chamissonis*, respectively (Sugier et al. 2017). Our results regarding spearmint's essential oil ratio and the effect of boron application on essential oil ratio are in agreement with previous studies.

3.2. Essential oil composition (%)

In the analysis of the essential oil at different boron doses (boron-free and 1/8 dose 8 L $decare^{-1}$) in spearmint (*Mentha spicata* L.), in the second year, 35 - 29 components were identified from the dried leaves. These components constituted respectively 96.71% and 99.31% of the average total essential oil in the dried leaves (average of the first and second harvests). The values of the components of the essential oil obtained from the leaves of the *Mentha spicata* L. species were determined in the specimens obtained in two harvests in the 2nd year. The values of the essential oil components of the *Mentha spicata* L. species are separately shown in (Table 3) (boron-free dose) and Table 4 (1/8 boron dose). The main components obtained from the dry leaves according to the average measures of the first and second harvest for boron-free dose are as follows: Carvone 54.57%, Limonene 9.68%, 1,8-cineole 8.88%, β-caryophyllene 3.88%, Myrcene 3.49%, Trans-Sabinenehydrate 2.41%, Carvacrol 1.21%. On the other hand, the main components obtained from the dry leaves according to the average measures of the first and second harvest are as follows for 1/8 boron dose, Carvone 56.07%, Limoneneby 14.2%, 1,8-cineole 6.80%, Myrcene 3.54%, β-caryophyllene 2.50%, Trans-Sabinenehydrate 2.32%, Carvacrol 1.60%. Other components are also shown in Tables 3 and 4. Figure 2 shows the comparison of the amounts of essential oil components for the boron-free dose and the 1/8 boron dose (%). As the boron dose was diluted the essential oil ratio increased, the essential oil components decreased, and some components were not obtained (Tables 3-4). Limonene, which was one of the main components obtained in the 1/8 boron dose, was found as 14.2% and higher than the values obtained in boron-free application (Table 4). According to the doses applied to the *Mentha spicata* L. species, the essential oil components varied to some extent. The top component in the *Mentha spicata* L. species was carvone by 55.12% without boron application, while it was the same but by 56.02% in boron application at the 1/8 dose in the first harvest. Boron with 1/8 dose had an increaser affect to carvone compound in the essential oil of spearmint. In the *Mentha species*, the composition of the essential oil determines its quality and flavor. This composition changes depending on ecological conditions, variety and harvest times. When the main components of essential oils are compared according to their harvest averages it was found out that the average values of 1,8-Cineole, trans-Sabinenehydrate, β-Caryophyllene components of the boron-free dose application were higher than of 1/8 boron dose application. Akgul (1993) stated that the most important components of *M. spicata* L. species are Carvone (40-70%) and Limonene (20%). Considering this statement of Akgul, it was seen that 1/8 boron dose had a significant effect on Limonene and Carvone components. The positive result on *M. spicata* L. strain increased as the dose of boron was diluted. The results obtained from the dose effect trials between boron-free dose and 1/8 boron dose are given in Table 5. According to pharmacopeia, in *M. piperita* oil, the rates should be as menthol by 50–78%, menthone by 10–30%, menthofuran by 2.5–5% and methyl acetate by 5–10%, while for *M. spicata* oil, the carvone ratio should be 42–67% (Wagner et al. 1984). Considering previous studies on essential oil component ratios, the menthol ratios of *M. piperita* species were found to be low (6.23–40.47%) in comparison to *M. arvensis* (66.20–72.29%). In *M. spicata* ssp. spicata, the carvone ratio varied in the range of 39.38–69.41%. The menthol ratios obtained in Adana were usually closer to or smaller than the lower limit of the range stated by researchers as 50–78%. The carvone ratio in *M. spicata* ssp. spicata varied in the range of 39.38–69.41%, which was within the range stated by researchers (Ozguven & Kırıcı 1999). The carvone ratio of spearmint leaves was found as 51.9–52.4 (Zheljazkov et al. 2014). The carvone ratio of spearmint leaves varied in the range of 39.38–69.41%, which was within the range specified by researchers (Singh et al. 1995; Wagner et al.

1984). The most important component for the oil of mint (*Mentha piperita* L.) was L-menthol, and it was found to be the highest occurring component in oils obtained from the tips and bottom parts (not the middle parts) by sun-drying (at least 30.50% – at most 42.58%). The ratio of this component increased from the tip to the bottom part, while the highest increase was in the bottom part material (Ozer 2012). The main components of essential oils included high rates of menthone and menthol in *M. piperita*, while these two compounds were found to be very low or non-existent in *M. spicata* and *M. villoso-nervata*. On the other hand, these two species contained higher ratios of carvone. The significant components of them were carvone, limonene and 1,8 cineol (Cam et al. 2012). Menthone was found to be 0.42% in the boron-free application and 0.48% in the 1/8 boron dose application. No L-menthol was obtained in the boron-free application and for the 1/8 boron dose application. Ratios of essential oil components in spearmint determined for different doses of N (0 da^{-1} N, 5 da^{-1} N and 10 kg da^{-1} N), the carvone ratio varied in the range of 49.70–61.50% (Buyukbayraktar 2014). The carvone and limonene ratios in the essential oil of spearmint were found as 4.52–60.54% and 9.44–51.90% respectively (Antal et al. 2011). The carvone and limonene ratio of the essential oils of spearmint leaves were found as 9.09–67.62% and 13.08–55.36% respectively (Kripanand et al. 2015). Our results regarding spearmint's essential oil composition and the effect of boron application on essential oil composition are in agreement with previous studies.

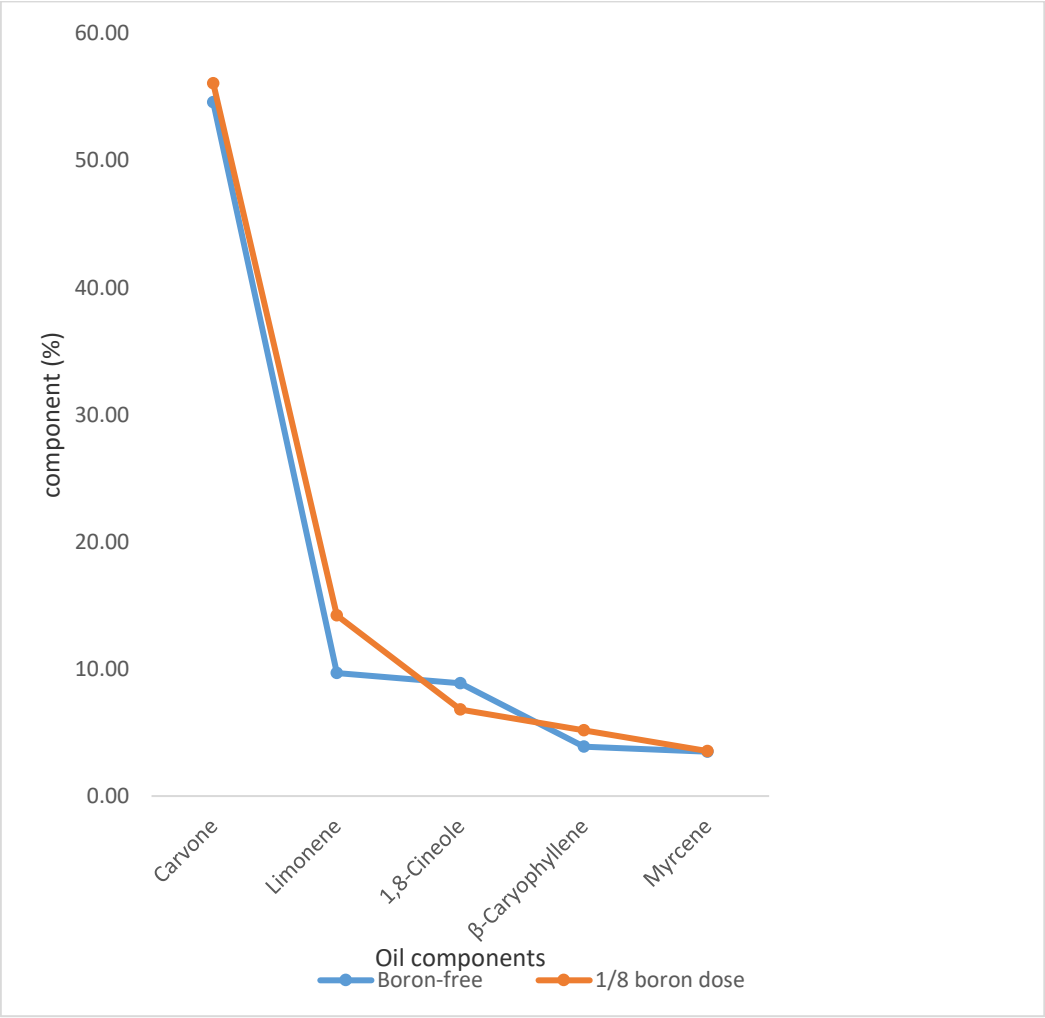

Figure 2- Comparison of the essential oil components of the spearmint for the 1/8 boron dose and the boron-free dose

Table 3- Effect of different doses of boron mineral on essential oil components (%) obtained from the leaves of Mentha spicata L. (Boron-free dose)

		Leaf					
S.no	*Component Name*	*RI*	*RT*	*1st Harvest*%*	*RT*	*2nd Harvest*%*	*Harvest Average*%*
1.	*α*-Pinene	1011	11.42	1.15±0.025	11.28	1.35±0.085	1.25±0.055
2.	*β*-Pinene	1096	14.84	1.52±0.030	14.41	1.65±0.050	1.58±0.040
3.	Sabinene	1082	15.43	0.99±0.130	13.12	0.78±0.088	0.88±0.109
4.	Myrcene	1123	17.24	3.43±0.102	14.85	3.56±0.097	3.49±0.099
5.	*α*-Terpinene	1141	18.02	0.26±0.060	18.54	0.22±0.160	0.24±0.110
6.	Limonene	1160	18.92	9.99±0.162	19.39	9.38±0.242	9.68±0.202
7.	1,8-Cineole	1171	19.43	8.81±0.126	18.10	8.95±0.020	8.88±0.073
8.	*cis-β*-Ocimene	1192	20.47	0.49±0.068	21.80	0.45±0.151	0.47±0.109
9.	*ϒ*-Terpinene	1204	20.92	0.44±0.040	21.42	0.38±0.090	0.41±0.065
10.	*trans-β*-Ocimene	1227	21.10	0.15±0.065	21.38	0.12±0.171	0.13±0.118
11.	Terpinolene	1241	22.52	0.17±0.105	23.24	0.15±0.155	0.16±0.130
12.	3-Octanol	1344	26.71	0.77±0.098	26.53	0.78±0.070	0.77±0.084
13.	Hexyl isovalerate	1397	28.74	0.18±0.087	30.12	0.12±0.183	0.15±0.135
14.	*trans*-Sabinene hydrate	1417	29.48	2.44±0.061	30.40	2.38±0.162	2.41±0.111
15.	Menthone	1425	29.76	0.41±0.075	28.90	0.44±0.128	0.42±0.101
16.	*cis-3*-hexenyl isovalerate	1439	30.20	0.23±0.095	31.11	0.18±0.075	0.20±0.085
17.	*β*-Bourbonene	1475	31.44	1.04±0.140	30.86	1.00±0.140	1.02±0.140
18.	Linalool	1490	32.05	0.11±0.085	18.89	0.08±0.075	0.09±0.080
19.	*β*-Elemene	1543	33.73	0.13±0.116	33.68	0.10±0.111	0.11±0.113
20.	*β*-Caryophyllene	1552	34.01	3.91±0.036	34.18	3.85±0.065	3.88±0.050
21.	*trans*-Dihydrocarvone	1567	34.52	0.32±0.050	34.27	0.34±0.096	0.33±0.073
22.	Dihydrocarvyl acetate	1618	36.10	0.48±0.086	36.78	0.38±0.104	0.43±0.095
23.	*α*-Humulene	1623	36.25	0.12±0.100	35.78	0.08±0.070	0.14±0.085
24.	Bicyclosesquiphellandrene	1625	36.34	0.23±0.079	36.98	0.18±0.07	0.20±0.074
25.	*α*-Terpineol	1642	36.84	0.25±0.088	36.08	0.24±0.090	0.24±0.089
26.	Borneol	1646	36.92	0.36±0.083	35.86	0.34±0.072	0.35±0.077
27.	Germacrene - D	1663	37.40	1.78±0.070	38.01	1.72±0.100	1.75±0.085
28.	Bicyclogermacrene	1686	38.15	0.38±0.090	37.85	0.33±0.110	0.35±0.100
29.	Carvone	1691	38.34	55.12±0.100	38.12	54.02±0.155	54.57±0.127
30.	*cis*-Carvyl acetate	1713	38.90	0.27±0.090	38.14	0.23±0.098	0.25±0.094
31.	*trans*-Carveol	1773	40.64	0.09±0.065	39.24	0.07±0.056	0.08±0.060
32.	*cis*-Carveol	1801	41.45	1.53±0.045	41.56	1.50±0.090	1.51±0.067
33.	*cis*-Jasmone	1892	43.8	0.46±0.095	43.65	0.43±0.095	0.44±0.095
34.	Caryophyllene oxide	1939	45.05	0.23±0.066	45.21	0.21±0.086	0.22±0.076
35.	Carvacrol	2129	49.71	1.23±0.065	49.76	1.20±0.075	1.21±0.070
	Total (%)			99.47		97.19	96.71

*Each value represents the mean ± standard deviation of triple analyses, RI: Retention indices; RT: Retention time (min)

Table 4- Effect of different doses of boron mineral on essential oil components (%) obtained from the leaves of Mentha spicata L. (1/8 boron dose)

		Leaf					
S.no	*Component Name*	*RI*	*RT*	*1st Harvest*%*	*RT*	*2nd Harvest*%*	*Harvest Average*%*
1.	*α*-Pinene	1011	11.40	1.17±0.055	11.41	1.22±0.070	1.19±0.062
2.	*β*-Pinene	1096	14.80	1.45±0.035	14.85	1.34±0.070	1.39±0.052
3.	Sabinene	1082	14.26	0.95±0.015	15.43	0.82±0.050	0.88±0.032
4.	Myrcene	1123	17.40	3.67±0.026	17.24	3.42±0.102	3.54±0.064
5.	*α*-Terpinene	1141	-	-	-	-	-
6.	Limonene	1160	18.29	14.22±0.070	18.91	14.18±0.072	14.2±0.071
7.	1,8-Cineole	1171	20.15	6.79±0.068	19.40	6.82±0.051	6.80±0.059
8.	*cis-β*-Ocimene	1192	21.10	0.28±0.040	20.36	0.22±0.070	0.25±0.055
9.	*ϒ*-Terpinene	1204	19.46	0.21±0.070	20.89	0.18±0.085	0.19±0.077
10.	*trans-β*-Ocimene	1227	-	-	-	-	-
11.	Terpinolene	1241	-	-	-	-	-
12.	3-Octanol	1344	26.12	0.41±0.055	26.69	0.46±0.080	0.43±0.067
13.	Hexyl isovalerate	1397	-	-	-	-	-
14.	*trans*-Sabinene hydrate	1417	29.21	2.38±0.090	29.42	2.27±0.055	2.32±0.072
15.	Menthone	1425	30.12	0.50±0.380	29.70	0.46±0.065	0.48±0.222
16.	*cis-3*-hexenyl isovalerate	1439	31.24	0.20±0.358	30.18	0.22±0.070	0.21±0.214
17.	*β*-Bourbonene	1475	30.24	0.67±0.020	31.44	0.72±0.070	0.69±0.045
18.	Linalool	1490	32.00	0.09±0.045	31.97	0.06±0.030	0.07±0.037
19.	*β*-Elemene	1543	-	-	-	-	-
20.	*β*-Caryophyllene	1552	34.21	2.55±0.085	34.00	2.46±0.097	2.50±0.910
21.	*trans*-Dihydrocarvone	1567	33.69	1.42±0.051	34.47	1.44±0.061	1.43±0.056
22.	Dihydrocarvyl acetate	1618	36.25	0.91±0.040	36.08	0.85±0.055	0.88±0.047
23.	*α*-Humulene	1623	36.30	0.11±0.070	36.25	0.14±0.110	0.12±0.090
24.	Bicyclosesquiphellandrene	1625	35.12	0.15±0.065	36.27	0.20±0.052	0.17±0.058
25.	*α*-Terpineol	1642	-	-	-	-	-
26.	Borneol	1646	36.10	0.31±0.050	36.92	0.28±0.090	0.29±0.070
27.	Germacrene - D	1663	37.09	1.14±0.072	37.40	1.22±0.070	1.18±0.071
28.	Bicyclogermacrene	1686	38.56	0.35±0.068	38.10	0.38±0.051	0.36±0.059
29.	Carvone	1691	38.71	56.02±0.080	38.26	56.12±0.080	56.07±0.080
30.	*cis*-Carvyl acetate	1713	38.24	0.41±0.070	38.89	0.38±0.090	0.29±0.080
31.	*trans*-Carveol	1773	40.21	0.14±0.090	40.57	0.17±0.098	0.15±0.094
32.	*cis*-Carveol	1801	40.75	1.01±0.111	41.36	1.04±0.125	1.02±0.118
33.	*cis*-Jasmone	1892	43.52	0.34±0.040	43.79	0.30±0.080	0.32±0.060
34.	Caryophyllene oxide	1939	45.12	0.30±0.061	45.01	0.28±0.153	0.29±0.107
35.	Carvacrol	2129	48.86	1.59±0.045	49.70	1.62±0.119	1.60±0.082
	Total (%)			99.74		99.27	99.31

Table 5- Comparison of the main components of essential oils of boron-free and 1/8 boron doses

Component name	Boron free harvest average	1/8 boron harvest average
Myrcene	3.49±0.099	3.54±0.064
Limonene	9.68±0.202	14.2±0.071
1,8-Cineole	8.88±0.073	6.80±0.059
trans-Sabinenehydrate	2.41±0.111	2.32±0.072
B-Caryophyllene	3.88±0.050	2.50±0.910
Carvone	54.57±0.127	56.07±0.080
Carvacrol	1.21±0.070	1.60±0.082

4. Conclusions

Considering the results of this study, the *Mentha spicata* L. species grown in the ecological conditions of Kutahya-Gediz is appropriate for this region. We may state that the 1/8 boron dose that was applied affected the essential oil ratio and its components. Limonene, 1,8- Cineole, and Carvone were determined to be the dominant essential oil components. At the 1/8 dose of boron application, the Limonene (14.22%) and Carvone (56.02%) ratios were found higher. The ratio of the essential oil was found to be the highest at 2.22% at the 1/8 boron dose ratio. After providing the spearmint plant with the 1/8 dose of boron, the compounds “trans-β-ocimene, terpinolene, Hexyl isovalerate, β-elemene, and α-terpineol” could not be obtained. It

is believed that the results obtained from this study will shed light on obtaining essential oils and volatile compounds of medicinal and aromatic plants in the future. The dose to be recommended is the 1/8 boron dose since it has the minimum toxic effect on the plants and a positive effect on the essential oil yield and quality as general.

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Effects of Modified Atmosphere Packaging and Methyl Jasmonate Treatments on Fruit Quality and Bioactive Compounds of Apricot Fruit during Cold Storage

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ABSTRACT
The study was carried out to investigate the effects of methyl jasmonate (MeJA) [0.5 and 1.0 mmol L^{-1}] and modified atmosphere packaging (MAP) treatments on weight loss, respiration rate, firmness, colour, soluble solids content (SSC), titratable acidity, vitamin C, total phenolics, flavonoids and antioxidant capacity (DPPH and FRAP assay) of apricot fruit (*Prunus armeniaca*) during cold storage. Fruit were stored at 0±0.5˚C and 90±5% relative humidity (RH) for 20 days, and analysis and measurements were performed at 5-day intervals. At the end of cold storage, the lowest weight loss was determined in fruit stored with the MAP following MeJA1 application. The lowest respiration rates were determined in fruits stored with the MAP following MeJA1 or MeJA2 treatment. The softening of fruit stored without the MAP or MAP was significantly delayed with the MeJA. The fruit stored without the MAP or MAP following MeJA2 treatment had the highest vitamin C at the end of storage period. MAP treatments had greater total phenolic and total flavonoids and antioxidant capacity than the treatment without MAP regardless of MeJA applications. At the end of storage, the highest total phenolic and antioxidant capacity were determined in fruits stored in the MAP following MeJA2 application. It was concluded that MAP and MeJA2 treatments could be used as an efficient postharvest tool to minimize quality losses throughout the cold storage period.

Keywords: Firmness, Phenolic, *Prunus armeniaca*, Respiration rate, Weight loss, Vitamin C

1. Introduction

Apricot is consumed both fresh and dried. It is also served to consumption as processed food like jam, marmalade, juice, nectar, jelly, pulp, frozen fruit and extrusion product (Asma 2007; Jannatizadeh et al. 2008). It is rich in minerals (boron, potassium, calcium, zinc, selenium and iron), provitamin A, vitamin B and vitamin C, sugars, organic acids, carotenoids and phenolic compounds and has a high antioxidant capacity (Sochor et al. 2010; Coşkun et al. 2013). With such rich nutritional attributes, extensive consumption of apricot is recommended against prostration, insomnia and stress and in degradation of body fats, for anaemic and anti-asthmatic effects and to reduce cholesterolaemia (Sochor et al. 2010). Turkey, is the world's leading apricot producer with a production amount of 750 000 tons, supports more than 20% of world total fresh apricot production and more than 56% of world dried apricot production in 2019 (Anonymous 2020; FAO 2020).

Apricot is a climacteric fruit. Fresh apricots can be stored in cold storages for 2-4 weeks depending on variety to prevent product build-up in markets during the harvest season and resultant quality losses in the phase of marketing (Ezzat et al. 2017). Even with the cold storage of the products, certain quantity of quality losses is evident. Modified atmosphere packaging (MAP) and plant growth regulators (PGRs such as 1-methylcyclopropene, salicylic acid, methyl jasmonate etc.) are commonly used as postharvest tools to minimize such losses (Dong et al. 2002; Moradinezhad & Jahani 2016; Ezzat et al. 2017). Rather than single use, combined use of MAP and PGRs yields better outcomes in preservation of quality attributes in medlar (Ozturk et al. 2019).

Stored fruit and vegetables with MAP treatments, a special ambient is generated around the product. Such a special ambient reduce oxygen (O_2) concentration and increase carbondioxide (CO_2) concentration through respiration process. Decreased O_2 and increased CO_2 lead to suppression of respiration rate of product. Previous studies reported significant contributions of MAP in the preservation of quality attributes of various fruit species including apricot (Pretel et al. 2000; Ozturk et al. 2019).

Several studies have recently been conducted about the effects of plant growth regulators on postharvest physiology of fruit (Dong et al. 2002; Ezzat et al. 2017; Ozturk et al. 2019). Researchers mostly focused on reduction of quality losses. Consumers

generally prefer to consume fruit species rich in vitamins, phenolic compounds and antioxidant activity. In this sense, methyl jasmonate (MeJA) was mostly studied in recent researches. MeJA promotes colour development in fruit, retard weight loss and flesh softening during the cold storage and shelf life (Kondo et al. 2001; Rudell et al. 2005; Balbontin et al. 2018). Number of studies about the effects of MeJA treatments on quality attributes of apricot is quite limited (Ezzat et al. 2017).

This study was conducted to investigate the effects of single MeJA +/- MAP treatments on fruit quality attributes and bioactive compounds of 'Precoce de Thyrinthe' apricot cultivar fruit throughout the cold storage period of 20 days.

2. Material and Methods

2.1. Plant materials

Fruit of 'Precoce de Tyrinthe' apricot (*Prunus armeniaca*) cultivar were used as the plant material of the study. Fruit were hand-harvested at commercial harvest maturity (15% SSC) from the Research and Application Orchard of Malatya Apricot Research Institute (38º19' N and 38º17' E). Harvested fruit (18 June 2018) were placed into 5 kg plastic box (39×29×21 cm, Plastas, Turkey) and transferred to laboratory with frigorific vehicle (10±1 °C and 75±5% RH) within 6 h. Then fruit with uniform maturity, size and colour and free of damage and defects were selected and defected fruit were discarded.

2.2. Experimental design and treatments

Experiments were conducted in randomized plots – factorial experimental design with 3 replications. Initially, fruit were divided into 3 groups. The first group was immersed into only distilled water as control treatment. The second group was immersed into 0.5 mmol L^{-1} (MeJA1) and the third group into 1.0 mmol L^{-1} (MeJA2) methyl jasmonate (Sigma-Aldrich, Germany) solutions for 1 min. Fruit were then dried on drying papers under laboratory conditions (21±1 °C and 80±5% RH) for 1 h. Triton X-100 (0.077%, Sigma-Aldrich, Germany) was used as a surfactant in MeJA solutions.

For each treatment, fruit were divided into two equal portions. The first group of fruit was placed into 5 kg plastic boxes in modified atmosphere packaging [Xtend® (815-AT 10/R, StePac, Tefen, Israel)] and the rest was placed into plastic boxes without MAP. For each treatment, a total of 24 boxes were used (12 with MAP (passive) and 12 without MAP). The O_2 and CO_2 concentrations of MAP were measured with a gas analyser (Abiss, France).

Fruits were initially subjected to pre-cooling with cold air at 4±0.5 °C and 90±5% RH for 24 h, then MAP-treated fruit were closed with plastic clips. Fruits were then stored at 0±0.5 °C and 90±5% RH for 20 days (d). Measurement and analyses were performed on 5, 10, 15 and 20th days of the storage period. For each treatment, 3 boxes were used in each measurement period. Each box represented a replication.

2.3. Methods

2.3.1. Weight loss

Initial fruit weight (Wi) was determined at the begging of closure (Day 0) with a digital scale (±0.01 g) (Radwag, Poland). Then, on day (d) 5, 10, 15 and 20, final fruit weight (Wf) was measured. The weight loss (WL) that occurs in fruit was based on the weight at the beginning of each measurement period and determined as a percentage through the equality given below (Equation 1).

$$WL = \frac{Wi - Wf}{Wi} \times 100 \quad \text{(Equation 1)}$$

2.3.2. Respiration rate and firmness

The 2 L airtight chambers were used to measure respiration rate of fruit. The chambers were fitted with a rubber septum and 5 fruit were sealed in each chamber at 20±1 °C temperature and 80±5% RH for 1 h. The chambers were then connected to a gas sensor (Vernier, Oregon, USA) and the amount of CO_2 produced by the fruit based on the weight and volume of fruit was considered as the respiration rate. Results were expressed in mL CO_2 kg^{-1} h^{-1} (Yarılgaç et al. 2019).

Ten fruits from each replication were used for firmness measurements. The measurements were made on two opposite sides of the equatorial part of the fruit through a portable digital durometer (nondestructive device, Agrosta® 100 Field, France) with a flat cylindrical penetrating tip (4.1 mm). The tip of the durometer was slightly and longitudinally pressed into the outer skin of the fruit, and the percentage (%) value on the screen was recorded. If the value is close to 100, the fruit is considered very firm, and close to 0 indicates that fruit was extremely soft (Ozturk et al. 2019).

2.3.3. Colour characteristics

L*, chroma and hue angles were measured by a colorimeter (Konica-Minolta, CR-400, Japan) in CIE (Commission Internationale de l'Eclairage system) colour system on 10 fruits. Then, the X, Y and Z values were converted into L*, a* and b* coordinates using the equations corresponding to illuminant D65 and standard observer 10°. The equation $C^*= (a^{*2}+b^{*2})^{1/2}$ was used for chroma and $h^\circ= \tan^{-1} b^*/a^*$ for hue angle.

2.3.4. Soluble solids content, titratable acidity and vitamin C

Ten fruits taken from each replication were first washed with distilled water. The fruit were chopped with a stainless-steel knife and cut into parts and homogenized by a blender (Model No. Promix HR2653 Philips, Turkey). Then the homogenate was filtered through a cheesecloth, and the juice was obtained. Soluble solids content (SSC) was measured with a portable digital refractometer (Atago PAL-1, USA) and expressed in %. For titratable acidity measurement, 10 mL juice was taken, and 10 mL distilled water was added on. Then 0.1 N NaOH (sodium hydroxide) was added until the pH of the solution reach to 8.2. Based on the amount of NaOH consumed in titration, titratable acidity was determined and expressed as g malic acid kg^{-1}.

For vitamin C measurement, 0.5 mL juice was taken, and 5 mL of 0.5% oxalic acid was added on it. The ascorbic acid test strip (Catalog no: 116981, Merck, Germany) was then taken from a collapsible sealed gas-tight tube. Reflectometer (Merck RQflex plus 10) was started. The test strip was plunged into the solution for 2 seconds, then removed from the solution. It was then held for 8 seconds, and reading was done at the end of the 15th second. Results were expressed as mg kg^{-1} (Ozturk et al. 2019).

2.3.5. Total phenolics, total flavonoids and antioxidant capacity

During each measurement period, 10 fruit were taken from each replication of each treatment. The fruit were washed with distilled water and sliced with a stainless-steel knife. Later, the fruit pulp was crumbled by a blender, and homogenized. About 30 mL of homogenate was taken and placed into a 50 mL falcon tube. The tubes were kept at -20 °C until the analyses.

Before the analyses, the frozen samples were dissolved under room temperature (21 °C). Pulp and juice were separated from each other by a centrifuge at 12 000×g at 4 °C for 35 min. The resultant filtrate was used to determine the total phenolics, total flavonoids and antioxidant activity.

Spectrophotometric measurements for bioactive compounds were performed in a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Total phenolics were measured according to the method of Beyhan et al. (2010) and was expressed as g kg^{-1} GAE (gallic acid equivalent) fresh weight (fw). Total flavonoids were measured according to the method of Zhishen et al. (1999) and was expressed as g kg^{-1} QE (quercetin equivalent) fw.

The antioxidant capacity of apricot fruit was determined according to two different procedures of 1.1-diphenyl-2-picryl-hydrazil (DPPH) (Blois 1958) and Ferric Ions (Fe^{+3}) Reducing Antioxidant Power (FRAP) (Benzie and Strain 1996), and the results were expressed as mmol kg^{-1} trolox equivalent (TE) fw.

2.4. Statistical analysis

Whether the data was normally distributed or not, it was checked by Kolmogrov-Smirnov Test. Homogeneity control of the group/subgroup variances was confirmed by Levene's test. After the variance analysis of the data, Tukey's multiple-comparison test was used to check whether there were significant differences ($P<0.05$) between the treatments or not. The statistical analyses were performed by using SAS software (SAS 9.1 version, USA).

3. Results

3.1. Weight loss

Weight losses were observed in apricot fruit throughout the cold storage. Considering the general average, MAP treatment significantly reduced weight loss, reducing the weight loss from 2.3% in non-MAP fruits to 0.7% (Figure 1 B). Effects of MeJA treatments varied with whether the fruit were treated with MAP or not. In 15 and 20th d of storage period, MeJA+MAP treated fruit yielded significantly lower weight losses than controls. On day 20, under MAP treatment, while weight loss in control treatment was 1.26%, it was found as 0.73% and 0.96% in MeJA1 and MeJA2 applications, respectively. However, significantly greater weight loss was observed in single MeJA-treated fruit than controls. Weight loss increased to 2.5% in control, 3.38% in MeJA2 and 3.59 in MeJA application at the end of the 20-day storage period (Figure 1 A).

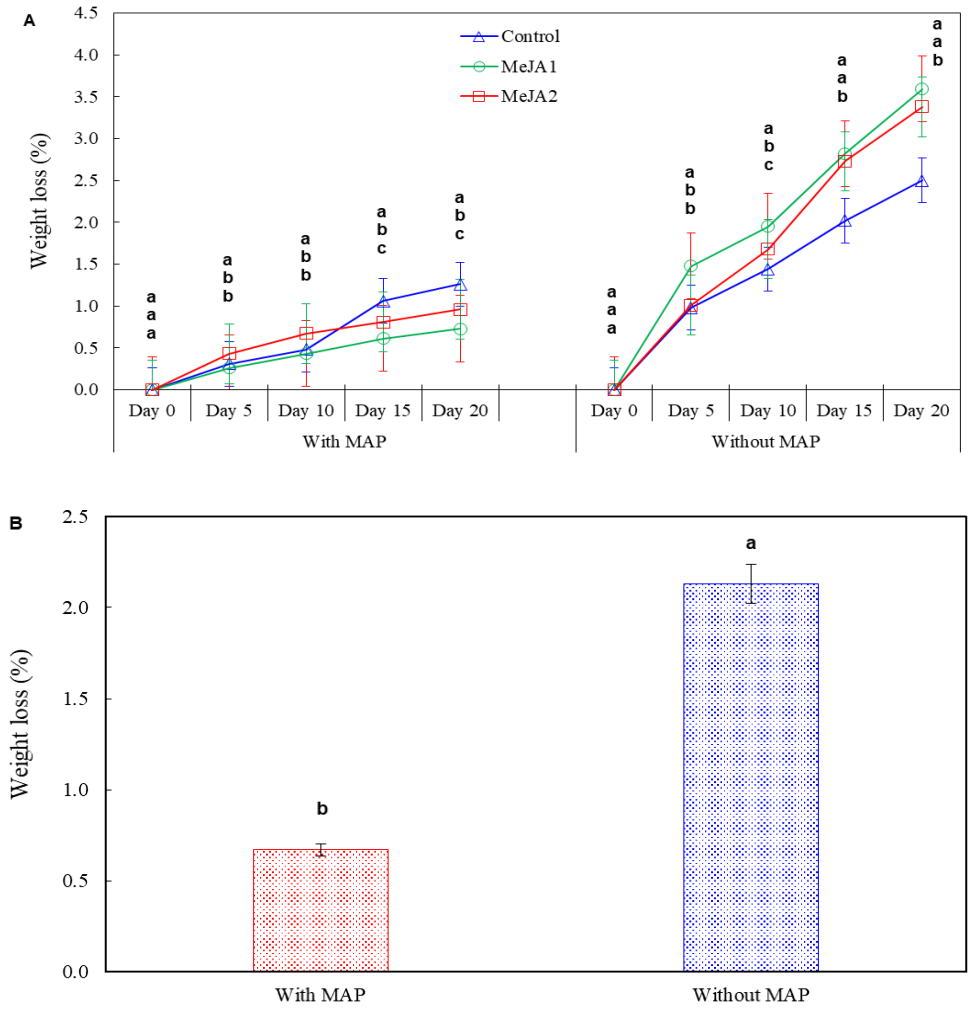

Figure 1- Effects of MeJA treatments (A) and MAP (B) on weight loss of fruit during the cold storage at 0 ± 0.5 °C and 90 ± 5% RH for 20 days. The means indicated with the same letter vertically in periods, and above the bars were not significant according to Tukey's test at P<0.05

3.2. Respiration rate and firmness

Throughout the storage period, changes of O_2 and CO_2 concentrations inside MAPs were shown in Figure 2. Considering the general averages, it was observed that MAP-treated fruit had significantly lower respiration rates (35.3 mL CO_2 kg^{-1} h^{-1}) than the untreated fruit (48.0 mL CO_2 kg^{-1} h^{-1}). The flesh firmness was 62.0% in fruits with the MAP and 55.5% in those without the MAP, and the difference between two treatments was statistically significant (Figure 3A). Considering the MAP x MeJA interactions, with or without the MAP, both MeJA treatment had lower respiration rates than control at all the dates of measurement (except for 5 and 15th d of MeJA1). After 20 days of storage period, respiration rates were decreased from 41.1 to 33.4, 26.8 and 26.6 mL CO_2 kg^{-1} h^{-1}, respectively, in control, MeJA1 and MeJA2 treatments. Without the MAP, respiration rates at the end of storage period were 51.9, 46.7 and 36.4 mL CO_2 kg^{-1} h^{-} in control, MeJA1 and MeJA2 treatments, respectively (Figure 3B). In with and without MeJA treatments, the O_2 in the MAP atmosphere similarly decreased and CO_2 content similarly increased during the storage process. A significant relationship between the respiratory rate of the products and the concentration of O_2 and CO_2 in the MAP atmosphere was not detected (Figure 2A, B, C).

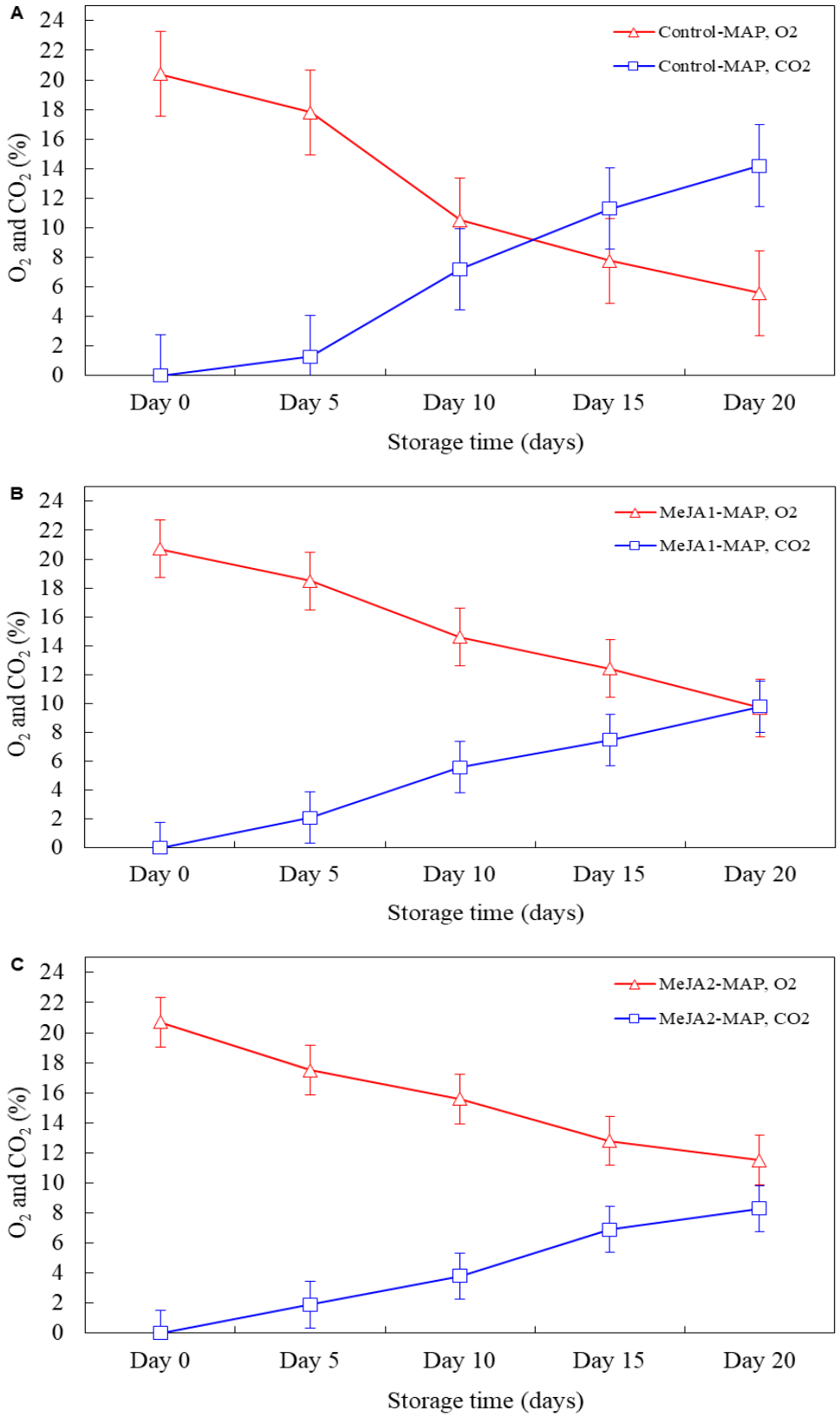

Figure 2- Changes of CO_2 and O_2 concentrations of control (A), MeJA1 (B) and MeJA2 (C) treatments inside MAP during the storage at 0±0.5 °C and 90±5% RH for 20 days

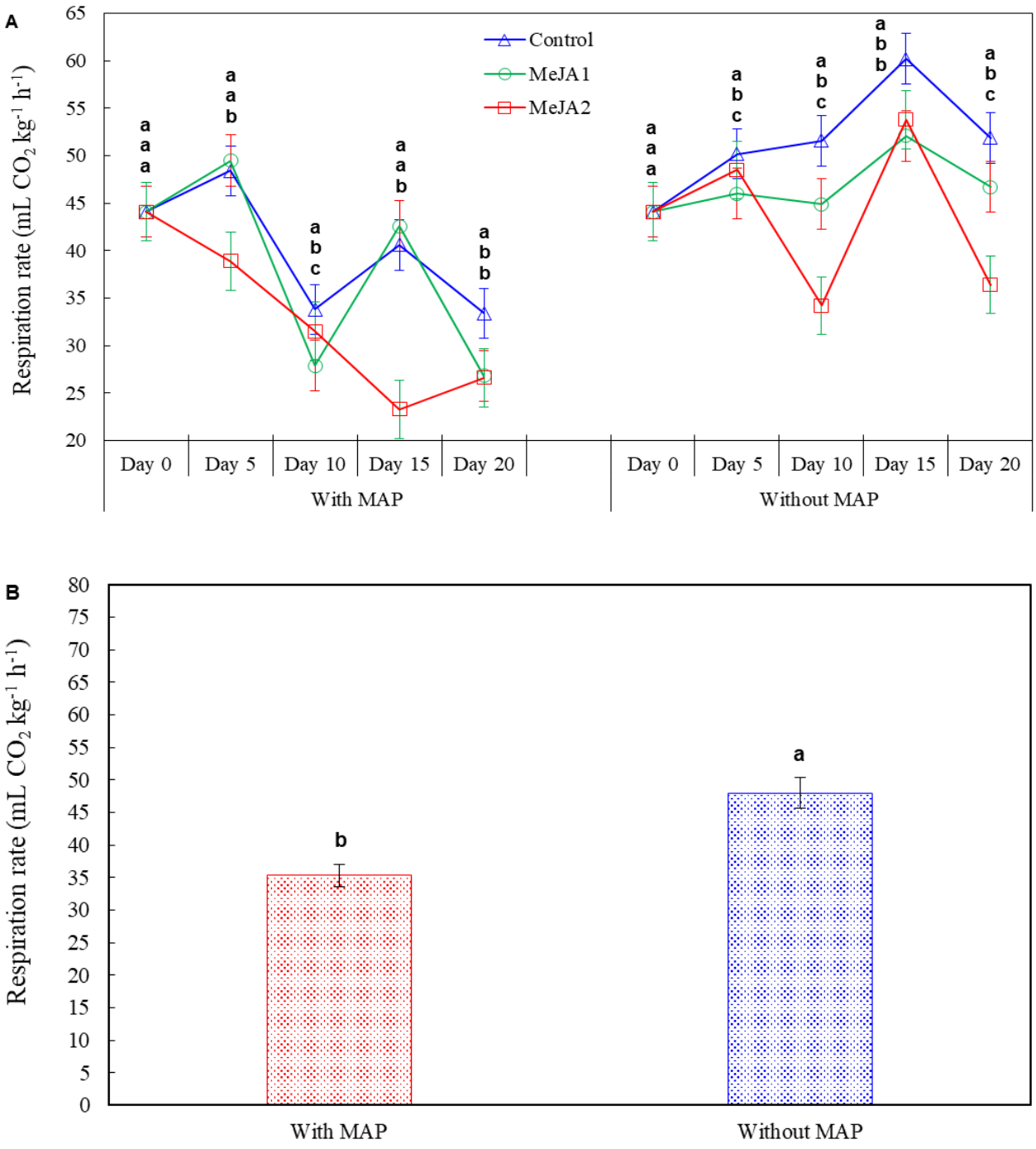

Figure 3- Effects of MeJA treatments (A) and MAP (B) on respiration rate of apricot fruit during the cold storage at 0 ± 0.5 °C and 90 ± 5% RH for 20 days. The means indicated with the same letter vertically in periods, and above the bars were not significant according to Tukey's test at P<0.05

Regardless of MAP treatments, both MeJA treatment had significantly greater firmness than the control at all the dates of measurement. At the end of storage with the MAP, firmness was 55.4, 62.2 and 61.6% in control, MeJA1 and MeJA2 treatments. Without the MAP, while the firmness of controls was 47.7%, the firmness values of fruit treated with MeJA1 and MeJA were 58.8% and 53.4%, respectively (Figure 4A-B).

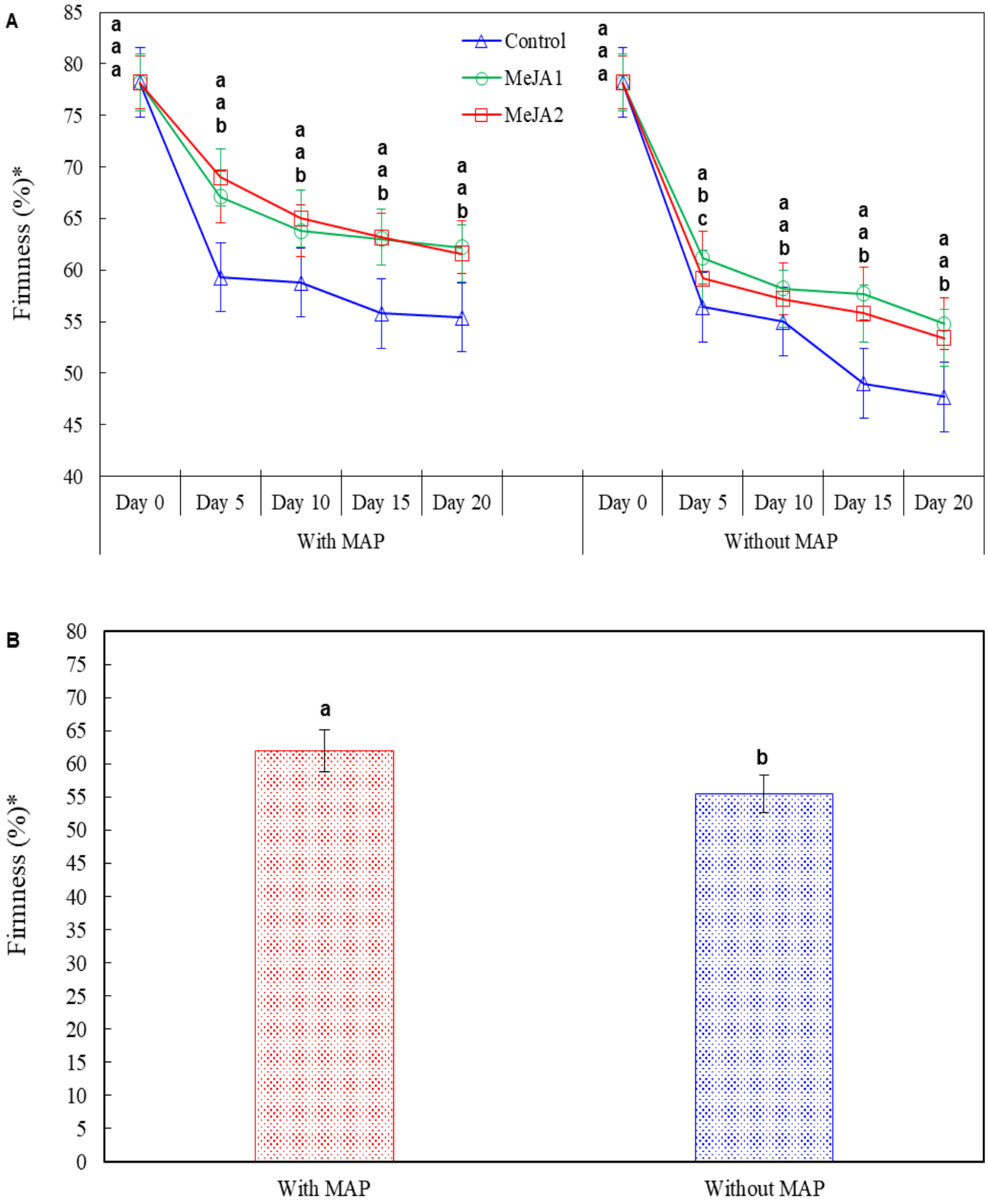

Figure 4- Effects of MeJA treatments (A) and MAP (B) on firmness of apricot fruit during the cold storage at 0 ± 0.5 °C and 90 ± 5% RH for 20 days. * The scale ranges from 0 to 100 for very soft to very firm surfaces. The means indicated with the same letter vertically in periods, and above the bars were not significant according to Tukey's test at P<0.05

3.3. Colour characteristics

Considering the general averages for colour parameters, it was observed that MAP-treated fruit had significantly lower hue angle (82.6), L* (64.2) and chroma values (52.1) than the untreated fruit which had hue angle of 84.7, 67.8 of L and 53.8 of

chroma values. Considering the MAP x MeJA interactions, with the MAP, the effects of MeJA treatments on L* values varied depending on the measurement dates. On the 20th day of storage period, both MeJA treatments had significantly greater L* values than the controls. At the end of the storage period, while L* value of controls was 62.5, those of MeJA1 and MeJA2 treatments was 66.0 and 65.5, respectively. Without MAP, no significant change was observed in the L* value due to MeJA, except for 15th d of storage period. During storage with the MAP, MeJA did not cause any significant change in chroma values of fruit colour. Without the MAP, the effect of MeJA on chroma differed according to storage period. At the end of storage period of 20 days, the chroma value, which was 51.8 in the controls, significantly increased to 53.7 in the MeJA2 treatment. Although there were exceptions depending on the storage periods, generally, there was no change in the hue values due to MeJA treatment with or without MAP (Table 1).

Table 1- Effects of MAP and MeJA treatments on L*, chroma and hue angle of apricot fruit during the cold storage at 0 ± 0.5 °C and 90 ± 5% RH for 20 days

MAP	*Treatments*	*L**					*Mean*
		Day 0	*Day 5*	*Day 10*	*Day 15*	*Day 20*	
With MAP	Control	69.1	66.7 a	61.9 b	64.9 a	62.5 b	64.2 b
	MeJA1	69.1	62.9 b	66.6 a	59.6 b	66.0 a	
	MeJA2	69.1	65.4 a	67.8 a	60.9 b	65.5 a	
Without MAP	Control	69.1	68.7 ns	68.7 ns	68.1 a	68.1 ns	67.8 a
	MeJA1	69.1	67.9 ns	68.7 ns	65.9 b	67.2 ns	
	MeJA2	69.1	67.8 ns	68.6 ns	65.8 b	68.1 ns	
		Chroma					
		Day 0	Day 5	Day 10	Day 15	Day 20	
With MAP	Control	56.0	52.2 ns	50.8 ns	50.6 ns	50.2 ns	52.1 b
	MeJA1	56.0	54.1 ns	52.1 ns	52.8 ns	50.9 ns	
	MeJA2	56.0	53.3 ns	53.5 ns	52.8 ns	51.9 ns	
Without MAP	Control	56.0	53.5 b	55.3 ns	52.2 b	51.8 b	53.8 a
	MeJA1	56.0	55.1 a	53.2 ns	54.6 a	52.7 b	
	MeJA2	56.0	54.7 a	53.9 ns	54.3 a	53.7 a	
		Hue angle					
		Day 0	Day 5	Day 10	Day 15	Day 20	
With MAP	Control	87.6	82.4 b	82.5 ns	82.2 ns	82.3 ns	82.6 b
	MeJA1	87.6	83.1 a	83.2 ns	81.8 ns	82.5 ns	
	MeJA2	87.6	83.8 a	83.7 ns	81.6 ns	82.6 ns	
Without MAP	Control	87.6	84.5 ns	86.7 a	84.1 ns	84.0 ns	84.7 a
	MeJA1	87.6	83.6 ns	84.8 b	83.7 ns	83.7 ns	
	MeJA2	87.6	85.2 ns	87.4 a	84.3 ns	84.1 ns	

ns: non-significant. The differences between the means indicated with the same lower-case letters in the same column were not significant according to Tukey's test

3.4. Soluble solids content (SSC), titratable acidity and vitamin C

Based on general mean values, it was observed that MAP-treated fruit had significantly higher SSC and vitamin C content and lower acidity values than the untreated fruit. Considering the MAP x MeJA interactions, it was observed that MeJA2 treatment with the MAP yielded significantly lower SSC than controls and MeJA1-treated fruit on day 15 and 20. At the end of storage period, SSC values in controls, MeJA1 and MeJA2-treated fruit were 18.5%, 18.2% and 17.9%, respectively. MeJA treatments without the MAP did not cause any significant change in SSC of fruit, except for 10th day of storage period. During the storage with the MAP, except for 20th day of storage, no significant change in titratable acidity between control and MeJA treatments was observed. On day 20 of storage period, MeJA1 (11.4 g malic acid kg^{-1}) and MeJA2 (11.1 g malic acid kg^{-1}) had greater acidity content than controls (10.5 g malic acid kg^{-1}). MeJA treatments without the MAP did not cause any significant change in acidity content of fruit, except for 5th day of storage. While the effect of only MeJA1 treatment was significant with the MAP treatment, both MeJA treatment caused a significant increase in vitamin C content without MAP. On day 20 of storage with the MAP, while the vitamin C was 76.6 mg kg^{-1} in the control, it was 83.0 mg kg^{-1} in the MeJA2 treatment (Table 2).

Table 2- Effects of MAP and MeJA treatments on soluble solids content (SSC), titratable acidity and vitamin C content of apricot fruit during the cold storage at 0 ± 0.5 °C and 90 ± 5% RH for 20 days

MAP	*Treatments*	*SSC (%)*					*Mean*
		Day 0	*Day 5*	*Day 10*	*Day 15*	*Day 20*	
With MAP	Control	15.5	16.9 ns	17.3 ns	17.3 a	18.5 a	
	MeJA1	15.5	17.4 ns	17.5 ns	17.6 a	18.2 a	17.4 a
	MeJA2	15.5	16.5 ns	16.6 ns	16.9 b	17.9 b	
Without MAP	Control	15.5	16.3 ns	16.3 a	16.4 ns	16.6 ns	
	MeJA1	15.5	15.7 ns	15.9 b	16.4 ns	17.1 ns	16.3 b
	MeJA2	15.5	16.1 ns	16.2 a	16.3 ns	16.3 ns	
		Titratable acidity (g malic acid kg^{-1})					
		Day 0	Day 5	Day 10	Day 15	Day 20	
With MAP	Control	15.1	12.2 ns	11.9 ns	11.4 ns	10.5 b	
	MeJA1	15.1	12.5 ns	12.4 ns	12.0 ns	11.4 a	11.9 b
	MeJA2	15.1	12.7 ns	12.3 ns	12.2 ns	11.1 a	
Without MAP	Control	15.1	13.7 b	13.1 ns	13.0 ns	12.8 ns	
	MeJA1	15.1	13.8 b	13.7 ns	13.4 ns	12.7 ns	13.4 a
	MeJA2	15.1	14.5 a	13.7 ns	13.0 ns	12.9 ns	
		Vitamin C (mg kg^{-1})					
		Day 0	Day 5	Day 10	Day 15	Day 20	
With MAP	Control	98.3	89.7 b	87.0 ns	79.7 b	76.0 b	
	MeJA1	98.3	90.3 b	88.3 ns	81.0 b	73.3 b	85.8 a
	MeJA2	98.3	94.0 a	89.0 ns	88.0 a	83.0 a	
Without MAP	Control	98.3	60.3 b	56.3 b	42.7 b	41.0 b	
	MeJA1	98.3	61.7 b	60.3 a	57.3 a	49.7 a	54.9 b
	MeJA2	98.3	66.3 a	60.3 a	52.3 a	50.3 a	

ns: non-significant. The differences between the means indicated with the same lower-case letters in the same column were not significant according to Tukey's test

3.5. Total phenolics, total flavonoids and antioxidant capacity

Considering the general means, it was observed that MAP-treated fruit had significantly greater total phenolics (36.9 g GAE kg^{-1}), total flavonoids (0.99 g QE kg^{-1}) and antioxidant capacity (5.24 mmol TE kg^{-1} in DPPH and 15.6 mmol TE kg^{-1} in FRAP assays) than the untreated fruit. MAP x MeJA interactions were also found to be significant for bioactive compounds (Table 3).

Considering the measurements data in the last measurement period of the cold storage, it was observed that MeJA+MAP treatments yielded significantly greater total phenolics and antioxidant capacity than controls. Similarly, MeJA2 treatments without MAP had significantly greater total phenolics and antioxidant capacity than controls and MeJA1-treated fruit. However, MeJA2 + MAP treatments and MeJA treatments without MAP yielded significantly lower total flavonoids than controls (Table 3). During the storage period, total phenolics of the MeJA2-treated fruit was higher than that of the controls at all measurement dates. On day 20 of storage period with MAP, while the amount total phenolics was 28.0 g GAE kg^{-1}, it was measured as 36.4 g GAE kg^{-1} in MeJA treatment. The effect of MeJA treatments on total phenolics differed depending on storage periods. At the end of storage with the MAP, while control fruit had total flavonoids of 0.99 g QE kg^{-1}, MeJA2 treated fruit had total flavonoids of 0.95 g QE kg^{-1}. At the end of storage without the MAP, while control fruit had total flavonoids of 0.91 g QE kg^{-1}, MeJA1 and MeJA2-treated fruit had total flavonoids of 0.93 and 0.76 g QE kg^{-1}, respectively. Although the effect of MeJA treatments on antioxidant capacity varied depending on the storage period, there were slight increases caused by MeJA treatments (Table 3).

Table 3- Effects of MAP and MeJA treatments on total phenolics, total flavonoids and antioxidant activity of apricot fruit during the cold storage at 0 ± 0.5 °C and 90 ± 5% RH for 20 days

MAP	*Treatments*	*Total phenolics (g GAE kg^{-1})*					Mean
		Day 0	*Day 5*	*Day 10*	*Day 15*	*Day 20*	
With MAP	Control	19.9	31.2 c	35.9 b	29.0 b	28.0 b	36.9 a
	MeJA1	19.9	36.0 b	39.8 b	32.3 b	34.7 a	
	MeJA2	19.9	39.5 a	61.8 a	38.7 a	36.4 a	
Without MAP	Control	19.9	20.2 b	22.38 b	30.1 b	24.1 b	26.2 b
	MeJA1	19.9	26.3 a	22.90 b	27.3 b	22.0 b	
	MeJA2	19.9	28.8 a	24.28 a	38.6 a	27.4 a	
		Total flavonoids (g QE kg^{-1})					
		Day 0	Day 5	Day 10	Day 15	Day 20	
With MAP	Control	0.56	0.88 b	1.18 a	0.85 b	0.99 a	0.99 a
	MeJA1	0.56	0.91 b	1.09 b	0.84 b	1.01 a	
	MeJA2	0.56	1.04 a	1.21 a	0.98 a	0.95 b	
Without MAP	Control	0.56	0.65 b	0.63 ^{ns}	0.79 b	0.91 a	0.76 b
	MeJA1	0.56	0.71 b	0.68 ^{ns}	0.87 a	0.73 b	
	MeJA2	0.56	0.84 a	0.64 ^{ns}	0.93 a	0.76 b	
		DPPH (mmol TE kg^{-1})					
		Day 0	Day 5	Day 10	Day 15	Day 20	
With MAP	Control	2.83	4.88 c	5.58 c	4.71 b	4.70 c	5.24 a
	MeJA1	2.83	5.37 b	6.40 b	4.91 b	6.29 b	
	MeJA2	2.83	6.15 a	7.16 a	5.42 a	7.06 a	
Without MAP	Control	2.83	3.69 b	2.94 c	4.48 b	3.69 b	4.01 b
	MeJA1	2.83	3.12 b	3.43 b	3.90 c	3.39 b	
	MeJA2	2.83	4.91 a	3.70 a	5.16 a	5.65 a	
		FRAP (mmol TE kg^{-1})					
		Day 0	Day 5	Day 10	Day 15	Day 20	
With MAP	Control	9.66	14.0 c	16.8 b	13.4 b	15.0 c	15.6 a
	MeJA1	9.66	14.8 b	17.3 a	14.6 a	16.0 b	
	MeJA2	9.66	15.9 a	17.3 a	15.1 a	17.2 a	
Without MAP	Control	9.66	10.5 b	10.5 b	13.5 b	13.2 c	12.7 b
	MeJA1	9.66	10.1 b	11.8 a	12.3 b	14.0 b	
	MeJA2	9.66	13.6 a	12.4 a	15.0 a	15.0 a	

ns: non-significant. The differences between the means indicated with the same lower-case letters in the same column were not significant according to Tukey's test

4. Discussion

Weight loss results in shrivelling, thus loss of allure and taste. In the present study, MAP treatments retarded weight loss throughout the storage period. MAP suppresses respiration rate, retards ripening and preserves high moisture levels with the ambient in which the fruit were placed (Muftuoğlu et al. 2012). Thusly, MAP-treated fruit had lower respiration rates than the untreated fruit. Several studies also reported retarded weight loss with MAP treatments (Muftuoğlu et al. 2012; Peano et al. 2014; Selcuk & Erkan 2015; Moradinezhad & Jahani 2016; Aglar et al. 2017). However, effects of MeJA on weight loss varied whether the fruit were placed into MAP. MeJA-treated fruit had lower weight loss values than the controls when they were placed into MAP, but had greater values when they were stored without MAP. MeJA might have contributed to cellular integrity of the fruit in MAP (Gonzalez-Aguilar et al. 2001; Ezzat et al. 2017). Thus, lower weight loss was experienced in MAP-treated fruit. Contrarily, Ozturk et al. (2019) reported that MeJA treatments did not have any extra contributions to weight loss of medlar fruit stored in MAP. Such a difference was attributed to differences in fruit species and treatments doses. In the current study, MeJA treatments suppressed respiration rate values of apricot fruit. Similar findings were also reported by previous researchers (Cao et al. 2009; Ezzat et al. 2017; Ozturk et al. 2019).

Flesh softening generates significant quality losses in apricot fruit. Therefore, firmer fruits are preferred in markets. In the present study, flesh softening was retarded with both MAP and MeJA treatments. MeJA had similar effects on MAP-treated and untreated fruit. Previous researchers also reported that flesh softening was retarded with MAP (Muftuoğlu et al. 2012; Selcuk & Erkan 2015) and MeJA (Rudell et al. 2005; Balbontin et al. 2018; Ozturk et al. 2019). MAP suppresses ethylene synthesis and retards ripening with low O_2 and high CO_2 concentration. It was reported that MeJA inhibited ethylene synthesis, thus reduced the activity of cell wall-hydrolyzing enzymes and retarded fruit softening (Ziosi et al. 2008). However, Kondo et al. (2001) reported that effects of MeJA on flesh softening were independent from ethylene. Contrarily, there are some other studies reporting insignificant effects of MeJA in maintenance of flesh firmness (Shafiq et al. 2011; Rehman et al. 2018). Differences in research findings were mostly attributed to differences in treatment times (preharvest or postharvest) and doses, fruit species and cultivars.

Fruit colour is an important sensorial quality attribute for consumers. In the present study, MAP-treated fruit had lower L* and chroma, but greater hue angle values. Present findings complied with the results of the studies reporting greater hue angle values (Peano et al. 2014) and lower L* and chroma values (Muftuoğlu et al. 2012) for MAP-treated apricot fruit. Although MeJA+MAP-treated fruit had significantly greater L* values, distinctive effects of MeJA treatments on colour parameters were not observed in this study. Contrarily, it was reported in some studies (Rudell et al. 2005; Saracoglu et al. 2017; Balbontin et al. 2018) that MeJA had positive effects on promotion of colour development. Lalel et al. (2003) reported that MeJA promoted chlorophyll degradation and carotenoid biosynthesis, thus contributed to promotion of colour development. Kondo (2005) also reported that effects of MeJA on colour development might vary based on respiratory pattern of fruit such as climacteric or not. Differences among findings of this study were probably resulted from the differences in studied species and cultivars, and treatment doses.

Today, while consumers prefer fruit rich in nutrients, vitamins and antioxidants, growers prefer to produce such fruits. However, the critical issue herein is postharvest prevention or minimization of losses in nutritional attributes of the fruit with appropriate methods or treatments. In the present study, SSC, vitamin C, total phenolics, total flavonoids and antioxidant capacity were better maintained with MAP treatments as compared to untreated fruit. Previous studies also reported better maintenance of bioactive compounds with MAP treatments (Serrano et al. 2005; Singh & Rao 2005; Ozturk et al. 2019).

Several researchers used MeJA to maintain postharvest fruit quality (Cao et al. 2009; Ezzat et al. 2017; Garcia-Pastor et al. 2019; Ozturk et al. 2019). In present study, MeJA treatments significantly retarded the losses in vitamin C, total phenolics and antioxidant capacity (both DPPH and FRAP) of apricot fruit. Greater MeJA concentration (1.0 mmol L^{-1}) was even found to be more effective in retarding losses in these parameters. Thusly, Rudell et al. (2005) reported that effects of MeJA might vary with the fruit species and cultivars, treatment times and doses. Phenolic compounds are natural compounds that make the greatest contribution to antioxidant capacity. The greater bioactive contents of MeJA-treated fruits were attributed to stimulant effect of MeJA on defense mechanism and phenol synthesis (Ali et al. 2007).

5. Conclusions

It was concluded based on present findings that MAP and MeJA treatments could be used as an efficient tool to prevent or minimize the quality losses throughout the cold storage period. It was also concluded that MeJA yielded better outcomes when combined with MAP. Further detailed research is recommended to be conducted for the best application time of MeJA (preharvest or postharvest) and method of application (spraying or dipping) for better maintenance of quality attributes of apricot fruit.

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Stabilities of Some Local Pea Lines

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ABSTRACT

This study was conducted to determine the stabilities and adaptation classes of edible pea lines, which will be cultivated in mild climate regions. Twenty local pea lines selected in previous breeding studies and 6 control varieties were used. The seeds of pea lines were sowed in 4 different locations in 2 sowing times. The experiment was conducted according to the Augmented design. Adaptation classes and stabilities of pea lines and varieties were determined based on fresh pod yield, fresh seed yield and dry seed yield. The mean of fresh pod yield was found as 1185.7 kg da^{-1}. Stable variety was not determined in terms of fresh pod yield among control varieties, which was conducted in the conclusion of evaluation as determining regression coefficient and average reliability. The average of varieties' fresh seed yield was 693.8 kg da^{-1}, Klein variety showed medium adaptation for all regions. It was found that 3 lines (B_{15}, B_{33}, and B_{36}) among used ones were placed at the same statistically group with this variety. The average of dry seed yield was 267.1 kg da^{-1} in the experiment, and Klein, Further, Green Pearl, and Lancet varieties were identified as stable varieties in the conclusion of stability analysis. B_6, B_{13}, B_{14}, B_{15}, B_{16}, B_{17}, B_{18}, B_{32}, B_{40} and B_{42} lines that involved in the same statistically group with these varieties identified were as stable. In the conclusion of the overall evaluation of the experiment, it was found that B_6 and B_{32} lines could be candidates for variety.

Keywords: Pea, Stability, Yield, Adaptation classes

1. Introduction

Pea is one of the most commonly used edible grain legumes. Thus, studies have been extensively carried out to investigate several aspects of pea cultivars in all over the world. Fresh pods and seeds of peas are used as fresh, frozen or canned vegetable and dry seeds or whole plant is used as forage (Akçin 1988). In addition, recently in the USA and also in many countries, the pea is used as modernist bakery products such as functional food, protein concentrates (55-60% protein) and isolates (85% protein), and as protein, folate and mineral enhancer in bread, pasta, cereals for breakfast, biscuits, crackers, energy bars, pressed cookies and processed meat products (URL-1). The largest cultivation of pea in the world takes place in Canada, where has the largest cultivation area in the world. In Canada, the pea is used for making bread because of due to the fiber-rich ingredient. The protein extracted from pea is used consumed as alternative source of protein by for the people who have soybean allergy as alternative source of protein and to enrichment of the animal rations. The cellulose of pea is used for fermented sausage and breakfast cereals and confectionery because of the swelling and water holding characteristics. In addition, pea starch is used in thickener, adhesive and carbon paper productions (Ratnayake et al. 2001). According to FAO 2018 statistics, dry pea cultivation area was 7.8 million ha, its yield was 171.8 kg da^{-1} and fresh pea cultivation area was 2.7 million ha, the yield was 773.6 kg da^{-1} in the world in 2018. The agriculture of pea is not widespread like chickpea, lentil and bean in Turkey. In Turkey, cultivation area of dry pea is 907 ha, production amount is 2603 tons, average yield is 287.0 kg da^{-1}; vegetable pea cultivation area is 10 917 ha, production amount is 107 344 tons, the average yield is 983.2 kg da^{-1} for fresh consumption. According to these statistics, Turkey takes the last ranks among countries, which cultivate pea. However, Anatolia, one of the origin centers of the pea, has suitable ecological conditions for pea cultivation. Pea agriculture becomes intense in the areas where are food industry regions in Turkey and its agriculture is maintained with Northern European countries varieties. In addition, local pea varieties were cultivated in the small family farms located in coastal regions with mild climate. Development of new varieties and determining the characteristics of local varieties are important to expand the pea cultivation. The environmental characteristics of a region and genotypic factors have significant impacts on yield performances of pea genotypes. Therefore, long-term studies should be conducted in several regions with different environmental characteristics. In these studies, both the differences between genotypes and genotype x environment interactions which come up because of showing different reactions of genotypes in different environments are analyzed.

The main objectives of plant breeding studies are developing highly productive varieties and making the new varieties available for producers. Therefore, studies primarily focused to develop advanced pea lines and determine the promising characteristics. The data obtained in these studies reveal the information on performances of varieties under different environmental conditions, and the findings are evaluated using specific statistical methods.

Stability analyses are applied to choose genotype when genotype x environment interactions indicated by variance analysis are significant. Stability is defined as to estimates of a change in environmental conditions, potential effects on genotypes (Kafa and Kırtok 1991). Becker (1981) defined biological stability as varieties showing a stable yield in different environments and as to agricultural stability as varieties in a certain environment has the specified level of efficiency in that environment. If genotype x environmental interactions are significant, the breeder should determine genotypes that do not show much variability in their productivity under changing environmental conditions, that is to say stable genotypes (Bozoğlu and Gülümser 2000).

Expansion of pea cultivation in our country as in developed countries of the world will make a significant contribution to food processing industry and agriculture. The most important duty of a breeder or an agronomist is to support this projection by ensuring the sustainability of local materials and developing new varieties suitable for the different regions of the country.

The pea is an important legume that can be used in crop rotation and sown as a winter crop in mild climate regions. Therefore, this study aimed to determine the stabilities of edible variety pea candidates, which have been determined as lines by choosing among local pea populations.

2. Material and Methods

The study was conducted in 8 different environments (Amasya, Samsun-Atakum, Samsun-Gelemen and, Tokat in 2 different sowing times winter and early spring) during 2015 and 2016. Some of the climatic data belonged to the environments where the experiments were carried out were presented in Figure 1 and 2. The data indicated that climates of Amasya and Tokat, Gelemen and Atakum are similar.

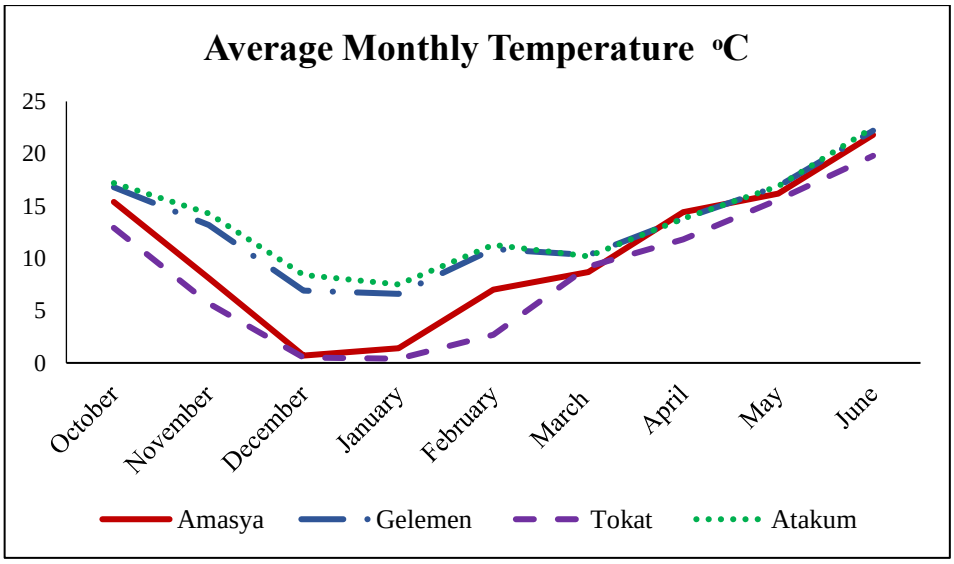

Figure 1- 2015-2016 temperature values in the locations where experiments are carried out

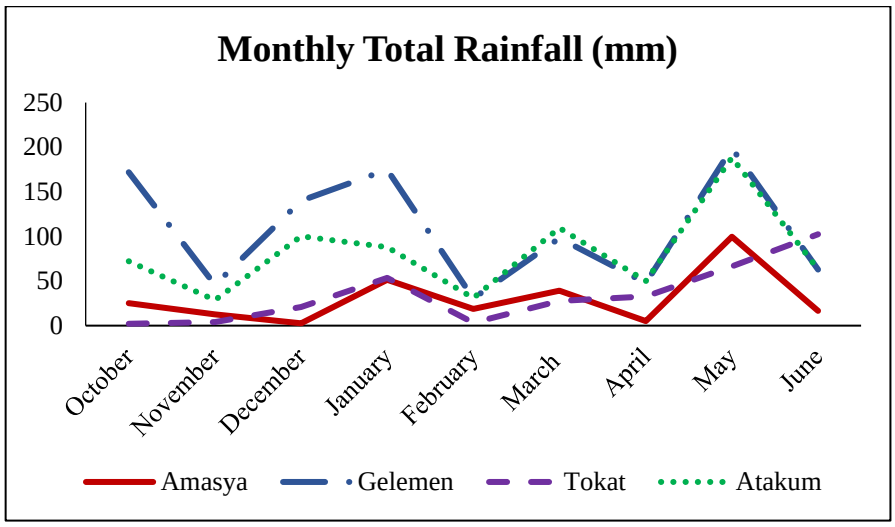

Figure 2- 2015-2016 rainfall values in the locations where experiments are carried out

Some differences were found out for the environments' soil characteristics. When Table 1 is analyzed, it is understood that insignificant variations were seen for soil type, pH and salinity, whereas there was no variation for potassium among regions. Significant variations were seen for lime and organic substance at other characteristics.

Table 1- Analysis results of soil properties of trial areas

Properties	*Amasya*	*Samsun-Gelemen*	*Samsun-Atakum*	*Tokat*
Saturation %	Clayey loam	Clayey loam	Clayey	Clayey loam
pH	Neutral	Slightly alkaline	Neutral	Slightly alkaline
Lime ($CaCO_3$)	High limy	Very low limy	Limy	Middle limy
% Total Salt %	Unsalted	Unsalted	Slightly salty	Unsalted
Phosphorus (P_2O_5 kg da^{-1})	Very high	Very high	Low	Very low
Potassium (K_2O kg da^{-1})	High	High	High	High
Organic matter %	Low	Low	Low	Very low

In the study 20 lines selected fresh and dry seed purpose fully with selection breeding among local pea materials (Karayel and Bozoğlu 2008) and 6 control varieties (Green Pearl, Sprinter, Further, Vilmoren, Lancet, and Klein) were used.

The experiments were carried out according to the Augmented Design in which the control varieties were placed in 3 blocks, and the lines were randomly placed in 3 blocks. The seeds of control varieties were sown at 60 cm row spacings on 4 rows with 4 m length, while the lines were sown on 2 rows at the same row spacings. The sowing dates for winter and early spring were given in Table 2. The experiments were carried out under rainfed conditions. Weed control was performed by hoeing when necessary. The pea was harvested as fresh and dry to determine the candidate varieties. The fresh and dry harvest dates were given in Table 2. Statistical analysis was carried out using SPSS software. Stability analyses were used to control varieties with regression model as Eberhart-Russell (1966) suggested and adaptation classes were applied as Arshad (1990) suggested. Varieties that average was higher than general average, regression coefficient (b) was equal to 1, regression deviation (S^2 b) variance was 0 or near 0 were defined as stable. Genotypes adaptation classes were defined as using experiments' general average ($\bar{x}$), regression coefficient (bi) and confidence limits (G.S= $\bar{x} \pm t \, . \, S \, \bar{x}$) determined for it (Bozoğlu & Gülümser 2000). These parameters were determined for control varieties, because Augmented Design was used for our experiment and at this design, only the control varieties values' variance analyses were done. The adaptation classes of control varieties and the lines those were placed at the same static group were decided regarding 5 % probability with LSD test grouping in the conclusion of variance analyses.

Table 2- Sowing dates and harvest date ranges of experiments

Location	*Winter sown date*	*Early spring sown date*	*Fresh harvest dates*	*Dry harvest dates*
Samsun-Gelemen	04.11.2015	04.03.2016	18.05.- 01.06.2016	20.06- 30.06.2016
Samsun-Atakum	20.11.2015	03.03.2016	20.05-18.06.2016	13.06-25.06.2016
Amasya	28.10.2015	09.03.2016	20.05-17.06.2016	16.06-24.06.2016
Tokat	05.11.2015	02.03.2016	15.04- 27.05.2016	20.05- 17.06.2016

3. Results and Discussion

Pea is a cool season legume plant. Total temperature demand of peas is 1600-2800 °C for full maturity; generally, it is sowed in autumn in mild climate regions, though it is sowed in early spring in regions where the winter is spent hard. Experiments were established in the same environment in two different planting times (winter and early spring) to create a different environment. Experiments were carried out at 8 environments with 6 control varieties and 30 lines. Their yield values and averages belonging to parameters required for stability and statistical grouping were given at Table 3.

Table 3- Stability parameters of yield values of pea genotypes grown in different environments

Genotypes	FPY (kg da^{-1})	RC (bi)	DR (S^2d)	FSY (kg da^{-1})	RC (bi)	DR (S^2d)	DSY (kg da^{-1})	RC (bi)	DR (S^2d)
G. Pearl	1155.8 de	0.52	156012	682.2 cd	0.40	62292	281.3 abc	1.10	2266
Sprinter	1359.5 cd	1.95	219404	695.8 cd	1.31	84656	313.8 a	1.7	2903
Further	1017.9 d-h	0.78	126933	570.7 e-h	0.42	28322	263.9 b-e	1.01	4281
Vilmoren	986.4 dfg	0.42	68844	744.4 c	1.11	28867	205.7 g-j	0.1	3293
Lancet	1503.5 bc	1.39	347737	788.6 bc	1.78	115909	306.8 ab	1.12	5068
Klein	1091.3 df	0.94	43318.3	681.0 cde	0.97	892.4	231.3 c-j	0.97	7387
Mean	1185.7	1		693.8	1		267.1	1	
CL	X±83.44	X±0.24		X±77.1	X±0.28		X±44.56	X±0.54	
B1	759.4 ij			410.3 ij			206.9 h-k		
B3	756.7 ij			471.4 hi			219.4 f-j		
B6	1734.3 a			975.9 a			320.9 a		
B10	811.5 hij			490.1g hi			243.4 jk		
B11	751.7 ij			484.6 ghi			271.7 f-j		
B13	782.8h ij			398.2 ij			170.4 jk		
B14	703.9 j			314.0 j			209.1 d-j		
B15	1057.0 df			535.5 fgh			295.5 a-d		
B16	769.0 hij			408.0 ij			223.1 e-j		
B17	642.1 j			376.6 ij			287.1 c-h		
B18	695.0 j			405.4 ij			248.9 b-f		
B19	784.0 hij			410.1 ij			290.0 hij		
B32	1587.6 ab			868.0 ab			298.9 c-i		
B33	909.9 e-i			548.5 fgh			211.2 d-jj		
B34	787.9 hij			595.3 d-g			241.7 ij		
B35	731.7 j			401.4 ij			204.7 jk		
B36	945.3 e-i			551.0 fgh			232.3 h-k		
B40	688.8 j			558.1 fgh			311.6 a		
B41	756.8 ij			331.5 j			241.5 h-k		
B42	969.6 d-i			533.4 fgh			247.7 d-j		
LSD	205.1			116.4			49.8		

RC: Regression Coefficient (bi); DR: Deviation from Regression (S^2d); CL: Confidence Limits; FPY: Fresh Pod Yield; FSY: Fresh Seed Yield; DSY: Dry Seed Yield, LSD: Least Significant Difference

3.1. Fresh pod yield

Pea is consumed as fresh seed, and sold with pods at local public markets in Turkey. Thus, the freshness of seeds after the harvest can conserve for a while. Therefore, the pod yield should also be determined. Since no graining is needed, the unit price is low. In our study, the average of fresh pod yield was 1185.7 kg da^{-1}, and as control varieties Sprinter and Lancet had higher values than the overall average. Regression coefficient of Lancet variety was nearer to 1. Sprinter and Lancet showed well adaptation to good environmental conditions as it can be seen from the stability graphic (Figure 3) given for fresh pod yield. The assessment conducted with mean confidence intervals and regression coefficients obtained using the fresh pod yield among the control varieties indicated no stable variety. Murtaza et al. (2007) stated fresh pod yield for pea was 276.7 - 525.5 kg da^{-1} (318.9 kg da^{-1} in control) for salicylic acid applications on pea seeds and leaves in Pakistan; Alam et al. (2010) stated it was 584.0 – 1144.0

kg da^{-1} for different fertilizer grade applications on pea in Bangladesh; Rasaei et al. (2012) stated it was 430.0- 910.0 kg da^{-1} for different irrigation dates and frequencies on pea in Iran; Gopinath and Mina (2011) stated it was 220.0- 724.0 kg da^{-1} for organic fertilizer applications on pea in India. According to the conclusion of multiple range test (LSD), it was found that B_6 (1734.3 kg da^{-1}) and B_{32} (909.9 kg da^{-1}) were placed at the same group with Lancet variety which showed well adaptation to good environmental conditions among 20 lines that was used (Table 2).

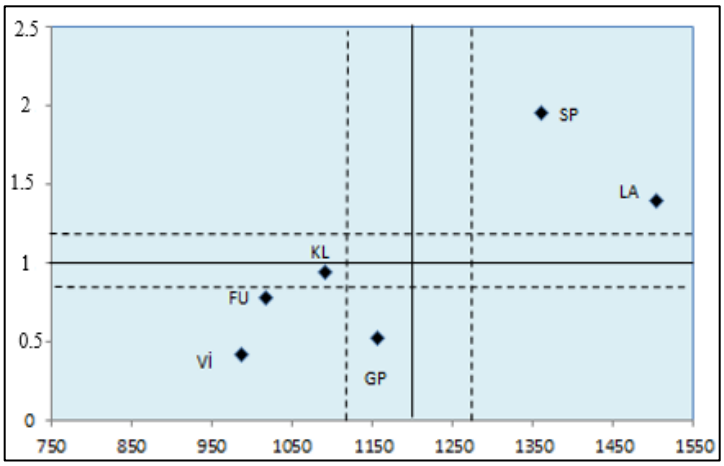

Figure 3- Stability graph of fresh pod yield of pea varieties

3.2. Fresh seed yield

Fresh pea seed is consumed both as fresh to cook directly and mostly canned in Turkey. The fresh seed yield was significantly different between genotypes and changed depending on the harvest dates. The seeds were lighter in early harvest, whereas peel pod was thinner and seed weight was heavier in the late harvest due to carbohydrate accumulation. Kalapchieva and Pevicharova (2009) reported that fresh seed yield ranged between 253.2 and 618.0 kg da^{-1} in 2000 and 2004 for Marsi variety, which is a new variety in Bulgaria. The difference in yield values can be attributed to the differences in the genotypes used and the growing environments. In our study, mean fresh seed yield for control varieties was 693.8 kg da^{-1}. The mean yield of Sprinter, Vilmoren and Lancet varieties was higher than the overall average yield value (Table 2). B_6 line gave the highest fresh seed yield as 975.9 kg da^{-1} among lines. It was determined that control varieties were different from that line statistically. B_{32} line came after B_6 with 868.0 kg da^{-1} fresh seed yield. That line placed at the same statistic group with Lancet which showed well adaptation to good environmental conditions (Figure 4). Calculated confidence limit for the average of experiment's fresh seed yield changed between 616.7-770.9 kg da^{-1}. Klein and Vilmoren showed medium adaptation to all environments, it was found that they were the most stable varieties namely. Only one line (B_{34}) placed at the same group with those varieties statistically.

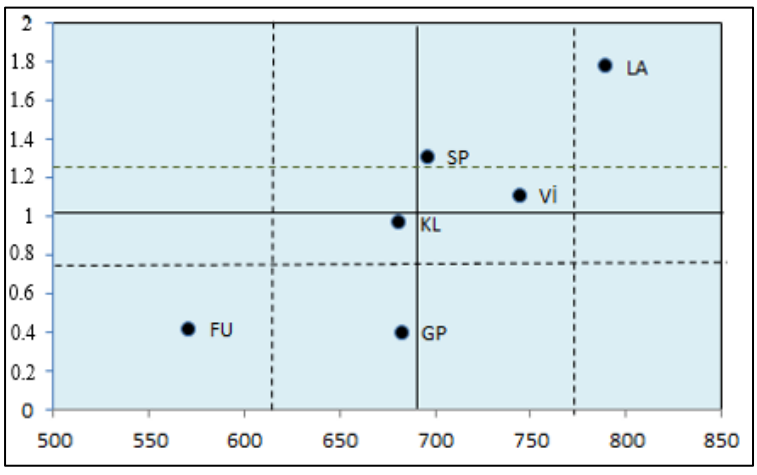

Figure 4- Stability graph of fresh seed yield of pea varieties

It was found that Klein and Vilmoren showed medium adaptation to all environments, namely stable, Lancet showed well adaptation to good environmental conditions, Green Pearl showed medium adaptation to bad environmental conditions. Among the lines only B_{34} line placed at the same statistic group with these varieties, and it was determined as stable line in terms of fresh seed.

3.3. Dry Seed Yield

High protein content of edible legumes consumed as dry seed come into prominence. Dry seed yield is an important trait even if pea is not consumed as dry seed. Various dry seed yields have been reported in studies conducted different ecological regions of Turkey. Öz & Karasu (2010) reported dry seed yield between 96.8 and 149.0 kg da^{-1} under ecological conditions of Bursa province, and the dry seed yield in İzmir ecology was between 143.0 and 349.0 kg da^{-1} (Alan & Geren 2012). The dry seed yield under Ankara ecology ranged from 190.9 to 276.4 kg da^{-1} (Kara & Ünver 1999). Dry seed yield recorded in this study was 267.1 kg da^{-1}, which was higher than the dry seed yields reported in literature and mean value in Turkey. Bozoğlu et al. (2007) found that dry seed yield was 212.4 kg da^{-1} averagely for winter sowing and 164.6 kg da^{-1} for in summer sowing in their study with 15 genotypes under Samsun's conditions. It was stated that Vilmoren and Sprinter placed at the first lines particularly in winter sowings. Except Vilmoren and Sprinter, all other control varieties were between confidence limit (222.54-311.66 kg da^{-1}) that is determined $P<0.05$ probability. This limit changed between 0.46-1.54 for regression coefficient. In this case Klein, Further, G. Pearl, and Lancet showed medium adaptation to all environments in terms of dry seed yield, namely they became stable varieties (Figure 5). It was found that all lines placed at the same group with stable varieties regarding applied LSD grouping.

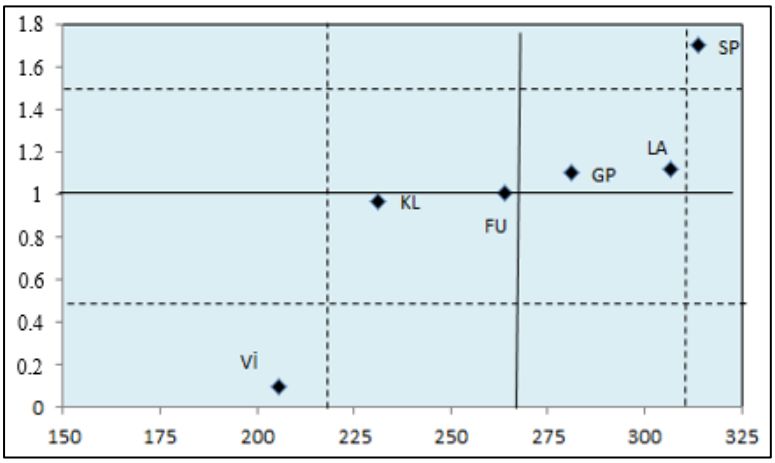

Figure 5- Stability graph of dry seed yield of pea varieties

4. Conclusions

The problem of coronavirus disease (COVID-19) that whole world threaten indicated the importance of health to all human being, and emphasized the necessity of living compatible with nature and sustainable use of natural sources. Seed is the most important and non-replaceable input of agricultural production. Domestic seeds, exist for hundreds of thousands of years in a geography are the most valuable sources of modern agriculture. Turkey is one of the germplasm centers of the pea. The aim of this study was determine the pea variety candidates by identifying the adaptation classes and the stabilities of local pea lines determined by the long-term field studies based on the yield characteristics. The experimental was carried out according to the Augmented design with 6 control varieties and 20 lines. Stability analyses were applied for control varieties. The data obtained in stability analysis were interpreted using LSD.

Although adaptation classes were same based on the regression coefficient the deviation, and the confidence limits for dry seed yield, it was found that multiple range test groups were different. It showed that lines and control varieties should be repeated as equal numbers, and a common stability parameter should be determined to make stabilities come up more precise.

The experiments carried out in 8 different environments indicated that the mean fresh pod yield, fresh seed yield and dry seed yield were 1188.7, 693.8 and 267.1 kg da^{-1}, respectively. Fresh pod and seed yield of B_6 and B_{32} lines were higher than those obtained from control pea varieties. The dry seed yield of B_6, B_{15}, B_{32} and B_{40} lines exceeded the overall average dry seed yield. The stability analysis revealed that B_6 and B_{32} lines are stable in terms of fresh pod and seed yield. Klein, Further, Green Pearl and Lancet varieties and B_6, B_{13}, B_{14}, B_{15}, B_{16}, B_{17}, B_{18}, B_{32}, B_{40} and B_{42} lines are stable for dry seed yield. Although some licensed varieties are available for the production of fresh seed in Turkey, no licensed local varieties are available for the production dry seed. The results of this study concluded that B_6 and B_{32} lines could be considered as the variety candidates in terms of yield parameters obtained.

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Effects of Growing Season and Ripening Stages on Transcription Level of Geranylgeranyl Reductase (*OeCHL P*) and Some Biochemical Properties in Some Important Olive Cultivars (*Olea europaea* L.)

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ABSTRACT

Transcription level of geranylgeranyl reductase (OeCHL P), and contents of α-tocopherol, oleuropein, hydroxytyrosol and tyrosol of seven olive cultivars obtained from Olive Germplasm Collection, Kemalpasa, Izmir were determined in two ripening stages (green and black fruit) in two consecutive years, 2017 and 2018.

Transcription level of OeCHL P was significantly affected by year, ripening stage and cultivar. The highest values were detected in green fruit of 'Uslu' in both years. In comparison to 2017, a significant increase in gene transcription was observed in 2018 independent of cultivar and ripening stage. The highest α-tocopherol and oleuropein content were obtained from 'Girit Zeytini'. The content of oleuropein decreased with ripening in all cultivars in both years. Tyrosol reached its highest and lowest values in 'Girit Zeytini' at black stage in 2018 and green stage in 2017, respectively. 'Girit Zeytini' stood out for both nutritional value and fruit size. The highest and lowest values of hydroxytyrosol content recorded in 'Girit Zeytini' (2017, black stage) and 'Izmir Sofralık' (2018, green stage), respectively. We also detected positive correlations between OeCHL P relative transcription level and tocopherol, tyrosol contents.

Our overall results indicated that olive CHL P plays an important role in regulation of tocopherol synthesis. A direct relationship was determined between OeCHL P and α-tocopherol, while there was an indirect link between others. These results revealed that more than one factor could affect the evaluated parameters.

Keywords: Gene expression, CHL P, Olive, Oleuropein, α-tocopherol, Tyrosol

1. Introduction

The origin of the olive is Anatolia. There are 97 selected cultivars or types originating from Turkey and 33 cultivars originating from other countries in the Olive Research Institute collection, Turkey (Ozkaya et al. 2004). The 108 olive cultivars grown in Turkey were registered. Thirty of these cultivars are common and others are grown in more limited areas (Efe et al. 2016).

Olive fruit ripening is a long process occurring within 6-8 months. Optimal harvest time varies according to the purpose of the utilization of olives. If olives are used for oil, the best harvesting time is considered between green and black stages. Phenolic compounds, e.g., hydroxytyrosol (HyT) and oleuropein (Ole) are responsible for its peculiar pungent taste and for its high stability in extra virgin olive (Psomiadou & Tsimidou 2002; Visioli et al. 2002).

Recently, some reference genes have been identified during the development and ripening periods of olive fruit. Within these genes, the NADPH-dependent geranylgeranyl reductase (*CHL P*) catalyzes formation of chlorophyll, tocopherols (TcP) and plastoquinone compounds, which are necessary for metabolic pathways associated with plant response to yield and stress, as well as the nutritional value of fruit (Muzzalupo et al. 2011). *CHL P* gene expression modulation follows a complex genetic network under developmental stages and stress conditions. It is reported that *CHL P* message is abundant in chlorophyll-containing tissue and flower organs, rarely in mesocarps and roots in peach genotypes (Giannino et al. 2004). *OeCHL P* transcripts were detected in various organs of olive plants (Threlfall & Whistance 1970; Bollivar et al. 1994; Keller et al. 1998; Tanaka et al. 1999; Munnè-Bosch & Alegre 2002). *CHL P* transcription in 'Coralea' olive cultivar fruits damaged by *Bactrocera oleae* pathogen was locally increased in specific cells. By examination of all the data, it was reported that *OeCHL P* gene

expression responds to biotic and abiotic stress factors in the early period and gene activity may be related to TcP activity under stress (Bruno et al. 2009).

The aim of this work was to study the transcription levels of *OeCHL P* gene during drupe ripening by comparing seven cultivars, to elucidate the possible correlation between *OeCHL P* mRNA level and phenolic compounds (Ole, HyT, and Ty) and α-TcP biomarkers.

2. Material and Methods

Fruit samples of seven cultivars were obtained from Olive Germplasm Collection, Kemalpasa, Izmir, Turkey (Table 1). The analyzes were carried out at Alata Horticultural Research Estitute, Erdemli, Mersin, Turkey in 2017 and 2018. Olive fruit samples were taken according to the scale of 0-7 based on ripening stages (Cebeci 2007). Green fruit (GF) samples were harvested at 127th day after anthesis (A), scale of 1. Black fruit (BF) samples were collected at 190th day, scale of 6. The container containing dry ice is used to transfer the harvested olive fruits rapidly from orchard to Alata Horticultural Research Enstitute Biotechnology Laboratory. Then fruits were transferred to liquid nitrogen (-196 °C) and were kept at -80 °C until analysis.

Table 1- List of the experimental olive cultivars

Cultivar name	*Origin*	*Use*
Cilli	Kemalpasa- Izmir	Green Table
Esek Zeytini (Odemis)	Odemiş- Izmir	Green Table
Girit Zeytini	Bodrum- Mugla	Oil
Izmir Sofralık	Izmir	Green Table
Nizip Yaglık	Gaziantep, Nizip	Oil, Table
Sinop No 5	Sinop	Table, Oil
Uslu	Akhisar	Black Table

Anthesis dates of cultivars were recorded before olive fruits were collected (Table 2). 'Uslu' and 'Sinop No 5' had the earliest anthesis date in 2017 (May, 13 and May, 15, respectively). Anthesis dates of 'Cilli', 'Esek Zeytini', 'Girit Zeytini', 'Nizip Yaglık' were May, 19. The latest anthesis date was in 'Izmir Sofralık (May, 20)' in 2017. The earliest anthesis date was in 'Uslu (May, 12)' and 'Sinop No 5 (May, 15)' again in 2018. Anthesis date of 'Cilli', 'Girit Zeytini', 'Nizip Yaglık' was May, 18. However, 'Esek Zeytini' was become to anthesis time two days early compared to 2017 (May, 17). Anthesis date of 'Izmir Sofralık' was not changed according to the previous year.

Table 2- The date of anthesis, green fruit (GF) and black fruit (BF) stages of the cultivars (sampling date)

Cultivars	*Anthesis 2017*	*GF Stage 2017*	*BF Stage 2017*	*Anthesis 2018*	*GF Stage 2018*	*BF Stage 2018*
Cilli	May,19	September, 25	November, 25	May, 18	September, 24	November, 24
Esek Zeytini (Odemis)	May,19	September, 25	November, 25	May, 17	September, 23	November, 23
Girit Zeytini	May,19	September, 25	November, 25	May, 18	September, 24	November, 24
Izmir Sofralık	May, 20	September, 26	November, 26	May, 20	September, 26	November, 26
Nizip Yaglık	May,19	September, 25	November, 25	May, 18	September, 24	November, 24
Sinop No 5	May,15	September, 21	November, 21	May, 15	September, 21	November, 21
Uslu	May, 13	September, 19	November, 19	May, 12	September, 14	November, 18

2.1. Transcript analysis in olive pericarp

Total RNA was isolated from pericarp tissues at two different developmental stages and processed separately. It was performed by using trizol (TRI®Reagent T9424 SIGMA) according to Avison (2007)s' protocol. The quality and quantity of total isolated RNA were controlled with a Nanodrop spectrophotometer (Thermo Fisher, Madison WI, USA), according to the manufacturer's instructions (Thermo Fisher). cDNA synthesis was performed from isolated RNA samples. The first sequence of cDNA synthesis was performed using High Reverse Transcriptase cDNA synthesis kit 200 rxn. Quantitative revers-transcription PCR (qRT-PCR) was performed on a Rotor-Gene 6000 Real-Time PCR (Qiagen, USA). The primer used for qRT-PCR analyses of the *OeCHL P* gene (GeneBank DQ424963) are Fw 5'-CCAAGGGAGGCATTTGTAGA-3' and Bw 5'-TGGATTCACAGCCAATTTCA-3'. 18S rRNA was used as a normalization control. The primer sequence of 18S rRNA was Fw 5'-AAACGGCTACCACATCCAAG-3' and Bw 5'-CCTCCAATGGATCCTCGTTA-3'. Amplification reactions were performed according to the procedures of Bruno et al. (2009). The results of qRT-PCR were analyzed via Opticon Monitor. Cycle threshold (CT) values were obtained from Genex Software (Bio-Rad) and data was analyzed through the 2^{-DDCT} method (Livak & Schmittgen 2001). The means of *OeCHL P* transcript levels were calculated from three biological repeats, obtained from three independent experiments.

2.2. Biochemical analysis in olive pericarp

The α-TcP analysis was performed by HPLC (High-Performance Liquid Chromatography, Shimadzu Corp, USA) according to Abidi (2000) and Ruperez et al. (2001). Five mg of dry sample was taken and 35 mL of each dioxane [n-hexane (1:1, v:v)] was extracted four times in mortar. Samples were cleaned by evaporation and centrifugation. The residue was dissolved in 10 mL of dioxane [n-hexane (3:97, v:v)]. This solution was analyzed in HPLC using a column filled with C18 Nucleosil 50 (4.6x250 mm) with dioxane (n-hexane (3:97, v: v) at a flow rate of 1.5 mL min^{-1}. α-TcP concentration was determined using a standard curve obtained from the commercial standard (Sigma-Aldrich, USA). α-TcP content was estimated at 295 nm_{ex} and 325 nm_{ex} using a fluorescent detector.

Ole, HyT, and Ty determined by HPLC according to Vinha et al. (2005). Each sample (1.5 g) was mixed with methanol. The methanolic extract was filtered, evaporated to dryness under reduced pressure (40 °C) and redissolved in methanol (4 mL) of which 20 µL were injected for HPLC analysis. 1.5 g of each sample were subjected to extraction. The methanolic extract obtained was taken to dryness under reduced pressure (40 °C) and redissolved in 50 mL of acidified water (pH 2 with HCl). The aqueous solution was passed through a C18 column, previously conditioned with 60 mL of methanol and 140 mL of acidified water (pH 2 with HCl). The loaded cartridge was washed with 60 mL of n-hexane to eliminate the lipid fraction and the retained phenolic compounds were then eluted with methanol (60 mL). The methanolic extract was concentrated to dryness under reduced pressure (40 °C) and redissolved in methanol (4 mL). The injection volume for HPLC analysis was 20 µL. When determining the phenolic content, the peak areas were calculated by multiplying by the dilution factor and the results were expressed as mg g^{-1} (dry weight). Hyt, Ty and Ole (Extrasynthese, France) were used as standard substances. Detection was carried out with a diode array detector, and chromatograms were recorded at 280 nm. The external standard method was used for the quantification of the individual phenolic compounds.

2.3. Statistical Analyses

Data were presented as mean±SD and subjected to 3-way ANOVA with randomized plot design with three replications for each parameter using JPM 5.0.1. software (SAS Institute, 1989) followed by LSD test (P<0.05). Additionally, possible correlations between parameters were determined.

3. Results and Discussion

OeCHL P transcription levels were estimated through RT-PCR analysis in seven cultivars harvested at two stages of drupes ripening. In 2017, 'Sinop No 5 (1.00)' was used as a sample calibrator in GF stages and 'Girit Zeytini (1.17)' was used in BF stages. In 2018, 'Uslu (2.96)' was used as a sample calibrator in GF stages and 'Esek Zeytini (2.95)' was used in BF stages. Transcription level of *OeCHL P* was significantly affected by year, ripening stage and cultivar. The highest values were detected in GFs of 'Uslu' in both years (3.01 in 2017 and 2.96 in 2018). The lowest *OeCHL P* gene expression levels detected in 'Sinop No 5', 'Nizip Yaglık', 'Girit Zeytini', 'Esek Zeytini' cultivars were changed according to ripening stage and years. 'Uslu' showed a stable structure by exhibiting the lowest expression level at the BF stages in both years. When the results of the two years are evaluated in general, almost all cultivars increased their *OeCHL P* expression levels in 2018 compared to the previous year (Figure 1).

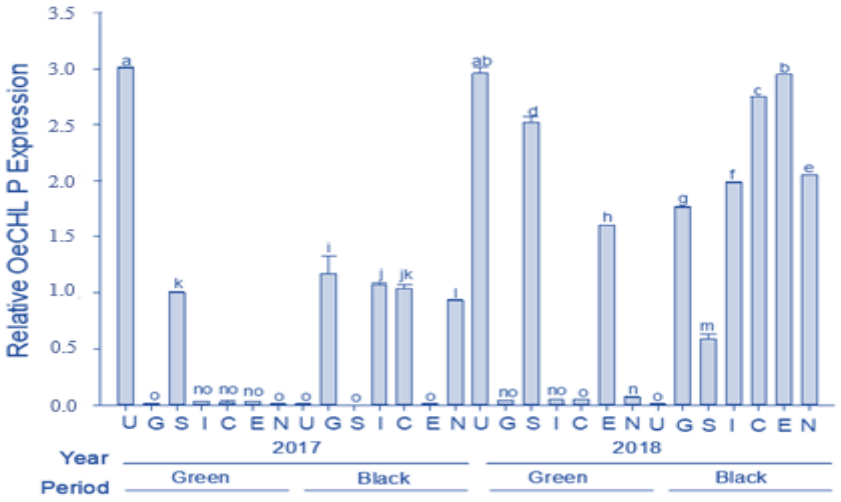

Figure 1- Transcript levels of *OeCHL P* gene in pericaps of cultivars (U: Uslu, G: Girit Zeytini, S: Sinop No 5, I: Izmir Sofralik, C: Cilli, E: Esek Zeytini, N: Nizip Yaglık) harvested at GF and BF ripening in the same cultivation areas (Kemalpasa, Izmir). Values represent the means of three independent biological replicates. Vertical bars show the relative *OeCHL P* level of cultivars. Significant differences between means are shown by different letters (P≤0.05)

OeCHL P gene expression responds to biotic and abiotic stress factors early and independently from the olive genotypes. The expression of *CHL P* was investigated in the different tissues. *CHL P* mRNAs in the cotyledon were induced by light but repressed by dark (Park et al. 2010; Zhou et al. 2013). Similar *CHL P* expression levels in cold stress condition have been also reported in peach (Giannino et al. 2004). Plants often encounter wounding and pathogen attacks because of the temperature changes (Hernandez et al. 2011). One of the main stress factors for the olive plants is the *Bactrocera oleae* (Burrack and Zalom, 2008). The average temperature and precipitation value of Kemalpasa location was recorded during experimental years from the 1st to the 12th month (Table 3). In 2018, olive fruit fly unexpectedly and intensely attacked and significantly increased the *OeCHL P* gene expression rate of cultivars compared to the previous year. Besides, olive fruit fly was variable and uncontrolled conditions such as early increased weather temperature and low precipitation compared to the previous year significantly affected the *OeCHL P* expression level of the cultivars. The same result was shown by Bruno et al. (2009). It was reported that *OeCHL P* gene expression level was 2.5 fold-higher in fruits attacked by *Bactrocera oleae* than healthy ones.

Table 3- The average temperature and precipitation values for the months of 2017 and 2018

Month	*Year*	*Average Temperature (°C)*	*Average Precipitation (mm)*	*Month*	*Year*	*Average Temperature (°C)*	*Average Precipitation (mm)*
1	2017	3.3	246.4	1	2018	6.1	81.8
2	2017	7.9	50.2	2	2018	9.8	119.6
3	2017	11.0	88.0	3	2018	13.0	58.4
4	2017	14.2	15.4	4	2018	17.2	10.0
5	2017	19.2	43.4	5	2018	21.5	26.2
6	2017	24.4	18.4	6	2018	24.1	46.8
7	2017	27.7	13.0	7	2018	27.3	24.0
8	2017	27.2	0.0	8	2018	27.4	6.6
9	2017	22.9	0.0	9	2018	23.1	6.6
10	2017	16.2	0.0	10	2018	16.6	61.6
11	2017	10.0	72.2	11	2018	12.4	83.0
12	2017	9.1	116.0	12	2018	8.7	108.4

The α-TcP levels of cultivars were significantly influenced by all factors and their interactions (Table 4). The highest α-TcP value was detected in 'Girit Zeytini' (2017, BF stage) as 362.14 mg kg^{-1} of total oil and this was followed by 'Izmir Sofralık' (2017, BF stage) as 349.74 mg kg^{-1} of total oil. The lowest α-TcP value was detected in 'Izmir Sofralık' (2017 and 2018, GFs stage) as 146.55 and 143.41 mg kg^{-1} of total oil, respectively. The content of α-TcP is decreased in 2018 in comparison to 2017 in all cultivars except for 'Esek Zeytini'.

CHL P is a minor component (Collakova and DellaPenna 2003) and required for synthesis of TcPs (Tanaka et al. 1999). α-Tocopherol comprises 90% of the total tocopherol content in olive oil (Paiva-Martins & Kritsakis 2017). Aguilera et al. (2005) reported that the amount of α-TcP sharply decreases in the earlier stages of ripening, but then maintains its concentration more or less constantly during ripening, with a further decrease at the latest ripening stage. But, Muzzalupo et al. (2011) reported that the biosynthesis of TcPs increased during the process of pericarp ripening. In our study, the amount of α-TcP decreased with the ripening in early ripening cultivars ('Uslu' and 'Sinop No 5') but increased in another medium late and latest ripening cultivars ('Cilli', 'Esek Zeytini (Odemis)', 'Girit Zeytini', 'Izmir Sofralık', and 'Nizip Yaglık'). Andrikopoulos & Hassapidou (1989) specified the α-TcP content in different types of Greek olive oil and found that virgin olive oil contained an average of 113.00 mg kg^{-1} of α-TcP. Bruno et al. (2009) determined that α-TcP content of 'Carolea' cultivar as 109.98 ug g^{-1} DW in GF stage and 151.44 ug g^{-1} DW in dark fruit. In our study, α-TcP content was found between 257.16-146.55 mg kg^{-1} of total oil in GF stage and 362.14-204.22 mg kg^{-1} of total oil in BF stage. Our α-TcP results are higher than those reported by Andrikopoulos & Hassapidou (1989) and Bruno et al. (2009).

The Ole content of cultivars were significantly influenced by all factors and their interactions except for year × ripening stage (Table 4). Fruit size (small or large drupe) affects the content of Ole (Amiot et al. 1989). The content of Ole in small drupe cultivars was greater than that of large drupes (Amiot et al. 1986). 'Girit Zeytini', which was quite small drupes cultivar, is determined as the highest cultivar of Ole content (65.67 mg g^{-1} DW at GFs in 2017, and 49.71 mg g^{-1} DW at BFs in 2017). The lowest Ole contents were detected in 'Cilli' as 3.73 and 2.99 mg g^{-1} DW at BFs in 2017 and 2018, respectively. When the results were evaulated, the content of Ole decreased with ripening in all cultivars in both years. The highest α-TcP and Ole content were obtained from 'Girit Zeytini'.

Table 4- α-Tocopherols (α-TcP), oleuropein (Ole), hydroxytyrosol (HyT) and tyrosol (Ty) content in pericarp of cultivars at different ripening stages

Year	*Ripening Stage*	*Cultivar*	*Parameters*			
			α-TcP (mg kg^{-1} oil)	*Ole (mg g^{-1} DW)*	*HyT (mg g^{-1} DW)*	*Ty (mg g^{-1} DW)*
2017	Green	Uslu	234.34±4.49^{j}	22.54±1.02^{m}	2.34±0.29^{cd}	1.75±0.61^{b}
		Girit Zeytini	257.16±0.58^{e}	65.67±0.34^{a}	2.24±0.24^{cd}	0.31±0.12^{efgh}
		Sinop No 5	247.66±0.15^{g}	26.55±1.34^{j}	1.53±0.38^{ghi}	0.56±0.18^{de}
		Izmir Sofralık	146.55±0.01^{t}	30.94±0.19^{h}	2.32±0.23^{cd}	0.15±0.09^{gh}
		Cilli	207.11±0.02^{op}	20.91±1.00^{n}	2.31±0.03^{cd}	1.49±0.63^{b}
		Esek Zeytini	204.27±0.03^{q}	29.45±0.39^{i}	2.08±0.03^{def}	0.01±0.00^{h}
		Nizip Yaglık	205.00±0.02^{pq}	46.48±0.67^{c}	2.14±0.01^{cde}	0.23±0.01^{fgh}
	Black	Uslu	213.23±0.19^{n}	5.58±0.58^{rs}	2.87±0.76^{ab}	0.50±0.08^{def}
		Girit Zeytini	362.14±0.65^{a}	49.71±0.45^{b}	3.00±0.94^{a}	1.66±0.39^{b}
		Sinop No 5	215.99±0.01^{m}	6.68±0.12^{q}	2.10±0.08^{cdef}	1.06±0.06^{c}
		Izmir Sofralık	349.75±0.45^{b}	18.39±0.20^{p}	2.15±0.10^{cde}	1.15±0.09^{c}
		Cilli	238.71±0.51^{i}	3.73±0.32^{t}	2.26±0.12^{cd}	1.55±0.16^{b}
		Esek Zeytini	208.15±1.75^{o}	19.32±0.06^{o}	2.15±0.02^{cde}	1.12±0.01^{c}
		Nizip YaGlık	236.09±0.03^{j}	35.74±0.66^{f}	3.02±0.02^{a}	1.14±0.01^{c}
2018	Green	Uslu	228.76±1.01^{k}	19.46±0.71^{o}	2.24±0.03^{abc}	1.06±0.05^{c}
		Girit Zeytini	255.66±0.10^{e}	31.19±0.07^{h}	2.02±0.04^{defg}	0.16±0.04^{gh}
		Sinop No 5	245.25±0.04^{h}	24.09±0.29^{l}	2.25±0.14^{cd}	0.30±0.05^{efgh}
		Izmir Sofralık	143.41±0.14^{u}	41.40±0.22^{e}	1.12±0.02^{i}	0.43±0.02^{defg}
		Cilli	198.17±0.01^{r}	22.77±0.51^{m}	1.72±0.29^{efgh}	0.22±0.06^{fgh}
		Esek Zeytini	222.41±1.47^{l}	43.11±0.39^{d}	1.32±0.12^{hi}	0.57±0.07^{de}
		Nizip Yağlık	175.13±4.33^{s}	33.96±0.63^{g}	1.61±0.36^{fghi}	0.09±0.08^{h}
	Black	Uslu	213.26±0.02^{n}	4.79±0.98^{s}	2.15±0.39^{cd}	0.27±0.17^{efgh}
		Girit Zeytini	297.86±0.16^{c}	25.13±0.12^{k}	2.30±0.04^{cd}	2.15±0.01^{a}
		Sinop No 5	204.22±0.49^{q}	5.90±0.11^{qr}	2.14±0.08^{cde}	1.04±0.02^{c}
		Izmir Sofralık	286.32±0.04^{d}	23.40±0.15^{lm}	2.17±0.07^{cde}	1.09±0.02^{c}
		Cilli	226.81±2.06^{k}	2.99±0.39^{t}	2.40±0.31^{bcd}	0.70±0.14^{d}
		Esek Zeytini	250.44±1.15^{f}	27.34±0.47^{j}	2.60± 0.62^{abc}	1.04±0.04^{c}
		Nizip Yaglık	234.72±1.63^{j}	23.86±0.19^{l}	2.46±0.22^{bcd}	0.48±0.02^{def}
3-way ANOVA						
Year			<0.0001	<0.0001	0.0001	<0.0001
Stage			<0.0001	<0.0001	<0.0001	<0.0001
Cultivar			<0.0001	<0.0001	0.0012	<0.0001
Year × Stage			<0.0001	0.6383	0.1667	0.6879
Year × Cultivar			<0.0001	<0.0001	0.0068	<0.0001
Stage × Cultivar			<0.0001	<0.0001	0.1227	<0.0001
Year × Stage × Cultivar			<0.0001	<0.0001	0.0002	0.0003

Data represent mean ± SD of three independent biological replicates. Significant differences between means are shown by different letters within each parameter (P≤0.05)

The Hyt content of cultivars were significantly influenced by all factors and their interactions except for year × stage and stage × cultivar interactions (Table 4). The highest Hyt content was found in 'Nizip Yaglık' and 'Girit Zeytini' (2017, BF stages) as 3.02 and 3.00 mg g^{-1} DW, respectively. The lowest contents were detected in 'Izmir Sofralık' as 1.12 mg g^{-1} DW and 'Esek Zeytini' as 1.32 mg g^{-1} DW at BF stages in 2018. The Ty contents of cultivars were significantly influenced by all factors and their interactions except for year × stage (Table 4). The highest Ty content was found in BFs of 'Girit Zeytini' as 2.15 and 1.66 mg g^{-1} DW in 2017 and 2018, respectively. The lowest content was found in Esek Zeytini as 0.01 mg/g DW at GF stage of 2017. When the Ty content of all cultivars were evaluated, Ty contents increased with ripening except for 'Uslu' in both years. Ty and HyT are phenolic products of hydrolyzed oleuropein (Montedoro et al. 1992; Tsimidou et al. 1992) and Ty content may be a harvest criterion. The HyT contents of cultivars ranged from 3.00 to 1.12 mg kg^{-1}. These values were quite higher than the findings (ranged from 0.006 to 0.057 mmol kg^{-1}) of Yousfi et al. (2006). The highest Ty content was determined in BFs of 'Girit Zeytini'. As a result of the evaluation of both years, the fastest conversion to tyrosol was in BFs of the 'Girit Zeytini'. The Ty contents of cultivars were increased with the ripening except 'Uslu'. 'Uslu' is earlier blooming cultivar and ripening period is short. Similar to results Bengana et al. (2013) stated that the Ty content of 'Chemlal' cultivar decreased as maturity progressed. Yıldırım et al. (2016) also reported that Ty content increased with the progress of maturity.

In the present study, a positive correlation was found between *OeCHL P* gene expression and α-TcP, and Ty. Muzzalupo et al. (2011) reported that a positive linear trend between *OeCHL P* relative transcription levels and α-TcP content during pericarp ripening. Furthermore, Boskou et al. (2004) reported that as the content of Ole content decreased with ripening, the content of HyT and Ty decreased in parallel. However, there was no connection between Ole and other parameters. But, a positive correlation was also found between α-TcP and HyT, Ty and between Hyt and Ty (Table 5).

Table 5- Correlation coefficients of the parameters obtained from two years averages

Variables	*OeCHL P*	*α-TcP*	*Ole*	*HyT*
α-TcP	0.3926**			
Ole	-0.1447	0.1080		
HyT	0.2005	0.3589*	-0.1216	
Ty	0.3926*	0.3926*	-0.2066	0.3497*

*P≤0.05; **P≤0.01

5. Conclusions

OeCHL P gene expression level varied between two consecutive years among the cultivars. Although these differences might be due to biotic and abiotic factors such as ripening stage, temperature, precipitation and *Bactrocera oleae*, some other factors, which have not been established yet, might be responsible as well. These results indicated that olive CHL P played an important role in response to the regulation of TcP synthesis. CHL P could be a good candidate gene for genetic improvement of plant growth and changing conditions. There was not a marked connection between oleuropein and other parameters. But, we suggest that oleuropein might be an important parameter for in determination of cultivars that can be utilized as functional products. On the other hand, Hyt can give an idea about the hydrolysis rate of oleuropein and Ty can be important criteria in determining the harvest date. Further studies need to be carried out, considering more factors and different ripening stages.

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Abbreviations	
BF(s)	Black Fruits
GF(s)	Green Fruits
HyT	Hydroxytyrosol
NADPH	Nicotinamide adenine dinucleotide phosphate
OeCHL P	*Olea europaea* geranylgeranyl reductase
Ole	Oleuropein
Phe(s)	Phenols
ROS	Reactive oxygen species
Ty	Tyrosol
α-TcP(s)	Tocopherol

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Effects of Different Milk-Tube Guidance Settings and Teat-Cup Types on the Dynamics of Teat-End Vacuum and Vacuum Fluctuations During Machine Milking

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ABSTRACT

The objective of this study was to determine the effects of milk tube guidance setting and teat cup type on the dynamics of teat-end vacuum and vacuum fluctuations in quarter individual milking systems. In order to meet this objective, a series of wet tests was conducted in the laboratory. Different teat cups; AMS, BIO and RVS along with the milk tube guidance settings namely; direct tube, half-half tube and Y-piece tube system with varying tube inside diameters were tested at different water flow rates. The data obtained from the wet-test measurements were used to calculate the teat-end vacuum and vacuum fluctuation at the teat-end in b and d- phase.

From the study conducted, it was found that the teat-end vacuum in BIO is always between the range of 32 and 42 kPa as recommended by DIN ISO 5707 (2010a) at any flow rate for all three types of connections. The teat-end vacuum for RVS was between 32-42 kPa range if the flow rate varies between 4 and 6 L min^{-1} for both, the direct and the half-half connection. The Y-piece connection meets DIN ISO 5707 (2010a) requirements once the flow rate changes between 2 and 6 L min^{-1}. The findings about milk tube inside diameter indicated that the use of 14 or 16 mm milk tube diameter for BIO will provide better teat-end vacuum if the recommended value of 10 mm is used. On the other hand, the use of 16 mm milk tube diameter was found to be appropriate for AMS as recommended by the manufacturer. For the RVS, the appropriate milk tube diameter should be 14 mm when statistical differences in b and d-phase are examined from the point of teat-end vacuum and vacuum fluctuations.

Keywords: Vacuum drops, Teat-end vacuum, Vacuum fluctuation, Quarter individual milking

1. Introduction

During the whole milking process, the udder is highly stressed. Therefore, the large-area spread of milking plants, number of udder diseases have strongly increased (Graff 2005). The modern milking technique is very concerned to work as animal-friendly as possible but nevertheless the damage and involved diseases of the udder cannot be completely eliminated.

The teat cup liner is of importance since it comes contact with the cow's teat. As a result of this, milking performance, udder and teat health are higly affected. Bobıć et al. (2018) worked on two different teat cup liners (different head diameter, length, body shape) with or without air vent. They investigated their efffects on teat condition score, somatic cell count. The results obtained from the study as a comparison with the control group indicated that teat cup liner type affetcs teat skin condition, somatic cell count, and therefore the risk of mastitis.

Ströbel et al. (2011) investigated the teat end vacuum condition in different automatic milking systems. For this purpose, three different milking systems were tested. Two of these systems had individually guided milk tubes. The teat cups of each system were constructed with air inlet at the end of each teat cup that allowed air entry into the milk tube.

In another study, Ströbel (2012) developed a vacuum control system for the teat-end vacuum. The concept for the vacuum control system indicated that it is possible to reduce the mean teat-end milking vacuum in the suction phase to 20 kPa at a flow rate of 0.25 L min^{-1} per udder quarter. At higher flow rates of 1.5 L min^{-1} and more per udder quarter, the teat-end vacuum is

similar to the machine vacuum with a mean value of approximately 30 kPa. Therefore, supplying a high teat-end vacuum at a high and a low teat-end vacuum at low milk flow rates was possible.

DIN ISO 5707 (2010a) indicates that a mean liner vacuum within the range 32 kPa to 42 kPa during the peak low period of milking for cows ensures that most cows will be milked quickly, gently and completely. Besier et al. (2016) additionally implied that this range of vacuum pressure is adequate for liner movement and a sufficient pressure on the teat can be provided during the massage d- phase. Besier et al. also mentioned that the teat-end vacuum should not be much higher than 42 kPa as it can cause damage of teat tissue mainly during periods of low milk. As a concluding remark, they stated that the most of the literature used in their review paper was from the 1960s, 1970s or 1980s, since there is no newer study and Thus, it is necessary to conduct new studies on the influence of vacuum drops and fluctuations.

Vacuum fluctuations can occur cyclic, irregular or in combination (Nyhan 1968; Tolle et al. 1977). Cyclic vacuum fluctuations occur in regular intervals during milking and the main reason is the liner movement and milk flow (Thiel et al. 1973) and this leads, due to a loss of flow, to a vacuum decline at the teat-end (Ordolff 1991; Mein 1992). On the other hand, irregular fluctuations are caused by liner slips, cluster changing or cluster fall off during milking and unplanned air admission into the vacuum system (Nyhan 1968; Thiel et al. 1973). If vacuum fluctuations occur in combination of irregular and cyclic form for a long milking duration, the risk for congestion of the teat tissue along with new infections was found to be increased (O'Callaghan et al. 1976; O'Shea et al. 1976). In this perspective, it is obvious that vacuum conditions at the teat-end are of importance to investigate in terms of for milking techniques and especially in quarter individual systems. It is important to determine the effects of different combination of teat-cups and milk tube connection alternatives in order to obtain the most stable and safe vacuum conditions at the teat-end. Hence, a study was conducted and the objective of this study was to determine the effects of milk tube guidance settings and different teat cup type on the dynamics of teat-end vacuum and vacuum fluctuations in quarter individual milking systems.

2. Material and Methods

The milking-system itself is a part of the laboratory milking parlour at Leibniz-Institut für Agrartechnik und Bioökonomie e.V. (ATB), Potsdam-Berlin/Germany. Figure 1 shows the set-up of the milking parlour laboratory which is basically a part of a conventional herringbone parlour. The wet-measurement-method as described in DIN ISO 6690 (2010b) was followed during the experiments and water that simulates milk flow was used during the measurements. The vacuum was measured at different locations in a milking-system with variable set-up in order to compare the vacuum behaviour of various milk tube and teat cup combinations. The different teat cup types are alternately, of four pieces each, clamped into the holder and in that way brought to udder level. Each artificial teat was placed into a teat cup and connected to a separate flow meter. The range and accuracy of each flow meter were 0.2 to 2.0 L min^{-1} per quarter and ± 2%. The sequential pulsation applied with the rate of 60 min^{-1} and the ratio of and 65:35, and these values were kept constant during the experiments. The vacuum pump of the experimental milking system was oil lubricated type with the capacity of 800 l at 50 kPa. Three different teat cups were used alternatively for the experiments and the basic specifications are tabulated in Table 1. Each teat cup was tested at a vacuum level as recommended by manufacturers.

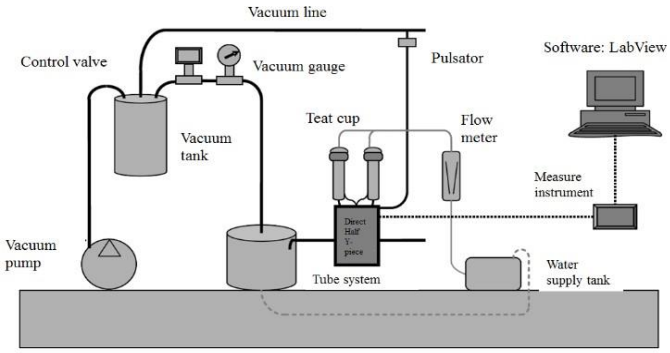

Figure 1- Schematic drawing of the laboratory milking parlor with connected milking-system (Rose 2006)

The BIO* teat cup consists of a silicon liner and is equipped with a periodic air inlet beneath the teat. Through pulse-cycle related rhythmic opening and closing of a valve the vacuum in the suction phase should be kept stable and as low as possible in the relief phase.

The RVS* teat cup liner is made of rubber. It has a constant and permanent air inlet above the teat. In contrast to the BIO teat cup, the air is inset at every suction phase as periodic air inlet to promote the milk flow. The AMS* teat cup uses a pulse independent and constantly disposable air inlet beneath the udder.

ж: Mention of company names or trademarks does not imply the endorsement of these products by the authors or their institutions. It is solely for scientific purposes.

During the experimental procedure three different tube connection systems were used as depicted in Figure 2. These tube connections were the direct tube, half-half and Y-piece tube. These tube connections were designed and manufactured at ATB.

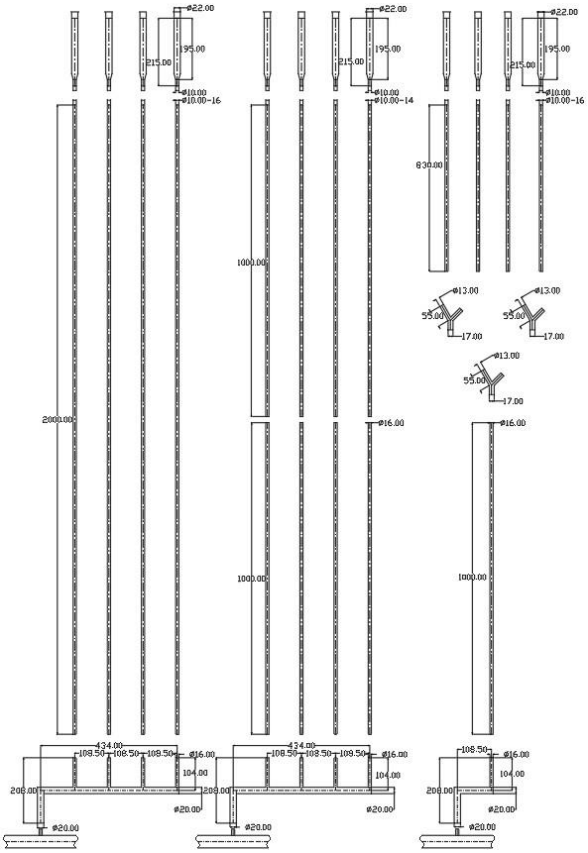

Figure 2- Technical drawing of the milk tube guidance settings. Direct tube system (left), Half-half tube system (middle), Y-piece tube system (right)

The simplest type is the direct tube guidance setting. In this type, teat cups are directly connected to the collecting piece by 2 m long tubes.

The half-half tube system consists two tubes, each 1 m long per cup at different tube inside diameters. The lower tube that is closer to the collecting piece is not modified during the whole test series and always has a tube inside diameter of 16 mm. If the upper part of the tube had a tube inside diameter of 16 mm as well, the experimental setup would be similar to the one of the direct tube system with 16 mm tube inside diameter. For this reason, only two tube inside diameters were tested for this tube guidance setting. The third set-up type uses special Y-pieces that bring together all four milk flows following 1 m long tube. Therefore, there is no collecting piece in this tube system.

The half-half tube system consists of two tubes, each one-meter-long at different inside diameters (e.g. 14 mm) for each teat cup. The lower tube that is closer to the collecting piece is not modified during the whole test series and always has a tube inside diameter of 16 mm. If the higher tube had a tube inside diameter of 16 mm as well, the experimental setup would be similar to the one of the direct tube system with 16 mm tube inside diameter. For this reason, only two different inside diameters were tested for this tube guidance setting. The third set-up type uses special Y-pieces so that all four milk flows merges after 1 m long tube with 16 mm inside diameter. To measure the vacuum levels in different location of the milking system four absolute pressure sensors manufactured by Keller Druckmesstechnik GmbH individually placed in a T-form test case made of brass and this T formed pieces were used the sensors to the milk and pulse tubes precisely. Accuracy of the sensor used was ± 0,5% and the maximum measurement frequency is 2 kHz. The sensors were directly connected to the A/D converter which transmitted the analog signals of the sensors to digital form and send them to the computer. The measurement rate adjusted to 500 Hz during the experiments. The data was stored in the computer with LabView software.

For each milk tube and teat cup combination tested at total flow rate ranging between 2 and 6 L min^{-1} (0.5 and 1.5 L min^{-1} per quarter). Three replications were achieved for each experiment and each measurement lasted 60 seconds and the data was consequently recorded every 2 msec with a measurement frequency of 500 Hz.

The first five measured pulse cycles were determined before every experimental procedure for the analysis of pulse progressions written as macro and evaluated as recommended by the DIN ISO standard (DIN ISO 6690, 2010b). At the same time, each of these five cycles was subdivided into its single pulse phases and the run-time was determined.

Furthermore, the vacuum fluctuations in each cycle were calculated. To estimate the fluctuation, the difference between the highest and the lowest value of every phase was identified as indicated in Equation 1. The results were averaged as well, so that a fluctuation value for the b- and d-phase was existent (DIN ISO 6690, 2010b).

$$VS_p = \frac{1}{n}\sum_{i=1}^{n}(VPmax_i - VPmin_i) \quad [1]$$

Where; VS_p is Vacuum fluctuation of the phase; VP_{max} is Maximum pressure in the pulse phase; VP_{min} is Minimum pressure in the pulse phase; n is Number of the consecutive pulse cycles (n=5)

The collected data with the help of software from LabView were transferred to SPSS® for the variance analysis and the Duncan's multiple range tests.

3. Results and Discussion

The data obtained from the experiments were evaluated based on ISO recommendation and the results are depicted for a direct tube connection in Figure 3, a half-half connection in Figure 4, y-piece connection in Figure 5 for three different milk tube diameters along with three different teat cups at different flow rates in b-phase. There are some common points in these three figures. One of them is that the general trend is the same even though the milk tube connection type is different. The teat-end vacuum in BIO teat cup is always between the range of 32 and 42 kPa at any flow and for all three types of connections. For RVS teat cup, the teat-end vacuum is between 32-42 kPa range if the flow rate varies between 4 and 6 L min^{-1} for both direct and half-half connection while Y-piece connection meets ISO requirements if the flow rate between 2 and 6 L min^{-1}. The trend in terms of teat-end vacuum for AMS is similar for all three types of connections. Only the flow rate that provides 32-42 kPa teat-end vacuum is 2 L min^{-1} for direct tube and half and half connection. On the other hand, once the milk flow starts, the teat-end vacuum for AMS is beyond the recommended range by ISO if Y-piece connection is used at any diameter except 2 L min^{-1} flow rate and at a diameter of 10 mm. The results from variance analysis carried out in b and d-phase for both, teat-end vacuum and fluctuations are as follows:

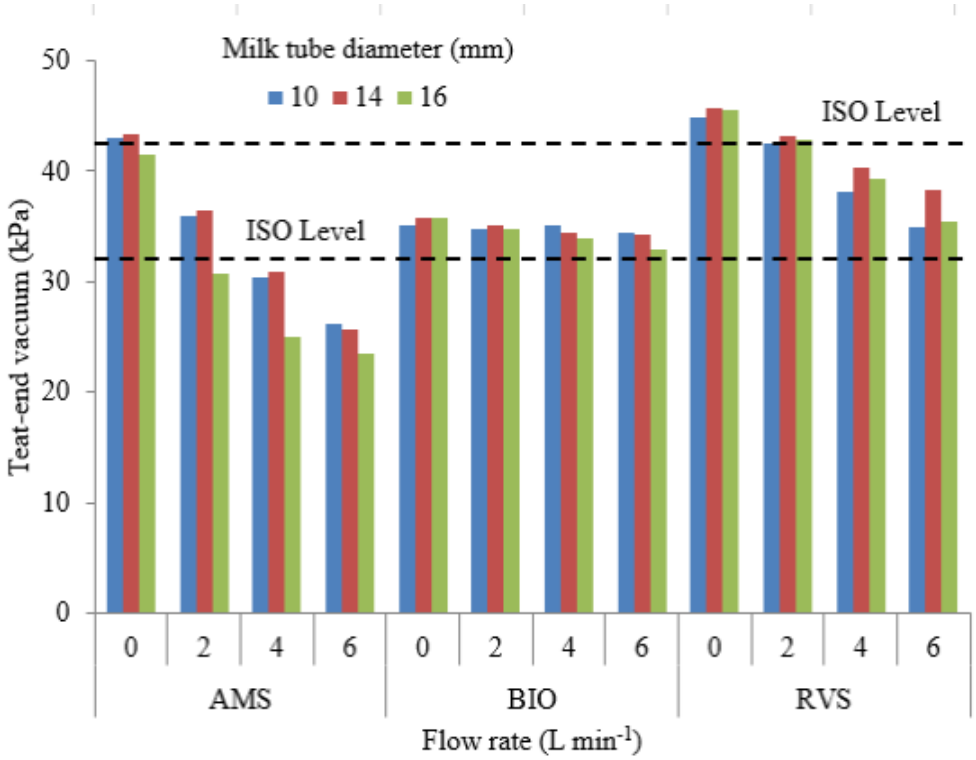

Figure 3- Effects of different flow rate and milk tube diameter on teat-end vacuum in different teat cups with a direct tube connection in b-phase

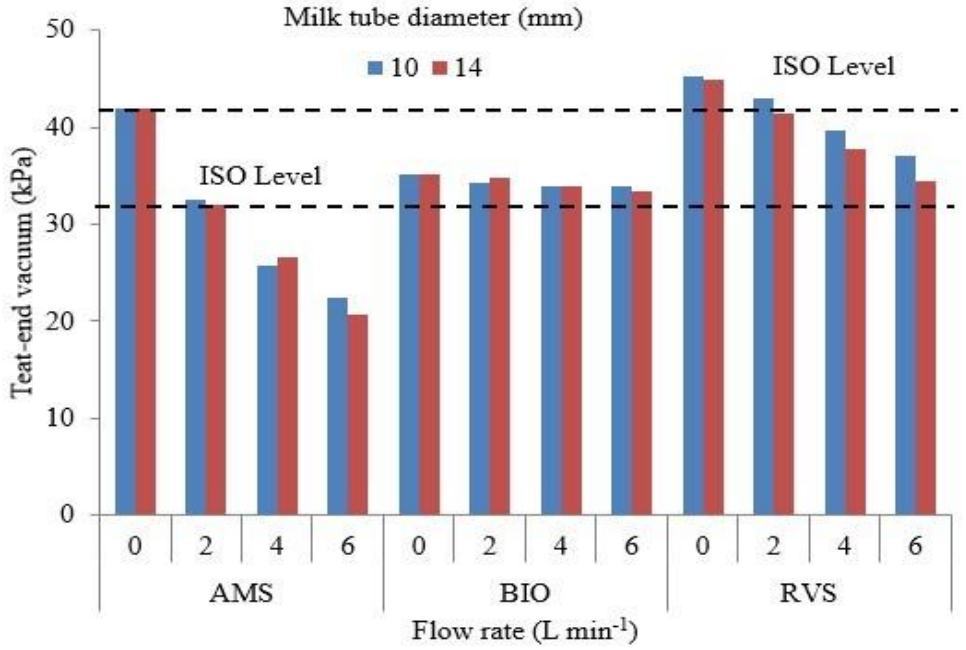

Figure 4- Effects of flow rate and milk tube diameter on teat-end vacuum in different teat cups with half-half connection in b-phase

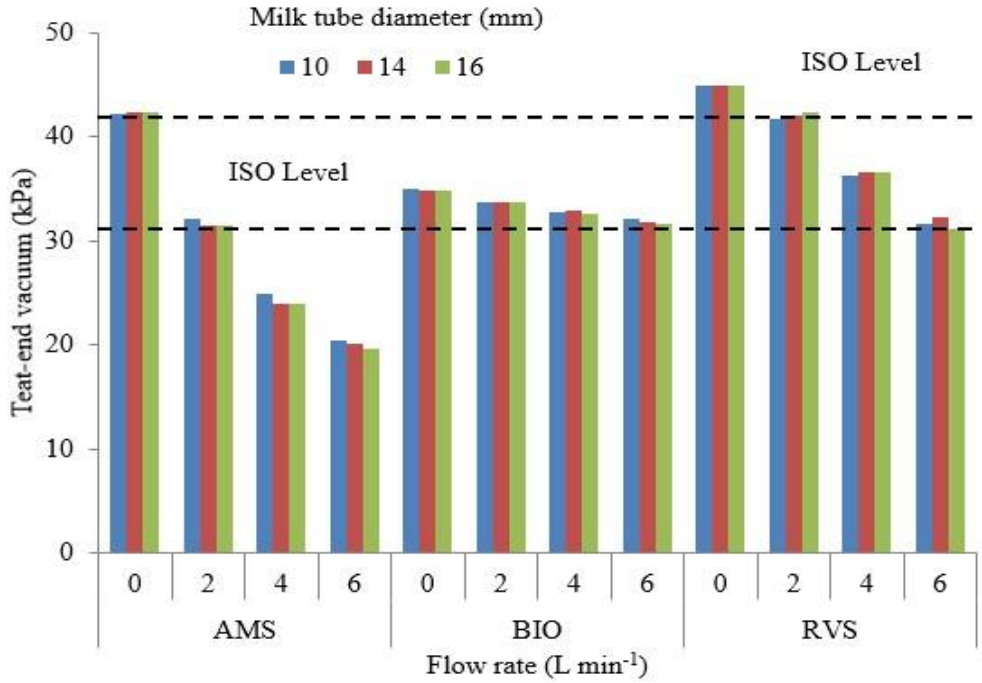

Figure 5- Effects of flow rate and milk tube diameter on teat-end vacuum in different teat cups with Y-piece connection in b-phase

The data collected for each milking system were not evaluated in the same pool, but separately since the recommended milking vacuum by the manufacturer for BIO was 35 kPa and the milk tube inside diameter was 10 mm while the recommend milking vacuum and milk tube diameter by the manufacturer was the same for AMS and RVS (45 kPa and 16 mm, respectively). The results from the analysis of teat-end vacuum in b and d-phase are tabulated in Table 2. As seen from the table, the main effect of tube connection type (direct, half-half and Y piece) along with flow rate and milk tube inside diameter and most of their possible (double and triple) interactions are significant at a probability level of 95%. Only the insignificant main effect is the diameter effect for AMS while triple interaction of TC x FR x DIA for BIO in b-phase and for RVS in d-phase are also insignificant at a probability level of 95%.

Table 1- Basic Specifications of the teat cups used in this study

Teat cup	Milking vacuum£	Liner material	Air inlet	Milk tube inside diameterΨ
AMS	45 kPa	Rubber	Free air inlet beneath the teat	16 mm
BIO	35 kPa	Silicon	Periodic air inlet beneath the teat	10 mm
RVS	45 kPa	Rubber	Periodic air inlet at mouthpiece	16 mm

£: Recommended milking vacuum and Ψ: recommended milk tube diameter by the manufacturers

Table 2- The results from variance analysis for the teat-end vacuum in b and d-phase in different milking systems

Factors		*F- value (P value)*					
		Teat-end vacuum in b-phase			*Teat-end vacuum in d-phase*		
		AMS	*BIO*	*RVS*	*AMS*	*BIO*	*RVS*
Tube conn.(Tc)	2	94.52 (<0.001)	189.26 (<0.001)	137.71 (<0.001)	54.22 (<0.001)	163.23 (<0.001)	93.14 (<0.001)
Flow rate (Fr)	3	1651.77 (<0.001)	240.87 (<0.001)	1032.14 (<0.001)	1292.39 (<0.001)	1186.37 (<0.001)	1407.73 (<0.001)
Diameter (Dia)	2	0.32 (0.721)	6.23 (<0.001)	113.65 (<0.001)	33.61 (<0.001)	597.03 (<0.001)	8.65 (<0.001)
Tc x Fr	6	27.84 (<0.001)	8.76 (<0.001)	38.13 (<0.001)	9.83 (<0.001)	13.68 (<0.001)	12.65 (<0.001)
Tc x Dia	4	58.97 (<0.001)	3.80 (0.007)	113.98 (<0.001)	9.48 (<0.001)	123.78 (<0.001)	3.11 (<0.001)
FR x Dia	6	16.49 (<0.001)	8.13 (<0.001)	3.50 (0.004)	8.64 (<0.001)	110.70 (<0.001)	3.08 (0.010)
Tc x Fr x Dia	12	23.52 (<0.001)	2.13 (0.025)	9.62 (<0.001)	2.28 (0.016)	13.72 (<0.001)	0.90 (0.551)
Error	72						
Total	108						

The results from the variance analysis as a consequence of 108 data evaluation for the vacuum fluctuations as it was the case for the teat-end vacuum are given in Table 3. As an opposite of teat-end vacuum evaluations, there are many insignificant factors that affect vacuum fluctuations in different milking systems. As the main factors, the effects of tube connection type and flow rate on vacuum fluctuations are significant if significance level of P is considered to be 95% except the tube connection type for BIO in d-phase. This means that the tube connection type for BIO does not affect the vacuum fluctuations in d-phase. Once the results are evaluated in terms of the effect of milk tube inside diameter, it can be said that the changes in diameter only affect the vacuum fluctuation in d-phase for both, AMS and BIO. The effects of milk tube inside diameter on vacuum fluctuations in b-phase for all milking systems along with the one in d-phase for RVS. The effects of double interactions of tube connection type and flow rate diameter is significant at a probability level of 95% except the tube connection and diameter interaction for BIO in b-phase. On the other hand, flow rate and diameter interaction is only significant in d- phase for two milking systems. The triple interaction of the factors is only significant in b-phase for AMS and RVS but it is only significant for BIO in d-phase at a probability level of 95%. The results obtained from the Duncan's multiple range tests for the main factors are tabulated in Table 4 throught Table 9.

Table 3- The results from variance analysis for the vacuum fluctuations in b and d-phase in different milking systems

Factors	*df*	*F- value (P value)*					
		Vacuum fluctuation in b-phase			*Vacuum fluctuation in d-phase*		
		AMS	*BIO*	*RVS*	*AMS*	*BIO*	*RVS*
Tube conn. (Tc)	2	15.49 (<0.001)	65.96 (<0.001)	6.16 (0.003)	40.11 (<0.001)	1.806 (0.172)	7.704 (<0.001)
Flow rate (Fr)	3	386.51 (<0.001)	594.54 (<0.001)	451.10 (<0.001)	343.87 (<0.001)	250.16 (<0.001)	314.94 (<0.001)
Diameter (Dia)	2	13.57 (0.721)	1.09 (0.340)	0.076 (0.926)	6.137 (0.003)	217.08 (<0.001)	0.994 (0.375)
Tc x Fr	6	3.71 (0.003)	4.91 (<0.001)	2.429 (0.034)	5.92 (<0.001)	10.08 (<0.001)	4.08 (0.001)
Tc x Dia	4	2.59 (0.043)	2.13 (0.086)	2.014 (0.101)	2.53 (0.047)	13.15 (<0.001)	2.52 (0.048)
Fr x Dia	6	1.21 (0.308)	0.92 (0.482)	0.624 (0.710)	6.53 (<0.001)	26.49 (<0.001)	2.78 (0.017)
Tc x Fr x Dia	12	0.718 (0.729)	3.21 (0.001)	2.386 (0.012)	1.51 (0.138)	13.54 (<0.001)	1.51 (0.140)
Error	72						
Total	108						

Table 4- The Duncan's multiple range test results$^{\zeta}$ for the effect of tube connection type on teat-end vacuum (kPa) in b and d-phase in different milking systems

Tube connection type	*Teat-end vacuum in b-phase*			*Teat-end vacuum in d-phase*		
	AMS	*BIO*	*RVS*	*AMS*	*BIO*	*RVS*
YP	29.54^{a}	33.29^{A}	$38.75^{a'}$	28.08^{d}	30.50^{D}	$37.76^{d'}$
HH	31.73^{b}	34.30^{B}	$38.41^{b'}$	29.23^{e}	31.46^{E}	$39.66^{e'}$
DT	32.74^{c}	34.71^{C}	$40.94^{c'}$	31.53^{f}	32.62^{F}	$40.03^{f'}$

$^{\zeta}$: Means with the same letter in the same column are not significantly different from each other for each column. YP: Y piece; HH: half-half and DT: direct tube connection. The gray shaded cells are the ones within the recommended range of 32-42 kPa by DIN ISO 5707

As seen from Table 4, the effect of tube connection type is significant for all milking systems considered in this study in both, b and d-phase in terms of teat-end vacuum. When the results were examined, it is seen that the teat-end vacuum in b-phase is within the 32-42 kPa range except for AMS when YP and HH connection types. For both types, the teat-end vacuum is lower than 32 kPa. It is also interesting to see that the direct tube connection provides the highest teat-end vacuum values for all milking systems in b-phase. This means that the lowest vacuum drops occur for all three milking systems and three connections. The reason for this can be attributed to the fact that HH and YP connection types require interim connection parts and milk tube diameter change between 10-16 mm. On the other hand, for DT tube connection, there is no interim part and tube is a single piece at a certain diameter and it is directly connected to the main milk line. This causes less frictional forces and vacuum with a lower loss affects teat-end. It could be also stated that the flow characteristics for all connection types change for three different milking system.

The effect of flow rate on teat-end vacuum in b and d-phase are tabulated in Table 5. The trend in teat-end vacuum for all milking systems in b and d-phase is the same. The teat-end vacuum goes down as the flow rate increases and the differences in each column for the same teat cup are significant in all cases.

Table 5- The Duncan's multiple range test results$^{\zeta}$ for the effect of flow rate on teat-end vacuum (kPa) in b and d-phase in different milking systems

Flow rate (L min^{-1})	*Teat-end vacuum in b-phase*			*Teat-end vacuum in d-phase*		
	AMS	*BIO*	*RVS*	*AMS*	*BIO*	*RVS*
0	41.59^{a}	35.25^{A}	$44.07^{a'}$	42.15^{e}	35.24^{E}	$44.86^{e'}$
2	33.03^{b}	34.40^{B}	$41.54^{b'}$	32.70^{f}	32.87^{F}	$41.86^{f'}$
4	27.25^{c}	33.71^{C}	$37.64^{c'}$	23.37^{g}	30.50^{G}	$37.53^{g'}$
6	23.48^{d}	33.02^{D}	$34.22^{d'}$	20.24^{h}	27.50^{H}	$32.34^{h'}$

$^{\zeta}$: Means with the same letter in the same column are not significantly different from each other for each column. The gray shaded cells are the ones within the recommended range of 32-42 kPa by DIN ISO 5707

This trend was observed in different studies. One of them is the study carried out by Haeussermann and Hartung (2010). In a field study, they investigated the amount of and variation at the teat-end vacuum during milking depending upon the milk flow rate.

Öz et al. (2010) used three different milking system to evaluate their preformances and one of them was BIO. From the study conducted they also concluded that teat-end vacuum in b-phase decreased with an increase in flow rate. At a flow rate of 4.8 L min^{-1}, they obtained a teat-end vacuum of 32.6 kPa in b-phase. This finding is supported with the the teat-end vacuum found in this study for BIO and it could be 33.43 kPa at 4.8 L min^{-1} by linear interpolation for the teat-end vacuum between the flow rate 4 and 6 L min^{-1}.

The results obtained for the teat-end vacuum from Duncan's multiple range tests for the milk tube diameter effect on teat-end vacuum are tabulated in Table 6.

Table 6- The Duncan's multiple range test results$^{\zeta}$ for the milk tube diameter on teat-end vacuum (kPa) in b and d-phase in different milking systems

Milk tube diameter (mm)	*Teat-end vacuum in b-phase*			*Teat-end vacuum in d-phase*		
	AMS	*BIO*	*RVS*	*AMS*	*BIO*	*RVS*
10	31.45^{a}	34.18^{A}	$40.15^{a'}$	28.30^{e}	29.18^{E}	$38.95^{e'}$
14	31.28^{a}	34.16^{A}	$40.02^{a'}$	29.49^{f}	32.86^{F}	$39.58^{f'}$
16	31.28^{a}	33.95^{B}	$37.93^{b'}$	31.03^{g}	32.72^{F}	$38.93^{e'}$

$^{\zeta}$: Means with the same letter in the same column are not significantly different from each other for each column.
The gray shaded cells are the ones within the recommended range of 32-42 kPa by DIN ISO 5707

It could be stated that the milk tube diameter does not affect the teat-end vacuum in b-phase for AMS while only 16 mm tube diameter is significant as compared to 10 and 14 mm. The use of 10 and 14 mm tube diameter does not differ the teat-end vacuum for BIO and RVS in b-phase statistically. The milk tube diameter effect on teat-end vacuum values in d-phase is statistically significant for AMS once the tube diameter increases from 10 to 16 mm. The teat-end vacuum in d-phase for 14 and 16 mm tube diameter is not statistically significant while the teat-end vacuum for 10 mm is the lowest. The teat-end vacuum for RVS as a result of using different tube diameters does not differ when the 10 and 16 mm tube diameters are used. Actually, the results from the Duncan's multiple range for the tube diameter should be evaluated based on the recommended diameters by the manufacturers and it should be reminded here that only BIO recommended 10 mm milk tube diameter while the other two manufacturers offer (AMS and RVS) the use of 16 mm milk tube diameter. It seems to be that the use of 14 mm milk tube diameter for BIO will provide better teat-end vacuum as compared to manufacturer's recommendation of 10 mm. On the other hand, the use of 16 mm milk tube diameter is considered to be appropriate for AMS since the differences in mik tube diameter in b-phase are not statistically significant while the highest teat-end vacuum value in d-phase can be obtained if the milk tube diameter of 16 mm used as it is significantly different than 10 and 14 mm. The appropriate milk tube diameter for RVS should be 14 mm when statistical differences in b and d-phase are examined. Hence, the recommended value by the company for RVS should be changed to 14 mm from 16 mm. The Duncan's multiple range tests for vacuum fluctuations are given in Table 7 thru 9. The effect of tube connection type on vacuum fluctuations can be seen from Table 7. Based on the results in Table 7, the Y piece connection should be recommended AMS since the vacuum fluctuations in d-phase is the lowest and statistically different than the other two connection types while the difference between Y-piece and direct tube connection is not significant in b-phase. The use of half-half tube connection results in the highest vacuum fluctuation in b-phase as compared to other two tube connections. The effects of flow rate as a result of Duncan's multiple range tests are given in Table 8. As seen from the table, vacuum fluctuation in both, b and d-phase increases as the flow rate is increased.

Table 7- The Duncan's multiple range test results$^{\zeta}$ for the effect of tube connection type on vacuum fluctuations (kPa) in b and d-phase in different milking systems

Tube connection type	*Vacuum fluctuation in b-phase*			*Vacuum fluctuation in d-phase*		
	AMS	*BIO*	*RVS*	*AMS*	*BIO*	*RVS*
YP	6.23^{a}	6.10^{A}	$5.64^{a'}$	4.31^{d}	4.02^{D}	$3.86^{d'}$
HH	7.51^{b}	4.67^{B}	$6.30^{b'}$	6.14^{e}	3.73^{D}	$4.44^{e'}$
DT	6.48^{a}	4.51^{B}	$6.25^{b'}$	5.29^{f}	3.94^{D}	$4.48^{e'}$

$^{\zeta}$: Means with the same letter in the same column are not significantly different from each other for each column. YP: Y piece; HH: half- half and DT: direct tube connection

Table 8- The Duncan's multiple range test results$^{\zeta}$ for the effect of different flow rates on vacuum fluctuations (kPa) in b and d-phase in different milking systems

Flow rate	*Vacuum fluctuationin b-phase*			*Vacuum fluctuation in d-phase*		
	AMS	*BIO*	*RVS*	*AMS*	*BIO*	*RVS*
0	1.27^{a}	1.51^{A}	$1.21^{a'}$	0.91^{a}	1.06^{A}	$0.74^{a'}$
2	6.64^{b}	3.94^{B}	$5.37^{b'}$	5.15^{b}	4.26^{B}	$4.06^{b'}$
4	9.03^{c}	6.42^{C}	$8.32^{c'}$	7.37^{c}	4.37^{B}	$6.07^{c'}$
6	10.00^{d}	8.50^{D}	$9.34^{d'}$	7.55^{c}	5.89^{C}	$6.17^{c'}$

$^{\zeta}$: Means with the same letter in the same column are not significantly different from each other for each column

Ströbel et al. (2011) as a results of using three different AMS system concluded that vacuum fluctuations were considerably higher in the tested AMSs as compared to modern conventional systems. The vacuum fluctuations in b-phase for all three AMS system ranged between 6.41 and 12.63 kPa range while the fluctuations ranged between 5.69 and 19.23 kPa at a flow rate of 4.8 L min^{-1} in d-phase. Rasmussen et al. (2006) also found similar trend in the study conducted with three different of automatic milking systems. At a flow range of 0.2 L min^{-1} fluctuations increased from 11 to 20.5 kPa for AMS system. For the other two systems fluctuations ranged between 15.6-23.3 kPa and 18.7-35.5 kPa and in both, the fluctuations increased with an increase in flow rate. In this study, the vacuum fluctuations in AMS are also found to be higher than BIO and RVS even though all of the systems achieve quarter individual milking.

The results from the Duncan's multiple range tests for the milk tube diameter effect on vacuum fluctuations in b and d-phase are tabulated in Table 9.

Table 9- The Duncan's multiple range test results$^{\zeta}$ for the milk tube diameter on vacuum fluctuations (kPa) in b and d-phase in different milking systems

Milk tube diameter (mm)	*Vacuum fluctuation in b-phase*			*Vacuum fluctuation in d-phase*		
	AMS	*BIO*	*RVS*	*AMS*	*BIO*	*RVS*
10	6.03^{a}	5.22^{A}	$6.01^{a'}$	5.59^{a}	5.79^{A}	$4.40^{a'}$
14	6.91^{b}	5.01^{A}	$6.08^{a'}$	5.27^{ab}	3.00^{B}	$4.19^{a'}$
16	7.27^{b}	5.04^{A}	$6.09^{a'}$	4.88^{b}	2.90^{B}	$4.19^{a'}$

$^{\zeta}$: Means with the same letter in the same column are not significantly different from each other for each column

It seems to be that the use of 14 or 16 mm tube diameter instead of recommended tube diameter of 10 mm by BIO would be appropriate since the differences between 14 and 16 mm tube diameter in b and d-phase are insignificant but the highest fluctuations occur at 10 mm tube diameter and difference than the other two diameters are statistically significant. This finding supports the findings and recommendation of 14 mm tube diameter in terms of teat-end vacuum values as given in Table 6. If the vacuum fluctuations are examined for AMS, it is seen that lowest ones are obtained at 10 mm in b-phase and 16 mm for the d-phase. On the other hand, the differences in milk tube diameter are not statistically significant for the RVS teat cup. This means that any diameter can be used for RVS if the vacuum fluctuations are considered in b and d-phase. The milk tube diameter for the RVS teat cup could be 14 mm.

4. Conclusions

The following conclusions were drawn from the study conducted:

- The teat-end vacuum in BIO teat cup is always between the range of 32 and 42 kPa as recommended by ISO at any flow and for all three types of connections.

- For RVS teat cup it could be stated that the teat-end vacuum is between 32-42 kPa range if the flow rate varies between 4 and 6 L min^{-1} for both direct and half-half connection while Y-piece connection meets ISO requirements if the flow rate changes between 2 and 6 L min^{-1}.

- The teat-end vacuum for AMS is similar for all three types of connections. Only the flow rate that provides 32-42 kPa vacuum range is 2 L min^{-1} for direct tube and half and half connection. With the start of milk flow, the teat-end vacuum for AMS is beyond the recommended range by ISO if Y-piece connection is used at any diameter.

- The main effect of tube connection type (direct, half-half and Y piece) along with flow rate and milk tube inside diameter and their possible (double and triple) interactions are significant on teat-end vacuum at a probability level of 95%.

- As an opposite of teat-end vacuum evaluations, there are many insignificant factors that affect vacuum fluctuations in different milking systems. As main factors, the effects of tube connection type and flow rate on vacuum fluctuations are significant at a probability level of 95% except the tube connection type for BIO in d-phase.

- It seems to be that the use of 14 or 16 mm milk tube diameter for BIO will provide better teat-end vacuum as compared to manufacturer's recommendation of 10 mm.

- The use of 16 mm milk tube diameter is considered to be appropriate for AMS and this was the recommended value by the manufacturer.

- The appropriate milk tube diameter for RVS should be 14 mm when statistical differences in b and d-phase are examined from the point of teat-end vacuum and vacuum fluctuations. Hence the recommended value for RVS should be changed to 14 mm from 16 mm.

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Effects on Performance, Egg Quality Criteria and Cholesterol Level of Adding Different Ratios Flaxseed Oil Instead of Sunflower Oil to Compound Feed of Laying Hens

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ABSTRACT
This study was conducted to examine the effect on performance, egg quality criteria and egg cholesterol level of adding different ratios of flaxseed oil instead of sunflower oil to the compound feed of laying hens. A total of 210, 30-week old Lohmann LSL laying hens were acquired for the study. The study groups consisted of a control group (5% sunflower oil (SO) + 0% flaxseed oil (FO) and trial 1 (4% SO + 1% FO), trial 2 (3% SO + 2% FO), trial 3 (2% SO + 3% FO), trial 4 (1% SO + 4% FO) and trial 5 (0% SO + 5% FO) groups. The study lasted for 8 weeks. The feed consumption was not different among the experimental groups. Feed utilization rate was higher in group 2 compared to those of other between 0-8 weeks ($P<0.001$). Between weeks 0 to 8, all trial groups were found have significantly higher levels of linoleic acid, one of the fatty acids found in yolk, compared to the control group ($P<0.001$). Additives were not found to affects levels of cholesterol in yolk, with no significant differences found between groups. In short, sunflower oil and flaxseed oil added to laying hen rations did not create any differences in terms of egg quality criteria or egg cholesterol levels, but higher levels of flaxseed oil added to the rations resulted in linearly higher levels of linolenic acid content of yolk, and use of the two oil additives together increased egg yields.

Keywords: Egg quality criteria, Laying hens performance, α-linolenic acid, Vegetable oils, Yolk cholesterol

1. Introduction

The need to provide a healthy and balanced diet to the world's growing population has made animal husbandry one of the most important economic sectors in countries. Quality foods have balanced amounts of nutrients such as protein, fat, carbohydrate, minerals and vitamins, which can be found in foods of vegetable and animal origin in varying amounts. Animal products such as meat, milk and eggs play an important role in human nutrition, and there has been a growing trend in recent years to pay more attention to the quality of the food we consume. The growing public demand for low-fat and low-cholesterol animal products has led to a number of studies into improving the quality of foods of animal origin (Choupani et al. 2013), and foremost among these foods are eggs.

Chicken eggs are one of the most important animal products for human nutrition. Similar to other farm animals, poultry use basic organic nutrients like carbohydrates, fats and proteins to meet their energy needs. In terms of level of use, carbohydrates come first, followed by fats (Özdoğan & Sarı 2001). In other words, compound feeds mostly rely on carbohydrates for energy. That said, carbohydrate-rich grains alone cannot meet the needs of animals with high energy needs such as poultry, and so energy-rich fats and oils are used to make up the shortfall in energy, and usually fats and oils that are not suitable for human consumption. All poultry feed must have a minimum fat content of 1% is it is to provide a sufficient amount of such essential fatty acids as linoleic and linolenic acids, to improve the taste of compound feeds and to prevent powdering, alongside other economic or nutritional considerations (Omidi et al. 2015).

Fats participating in the rations are divided into two main groups, being either saturated or unsaturated (Çakmakçı & Kahyaoğlu 2012). Of the saturated fatty acids, acetic and butyric acids are formed as the final products of carbohydrates; capric, caproic and caprylic acids are found in vegetable oils, and in very small concentrations, in butter; lauric acid is found in coconut oil, cinnamon, palm kernel, and bay leaf; myristic acid is found in coconut oil and palm kernel; and palmitic and stearic acids are found in all animal fats and vegetable oils (Ergün et al. 2014). Of the unsaturated fatty acids, palmitoleic acid and oleic acid are found in vegetable oils and animal fats; linoleic acid is found in sunflower seeds, soybeans, corn, peanuts, and cotton seeds;

linolenic acid is found in flaxseed oil and fish oil; and arachidonic acid is found in peanuts and animal phospholipids (Ergün et al. 2014).

To obtain eggs rich in linoleic and linolenic fatty acids, which are needed by humans but have to be obtained through foods because the human body cannot synthesize them, vegetable oils rich in these fatty acids are added to the rations of laying eggs. Sunflower oil and flaxseed oil are the two most common oils used for this purpose.

Sunflower oil is rich in linoleic acid (omega 6) but poor in linolenic acid (omega 3). Flaxseed oil is one of the richest sources of α-linolenic acid (ALA)-a type of omega-3 (n-3) fatty acid that accounts for around 55% of all fatty acids (İşleroğlu et al. 2005). The fat content and quality of flaxseed varies depending on the plant variety and hereditary characteristics. Moreover, environmental factors such as temperature, soil conditions, cultural practices and plant diseases can affect both the fat content and quality. The greatest variation in the composition of fatty acids can be observed in oleic acid (14-60%), linoleic acid (3-21%) and linolenic acid (31-72%) (İşleroğlu et al. 2005). Zelenka et al. (2007) report that some flax varieties are rich in linolenic acids, whereas others are rich in linoleic acids.

Eseceli & Kahraman (2003) found that adding vitamins E and C to hen rations with sunflower oil and fish oil increased some performance parameters, but the qualities of the source of oil in the ration had a more significant effect on performance compared to the addition of vitamins E and C. Mazalli et al. (2004) fed laying hens mixed rations with 3% animal fats and vegetable oils, including sunflower oil and flaxseed oil, as well as ground flaxseed treated with two different levels of vitamin E (12 IU/kg and 100 IU/kg), and reported that the treatment did not produce any significant effects on performance or egg quality criteria.

Cholesterol is a natural yolk component, and an egg weighing 60 to 62 g is expected to contain 200 to 220 mg of cholesterol under normal conditions, but studies by different researchers report a range of 122 to 408 mg (Jacob & Miles 2000; Vorlova et al. 2001; Yegani & Nilipour 2003; Raj Manohar 2015; Rakıcıoğlu 2016). In a study on functional egg production, 2% flaxseed oil added to laying hen rations was found to lower the cholesterol level of eggs significantly (Küreç 2009).

The present study investigates the effects on performance, egg quality criteria and cholesterol of using varying rates of flaxseed oil rather than sunflower oil in layer rations, the former of which has greater linolenic acid content than the latter.

2. Material and Methods

2.1. Animals, rations and experimental design

The animal materials used in the study were 210 Lohmann LSL type white egg-laying hens aged 30 weeks, purchased free of disease from a private poultry business in Adana, Turkey. The raw materials for the feeds used in the study were bought on the open market in Van. The cold-pressed flaxseed oil used in the trial was obtained from the plant products company Doğa Bitki Ürünleri Gıda Ticaret Ltd. Şti. in Antalya.

The trial was conducted at the Laying Hens Trial Unit of the Van Yüzüncü Yıl University Research and Application Farm. The hens were kept in two-tier battery cages for laying hens for the trial. The site was illuminated with fluorescent lights for 16 hours a day during the trial and kept dark for 8 hours a day.

The 210 hens used in the study were divided into six groups of 35 hens each, and each group was further divided into five subgroups consisting of seven hens. The hens were group-fed ad libitum, keeping trial feed containing approximately 18.5% crude protein and 2800 kcal/kg metabolic energy in the feeders at all times during the egg laying period, taking care to keep all groups under identical conditions. Fresh water was provided continuously using a drip watering system. The oil content of the feeds was 5% for all groups. In the control group, all of this was sunflower oil, whereas in the trial groups, sunflower and flaxseed oils were used in different ratios, with one trial group receiving only 5% flaxseed oil. The trial lasted for 8 weeks. Table 1 reports the treatments for all groups.

Table 1- Groups and treatments (Total oil content of the ration 5%)

Groups	*Sunflower content of the ration (%)*	*Flaxseed content of the ration (%)*
Group 1 (Control)	5	0
Group 2 (Trial 1)	4	1
Group 3 (Trial 2)	3	2
Group 4 (Trial 3)	2	3
Group 5 (Trial 4)	1	4
Group 6 (Trial 5)	0	5

Isonitrogenic and isocaloric rations were prepared in the compound feed preparation unit of the poultry house for laying hens of the Van Yüzüncü Yıl University Research and Application Farm. The nutrient requirements of laying hens were determined according to NRC (NRC 1994). The compositions and nutrient contents of the feeds were reported in the Table 2 and fatty acid

composition of the sunflower and flaxseed oils used in the trial (%) are reported in Table 3. The chemical compositions of the trial feeds were determined using the methods explained in AOAC (1984), in the laboratory of the Department of Animal Feeding and Feeding Diseases of the Van Yüzüncü Yıl University Faculty of Veterinary Medicine. The metabolizable energy (ME), calcium, available phosphorus, methionine+cysteine, lysine and sugar values of the ration used in the trial was determined by calculation (Jurgens 1996). Starch analysis was made according to Karabulut & Canbolat (2005). The metabolic energy contents of sunflower and flax oil included in the rations were determined as 8840 and 8842 kcal/kg, respectively. Thus, while the participation rate of the raw materials in the experimental rations did not change, only the fat ratios were changed gradually (Table 1). Trial rations were prepared by adding oil sources at different rates to a basic ration (Jurgens 1996). Fatty acid compositions of the sunflower and flaxseed oils, and the yolks samples were performed according to Cherian & Quezada (2016) by using GC-FID device.

Table 2- Components and chemical composition of the ration used in the trial, %

Ingredients	*Basic ration*
Yellow corn	45.05
Wheat	5.00
Wheat bran	1.50
Sunflower oil	5.00
Flaxseed oil	0.00
Soybean meal (48%)	15.05
Sunflower meal (36%)	15.15
Meat and bone meal	2.50
Dicalcium phosphate	4.00
Limestone	5.90
Antioxidant*	0.10
Salt	0.40
Vitamin mix**	0.175
Mineral mix***	0.175
Total	100
Chemical composition	
Dry matter (%)	91
Crude protein (%)	18.5
Metabolic energy (kcal/kg)#	2800
Crude fat (%)	6.53
Crude ash (%)	13.93
Crude fiber (%)	3.97
Calcium (%)#	3.62
Available P (%)#	0.93
Methionine + cysteine (%) #	0.65
Lysine (%) #	0.86
Starch (%)	34.37
Sugar (g/100 g)#	3.24

*: Contains 10.000 mg of antioxidant (Ethoxyquin E324, BHA E320, E330) per kilogram; **: Contains 12.000.000 IU Vitamin A, 2.500.000 IU Vitamin D_3, 30.000 mg Vitamin E, 4.000 mg Vitamin K_3, 3.000 mg Vitamin B_1, 6.000 mg Vitamin B_2, 30.000 mg Vitamin B_3, 10.000 mg Vitamin B, 5.000 mg Vitamin B_6, 15 mg Vitamin B_{12}, 1.000 mg Folic acid, 50 mg D-Biotin H_2, 50.000 mg Vitamin C, 300.000 mg Choline, 3.000 mg Canthaxanthin, 1.500 mg apo ester and 10.000 mg antioxidant per 1.75 kg; ***: Contains 80.000 mg Mn, 60.000 mg Fe, 60.000 mg Zn, 5.000 mg Cu, 2.000 mg I, 500 mg Co and 150 mg Se per 1.75 kg; #: Calculated value (Jurgens 1996)

Table 3- Fatty acid composition of the sunflower and flaxseed oils used in the trial

Number of carbons	*Chemical name*	*Flaxseed oil (%)*	*Sunflower oil (%)*
C14:0	Myristic acid	-	0.07
C16:0	Palmitic acid	5.0	6.5
C16:1n-9	Palmitoleic acid	0.2	0.1
C17:0	Margaric acid	0.1	0.05
C17:1	Heptadecenoic acid	0.1	0.05
C18:0	Stearic acid	2.6	3.8
C18:1n-9	Oleic acid	46	27.6
C18:2n-6	Linoleic acid	19	60.5
C18:3n-3	Linolenic acid	25	0.1
C20:0	Arachidic acid	0.5	0.2
C22:0	Behenic acid	0.3	0.8
C24:0	Lignoceric acid	-	0.3

2.2. Performance parameters

The hens in each subgroup were group-fed, and average feed consumption by each subgroup was measured with weekly weighing. Daily egg yield records were kept for each subgroup. Feed conversion rate was calculated from feed consumption and egg mass values as amount of feed consumed (g)/egg mass (g).

2.3. Egg quality characteristics

Every two weeks and for a total of four times throughout the duration of the study, 20 eggs were selected at random from each group (4 from each subgroup), making a total of 120 eggs, and egg weight, eggshell thickness, egg shape index, albumen index, and Haugh unit parameters were examined.

Once a week, all eggs from each group were weighed after being kept at room temperature for 24 hours. Samples were taken from the pointy, blunt, and middle sections of broken eggshells, and their thicknesses were measured using a micrometer after removing the membrane. The arithmetic mean of these values was recorded as eggshell thickness. The egg width and length were measured with a digital caliper and egg shape index was calculated from the data (Card & Nesheim 1972).

Shape index = (egg width/egg length) x100

The eggs were cracked on a glass table. The heights of yolk and albumen were measured using a tripod micrometer, while yolk diameter and albumen width were measured using a caliper, after waiting for 10 minutes to avoid large changes. These values were used to calculate the albumen index and Haugh unit based on the following equations (Şenköylü 2001):

Albumen index = (height of the thick albumen/(width+length/2)) x100

Haugh unit = 100 Log (height of the thick albumen + 7.57 – 1.7 egg weight$^{0.37}$)

2.4. Yolk cholesterol content

Following the trial, 15 eggs from each group (three from each subgroup) were boiled, and the cholesterol content of their yolks was examined (Boehringer Mainheim Gmbh Biochemica 1989; Küçükersan 2004).

For cholesterol analysis, yolks were mashed and homogenized. 0.1 g of yolk were put in a glass tube, and 4 mLof isopropyl alcohol were added. The mixture was mixed in a vortex until it became homogeneous, and filtered through a filter paper. For the cholesterol kit, glass tubes were marked sample, standard, and blind. 2 mL of cholesterol kit were added to all tubes, which were then kept in a 37 ºC water bath for 2 minutes. At the end of this period, 0.02 mL (20µl) kit was added to the sample tubes, 0.02 ml cholesterol standard was added to the standard tube, and 0.02 mL water was added to the blind tube. All tubes were kept in a 37 ºC water bath for 10 minutes. Results were read on the spectrophotometer (Schimadzu UV-1201V) at a wavelength of 520 nm. The optic density (OD) values read were evaluated using the following formulas:

$$\text{Cholesterol content of the extract (mg/g)} = \frac{\text{Sample OD}}{\text{Standard OD}} \times \text{Concentration of the standard}$$

$$\text{Yolk cholesterol content (mg/g)} = \frac{(\text{Cholesterol content of the extract} \times 100) \times 4}{\text{Sample weight (g)}}$$

2.5. Statistical analysis

All of the data obtained during the study was subjected to a variance analysis (SAS 1982). Inter-group differences were examined using Duncan's Multiple Comparison test (Steel & Torrie 1980).

3. Results

Looking at trial data for the entire 8-week period, no statistically significant differences were found between the groups in terms of feed consumption (Table 4). Trial group 2 had a higher feed utilization rate compared to the control group for weeks 0 to 8 ($P<0.001$, Table 4). Trial groups 3 and 5, in particular, were found to have feed utilization rates that were very similar to one another and better than other trial groups at 6. weeks. In general, mean egg yields was considerably higher in the intervention groups when compared to the control group in weeks both 5 and 0-8 ($P<0.05$, $P<0.001$, Table 4). Only egg yield in trial group 2 was similar to that of the control group in weeks both 5 and 0-8. In terms of average egg weights, statistically significant differences were found between groups at weeks both 7 and 0-8. Trial 2 group had higher egg weight than other groups in 0-8 weeks period. Egg quality criteria for trial groups given in Table 5. An overall analysis of the findings of the study of egg quality criteria identified no statistically significant differences between the groups. Total fatty acid content of yolks for groups are given

in Table 6. The present study found significant differences between the groups (P<0.001) in terms the total fatty acid content of the eggs, with the exception of linoleic acid content. Linoleic acid levels may fluctuate depending on the amount of sunflower oil used in trials. Yolk cholesterol content of groups are given in Table 7. No significant differences were found between the groups in terms of egg cholesterol content.

Table 4- Performance parameters for trial groups

	Feed consumption, g/day/hen						
Weeks	*Control*	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*	*Trial 5*	P
1	116.18±1.80	120.80±3.91	118.38±1.16	116.78±0.84	119.62±1.25	118.32±1.30	0.599
2	113.61±4.82	116.00±2.61	118.59±4.09	124.41±3.76	114.21±3.90	120.46±3.55	0.362
3	110.62±10.58	114.08±1.58	111.48±2.43	116.04±2.79	113.68±1.27	109.02±2.86	0.915
4	121.62±4.67	118.41±1.86	121.00±2.66	123.28±6.73	117.64±1.89	121.81±2.43	0.894
5	119.43±1.98	119.58±2.54	116.44±2.04	118.53±3.79	118.42±1.78	118.25±2.45	0.960
6	124.48±1.90	118.25±4.01	121.26±4.20	114.38±5.26	118.25±1.28	106.40±5.32	0.060
7	120.09±7.75	118.48±1.58	120.48±4.66	119.31±3.24	118.28±1.97	111.36±3.54	0.692
8	125.90±1.79	123.90±1.94	116.02±2.76	123.25±3.10	120.77±0.77	126.63±3.67	0.069
0-8	119.56±1.02	117.52±0.75	117.08±1.01	117.94±0.86	117.61±0.73	116.67±1.17	0.599
	Feed conversion rate, kg feed/kg egg						
1	2.21±0.03	2.42±0.14	2.39±0.07	2.25±0.11	2.37±0.14	2.40±0.15	0.729
2	2.27±0.08	2.21±0.03	2.48±0.10	2.34±0.12	2.19±0.03	2.27±0.06	0.147
3	2.14±0.13	2.16±0.09	2.17±0.08	2.03±0.05	2.23±0.15	1.99±0.05	0.547
4	2.37± 0.12	2.13±0.06	2.33±0.10	2.23±0.05	2.20±0.05	2.22±0.05	0.269
5	2.20±0.05	2.17±0.07	2.24±0.06	2.10±0.08	2.19±0.05	2.12±0.04	0.605
6	$2.22±0.02^{b}$	$2.07±0.06^{c}$	$2.42±0.04^{a}$	$2.05±0.05^{c}$	$2.12±0.03^{bc}$	$2.05±0.07^{c}$	0.001***
7	2.13±0.15	2.06±0.07	2.35±0.07	2.25±0.07	2.11±0.03	2.55±0.25	0.117
8	2.19±0.03	2.19±0.05	2.23±0.06	2.26±0.03	2.21±0.09	2.37±0.08	0.329
0-8	$2.21±0.03^{b}$	$2.17±0.03^{b}$	$2.33±0.03^{a}$	$2.19±0.03^{b}$	$2.20±0.05^{b}$	$2.25±0.05^{ab}$	0.020*
	Egg yield, %						
1	87.76±1.44	91.84±1.18	85.71±2.58	93.37±2.26	92.35±1.93	90.31±1.74	0.089
2	85.20±1.74	92.35±2.10	88.44±1.36	90.31±2.26	89.80±2.89	91.43±0.76	0.251
3	88.27±2.68	96.94±1.02	88.98±2.47	96.60±1.36	87.25±6.68	94.29±1.00	0.271
4	90.48±3.40	95.92±1.18	86.74±5.11	92.25±1.19	91.84±2.58	94.70±1.53	0.270
5	$83.67±2.04^{b}$	$96.43±0.51^{a}$	$89.79±2.96^{ab}$	$94.90±3.06^{a}$	$91.84±2.76^{a}$	$96.33±0.76^{a}$	0.025*
6	90.31±3.37	94.69±2.29	88.78±3.17	94.39±2.93	93.47±3.06	89.39±2.92	0.561
7	88.98±3.33	92.35±3.16	88.16±3.62	86.39±1.80	93.88±2.14	92.86±1.02	0.527
8	87.24±2.93	93.37±3.94	87.24±3.67	93.37±1.28	94.96±0.00	92.86±3.17	0.178
0-8	$87.95±0.94^{b}$	$94.09±0.84^{a}$	$88.06±1.02^{b}$	$92.61±0.81^{a}$	$92.13±1.12^{a}$	$92.83±0.71^{a}$	0.001***
	Egg weight, g						
1	56.94±0.61	57.30±0.79	58.43±0.68	56.66±0.51	57.13±0.64	56.95±0.66	0.459
2	58.30±0.67	58.54±0.71	59.43±0.70	57.54±0.53	58.42±0.61	57.65±0.60	0.352
3	58.07±0.54	58.58±0.69	59.65±0.69	58.33±0.58	58.50±0.65	58.11±1.01	0.662
4	58.87±0.58	58.60±0.72	59.58±0.65	57.80±0.66	58.84±0.71	58.54±0.66	0.589
5	59.95±0.79	59.41±0.72	60.23±0.68	58.84±0.66	59.68±0.62	58.79±0.78	0.652
6	59.64±0.74	58.99±0.65	59.75±0.62	59.85±0.78	59.96±0.61	58.34±0.81	0.589
7	$61.10±0.69^{ab}$	$59.59±0.68^{bc}$	$61.95±0.80^{a}$	$59.64±0.71^{bc}$	$59.85±0.72^{abc}$	$58.28±0.86^{c}$	0.019*
8	60.46±0.64	60.65±0.78	61.53±0.79	59.83±0.78	59.60±0.65	58.81±0.63	0.140
0-8	$59.19±0.25^{b}$	$58.98±0.26^{b}$	$60.06±0.26^{a}$	$58.51±0.24^{bc}$	$59.03±0.24^{b}$	$58.17±0.27^{c}$	0.001***

Values with different letters in the same row were found to be different from one another; (*): P<0.05, (***): P<0.001

Tablo 5- Egg quality criteria for trial groups

	Shape index						
Weeks	*Control*	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*	*Trial 5*	P
2	76.31±0.23[a]	76.49±0.43[a]	76.14±0.43[a]	76.12±0.51[a]	74.68±0.15[b]	75.41±0.39[ab]	0.022*
4	76.32±0.33	74.86±0.86	75.94±0.28	75.14±0.42	75.77±0.58	75.45±0.45	0.406
6	75.80±0.29	75.51±0.57	76.28±0.46	76.64±0.53	75.22±0.19	76.00±0.83	0.458
8	75.24±0.24	75.26±0.36	76.55±0.62	76.25±0.62	76.92±0.54	75.48±0.46	0.093
	Eggshell thickness, mm×100						
2	34.60 ±0.71	34.72 ±0.30	34.88 ±0.26	35.04 ±0.35	34.84 ±0.41	34.96 ±0.80	0.992
4	33.48 ±0.74	32.92 ±0.68	33.04 ±0.35	32.68 ±0.44	33.40 ±0.82	34.16 ±0.59	0.624
6	33.68 ±0.38	32.80 ± 0.90	34.32 ±0.76	34.40 ±0.62	34.08 ±0.22	34.24 ±0.61	0.472
8	34.24 ±0.64	34.20 ±0.37	33.28 ±0.44	33.96 ±0.57	34.32 ±0.37	34.76 ±0.82	0.582
	Albumen index						
2	7.57 ±0.27	8.13 ±0.25	7.71 ±0.33	7.86 ±0.37	8.15 ±0.28	7.65 ± 0.27	0.621
4	8.67 ±0.42	8.98 ±0.33	8.74 ±0.51	8.81 ±0.26	8.85 ±0.32	8.66 ±0.36	0.988
6	8.65 ± 0.23	9.96 ±0.17	9.66 ±0.45	9.40 ±0.30	9.05 ±0.50	9.27 ± 0.37	0.182
8	8.69 ±0.39	9.62 ± 0.42	8.95 ±0.27	9.10 ±0.25	9.68 ±0.41	9.17 ±0.33	0.344
	Yolk index						
2	55.64±0.68	54.16±0.33	55.79±0.72	56.53±0.79	54.41±0.54	55.54±0.86	0.160
4	54.34±0.36	54.70±0.82	55.17±0.51	54.80±0.28	53.71±0.45	55.40±0.51	0.271
6	53.82±0.53	53.41±0.62	53.54±0.60	53.54±0.65	53.25±0.34	53.95± 0.19	0.935
8	52.73± 0.75	52.20±0.78	52.12±0.46	53.70±0.95	52.88±0.35	52.96±0.35	0.579
	Haugh unit						
2	79.52±1.48	79.90± 1.64	78.60±1.55	79.37±1.95	80.52± 1.06	77.66±0.97	0.801
4	83.14±1.74	82.80±1.52	82.79± 2.04	83.44± 1.26	84.29±0.79	82.34± 1.14	0.954
6	82.89±0.99	87.86±0.70	86.74±1.88	85.53±1.06	83.45±1.39	84.44± 0.99	0.063
8	83.01±1.42	85.96±1.62	84.39± 1.11	84.13± 1.10	86.88± 1.53	85.10± 1.29	0.424

Values with different letters in the same row were found to be different from one another; (*): P<0.05

Table 6- Total fatty acid content of yolks in groups (%)

Fatty acids	*Control*	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*	*Trial 5*	P
Palmitic acid (C16:0)	23.75 ±0.64[a]	23.56 ±0.37[a]	24.49 ±0.42[a]	23.25 ±0.51[a]	23.15 ±0.40[a]	21.80 ±0.24[b]	0.005**
Oleic acid (C18:1n-9)	41.14 ±1.70[c]	42.33 ±0.51[c]	42.74 ±0.91[c]	44.17 ±1.04[bc]	46.60 ±1.00[b]	49.69 ±0.72[a]	0.001***
Linoleic acid (C18:2n-6)	18.07 ±6.07	19.86 ±0.96	18.37 ±1.35	18.03 ±1.21	14.78 ±0.45	13.68 ±0.43	0.355
Linolenic acid (C18:3n-3)	0.25 ±0.04[e]	0.82 ±0.79[d]	1.55 ±0.17[c]	2.38 ±0.27[b]	3.00 ±0.24[a]	3.34 ±0.17[a]	0.001***

Values with different letters in the same row were found to be different from one another; (**): P<0.01; (***): P<0.001

Table 7- Yolk cholesterol content of groups (mg/egg)

Control	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*	*Trial 5*	P
244.31 ± 16.69	239.51 ± 12.92	245.52 ± 30.63	217.98 ± 8.08	223.38 ± 11.39	247.23 ± 12.76	0.694

4. Discussion and Conclusions

The findings concerning feed consumption are consistent with the findings of some previous studies in literature. Küreç (2009) found that adding flaxseed oil to rations had no significant effect on feed consumption in the first week but had a significant effect in the second week. The same study reported that adding 1-2% flaxseed oil to the ration increased feed consumption when compared to the control group, whereas adding 4% flaxseed oil resulted in a significant decrease in feed consumption. In a similar study, Küçükersan (2004) found statistically significant differences in feed consumption in weeks 24, 26, 34 and 36, but no significant differences in the other weeks. Feed utilization rate findings are consistent with the findings of Küçükersan (2004), but slightly higher than those reported by Ezhil Valavan et al. (2006) who reported no statistically significant differences in feed efficiency as a result of adding various feed sources. The feed utilization figures reported by Ahmad et al. (2013) were slightly higher than those in the present study; and the feed utilization rates obtained in the present study were slightly higher than those

reported by Kahraman et al. (2004). In a study conducted with 256 laying hens fed rations containing different amounts flaxseed oil (0%, 5%, 10% and 15%), Sarı et al. (2002) found that adding flaxseed oil resulted in improved feed utilization rates.

The overall findings of the present study concerning egg yield are similar to those reported by Küçükersan (2004), Ezhil Valavan et al. (2006) and Gürbüz et al. (2012). In a similar study, Balevi & Coskun (2003) found that egg yield at the end of 56 days was 69.96% for the group fed a ration containing sunflower oil, and 76.81% for the group fed a ration containing flaxseed oil. The egg yield figures reported Balevi & Coşkun (2003) are lower than those obtained in the present study.

Consistent with the egg weight findings presented here, Kahraman et al (2004) report that adding different sources of oil to rations at varying amounts increases egg weight. Petrovic et al (2012) added 1%, 2%, 3% and 4% flaxseed oil to the compound feed of laying hens, and found that the average egg weights for these groups were 62.5, 60.5, 60.6 and 61.4 g, respectively, compared to 60.4 g for the control group. In the present study, significant differences were found between the groups in week 8 only, but no significant effect was observed compared to the control group. Differences among trial groups may be attributed to linoleic acid contents of the rations with high levels of sunflower oil. Mazalli et al. (2004) added 3% sunflower, flaxseed and fish oils to the rations, as well as two different amounts of vitamin E (12 IU/kg and 100 IU/kg) and found that the treatments did not affect egg weights.

The shape index of the eggs was found to be close to 76, which shows, on the basis of the values reported by Küreç (2009), that the eggs can be classified as round. The finding that additives had no significant effect on the shape index is consistent with the findings of Küçükersan (2004), Küreç (2009) and Oğuz et al. (2016). Similarly, no significant differences were noted between the groups in terms of eggshell thickness, albumen index, yolk index and Haugh unit, which is consistent with previous studies in literature (Küçükersan 2004; Mazalli et al. 2004; Artan 2015; Yassein et al. 2015).

The linolenic acid content, in particular, was observed to increase linearly as the amount of flaxseed oil was increased in the trial groups, when compared to the control group. This finding is consistent with the findings reported by Herkel et al. (2016) in a study their study of flaxseed oil use. What is significant here is that as the amount of flaxseed oil was increased, so did the linolenic fatty acid content by a corresponding amount, indicating transfer to the eggs.

The results obtained in cholesterol values were similar to the results reported by Küçükersan (2004). In a study involving laying hens fed rations containing different amounts of whole flaxseed, Sarı et al. (2002) found that the groups fed rations containing 5%, 10% and 15% flaxseed had lower levels of yolk cholesterol than the control group (0% flaxseed in the ration). The difference here may be attributed to the use of flaxseed.

In conclusion, sunflower oil and flaxseed oil, when added to laying hen rations, produced no difference in egg quality criteria or egg cholesterol levels, while higher ratios of flaxseed oil added to the rations resulted in linearly higher levels of linolenic acid content in the yolk, while use of the two oil additives together increased egg yield. To obtain beneficial effects in the egg yield and linolenic acid content of eggs, it is recommended that a minimum of 1% flaxseed oil should be added to laying hen rations, taking the results of an economic analysis into account.

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In vitro Regeneration Studies of *Vuralia turcica* Using Unpollinated Ovaries

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ABSTRACT
Vuralia turcica is an endemic plant species with a polycarpellary gynoecium and has the ability to survive in salt stress. However, the maintenance of this species is currently challenging because of climatic changes in the plant's habitat, intensive agricultural purposes, grazing, and the like. Gynogenesis is a promising method for micropropagation and a useful technic for genetic engineering. The objective of this study was to analyze the gynogenesis of *V. turcica*. The experiments described here were implemented during the flowering time of *V. turcica* (spring) using unpollinated ovaries. Modified MS and B5 induction media and different plant growth regulators (BAP, GA_3, 2,4-D, and KIN) were compared. The comparison of both media (MS and B5) showed that MS medium supplemented with 2,4-D and KIN was the best medium for *in vitro* unfertilized ovary culture and gave the best result of regenerated plants per 30 ovaries, and 92% callus was obtained. Only the control groups showed very low scores or no plants. From plant regeneration and subcultures, a total of 60 plants were produced, all green. These results are of interest to the *in vitro* conservation of endangered plant species like *V. turcica*.

Keywords: B5, legumes, MS, pure lines, ovary culture

1. Introduction

Plant tissue culture techniques are of the most important for the study and production of plants. The two most essential factors mainly affecting the track of study are the origin of the explant and culture medium. The literature of plant tissue culture may not apply to all results of a study because of differences in age, cell origin, and growth rate, as they may be associated with characteristics of a line (Gamborg et al. 1976; Bednarek & Orlowska 2020). Also, haploidization as a kind of plant tissue culture is particularly important for producing pure lines. Anther and pollen (Androgenesis), as well as unfertilized ovary and ovule culture (Gynogenesis), allows the study of haploid plants (Sibi et al. 2001; Hazarika & Chaturvedi 2013). Gynogenesis is the regeneration of plants from unfertilized cells of the female gametophyte. Explant sources for *in vitro* haploid plant production include blooming flowers, unpollinated ovaries, and ovules. Ovules attached to the placenta may respond more efficiently than their isolated counterparts. Induced haploids are mostly originated from the unfertilized egg cell (Bhojwani & Razdan 1996).

Ovary culture has many applications, including embryo development, fruit development, physiology, the influence of phytohormones on the formation of parthenogenetic fruits, and the role of floral organs on fruit development, the stimulus of pollination in apomictic plants. Ovary culture may also be used in the production of hybrids, as it may overcome the problems such as the inability of pollen germination on stigma, insufficient development of pollen tube, and immature flower abscission. It is also used in inducing polyembryony, which results in many shoots (Van Tuyl et al. 1991).

Vuralia turcica (Tan et al. 1983), Uysal et al. (2014) is a rhizomatous plant species endemic to the region of Eber and Aksehir lakes in Turkey, belonging to the Fabaceae family, locally named as "Piyan." It has the significant property of usually having 3 (2 or 4 also being possible) ovaries within a flower bud, which makes the *V. turcica* unique in the Fabaceae family (Vural 2009) (Figure 1). Naturally, it only has two main subpopulations, which are found at protected properties and is under the danger of extinction due to the conversion of its habitat into agricultural fields, the intervention of nearby wetlands, and the change of suitable microclimate (Vural 2009). For clonal propagation, somatic cells are beneficial and reused mostly for the initiation of *in vitro* embryogenesis. In the case of the unfertilized ovule or ovary culture, embryogenesis occurs in the embryo sac cells or the somatic cells, or both (Reiser & Fischer 1993).

In the present study, *V. turcica* was grown from unfertilized ovary explants, which were taken from Nezahat Gökyiğit Botanical Garden (NGBG) of Istanbul in Turkey. It is known from previous studies that micropropagation of *V. turcica* using its diploid explants such as rhizome, leaf, and epicotyl has already been conducted (Cenkci et al. 2009; Karadag et al. 2013). However, plant propagation using unfertilized ovary explants of *V. turcica* is the first attempt in this study. In the present study, the effects of culture conditions on cultured unfertilized ovaries of *V. turcica* were investigated.

Figure 1- Flower and bud structure of *V. turcica*

2. Material and Methods

2.1. Preparation of media

Murashige and Skoog (MS) (1962) (Caisson Labs, USA), and Gamborg's B5 (B5) (1976) (Caisson Labs, USA) were used as primary media and different plant growth regulators, and concentrations as 0.5 mg L^{-1} Kinetin (KIN; Sigma-Aldrich, USA) + 0.5 mg L^{-1} 2,4-Dichlorophenoxyacetic Acid (2,4-D; Sigma-Aldrich, USA) (Srivastava et al. 2009), 1 mg L^{-1} 6-Benzylaminopurine (BAP; Sigma-Aldrich, USA) + 0.1 mg L^{-1} α-Naphthaleneacetic Acid (NAA; Sigma-Aldrich, USA) (Chand & Basu 1998) and 1 mg L^{-1} 6-Benzylaminopurine + 0.2 mg L^{-1} Gibberellic Acid (GA_3; Sigma-Aldrich, USA) (Keller 1990) were tested with control (PGR-free) group for both media. Sucrose and agar in both media were added as 3% and 0.7%, respectively. The pH of the media was adjusted to 5.7-5.8. Following autoclave at 121 °C (1 atm for 30 min), the media poured sterilized plastic Petri dishes (15 x 60 mm).

2.2. Ovary culture

The stock plants (*V. turcica*) were cultivated in a field located in NGBG under controlled conditions. The flower buds were harvested from the field at anthesis and subjected to sterilization with 10% bleach (10% brand bleach, 90% dH_2O, and 1-2 drops Tween 20) for 10 minutes and later on immersed in 70% ethanol for 5 minutes (adding fresh ethanol three times). Flower buds were rinsed three times with distilled water. Flower buds' gynoecium (ovarium) was picked up with tweezers by cutting flower petals and anthers. Two or three gynaecia (ovary) were collected per flower. The gynoecia were excised from the style part to separate ovary to be used as in the experiments. Six ovaries were placed on each Petri dish with a culture medium. Thirty ovaries were cultured for each experiment. Each experiment was conducted with five replicates.

2.3. Culturing stage

The Petri dishes were placed in a growth chamber at 26 °C and 70% relative humidity, and 16/8 h light/dark photoperiod hours (light intensity is 8000 lux). The cultures were observed and photographed every second day. Morphogenetic responses were observed after a month of culture. Calli were detected within a month from the surface of ovary explants. The color of the calli varies from white to green; their surface showed structures such as the formation of shoots, and the calli were mostly regenerative. For plant regeneration, somatic embryos were transferred to a fresh MS medium without PGR, which was reported as a differentiation medium for *V. turcica* embryos.

2.4. Observations

The data were compiled according to the response of cultured ovaries on MS and B5 media supplemented with different concentrations and combinations of hormones given in Table 1. The percentage responses were provided using total cultured ovaries and on responding ovaries. For each explant, regeneration percentages were determined by [(the number of regenerated plants ÷ number of cultivated explants) x 100] equation.

Table 1- Methods of embryo induction by the culture of unpollinated ovaries

Culture media (mg L^{-1})	*References*	*Mode of inoculation*	*Type of induction medium*	
			MS	*B5*
			Total number of cultured ovaries	
Control (PGR-free)	-	Ovary culture	30	30
0.5 KIN + 0.5 2,4-D	Srivastava et al. (2009)	Ovary culture	30	30
1 BAP + 0.1 NAA	Chand & Basu (1998)	Ovary culture	30	30
1 BAP + 0.2 GA_3	Keller (1990)	Ovary culture	30	30

Abbreviations and legend: 2,4-D (2,4-dichlorophenoxyacetic acid); NAA (naphthalene acetic acid); KIN (kinetin); GA_3 (gibberellic acid); BAP (6-benzyl amino purine); MS (Murashige-Skoog medium); B5 (Gamborg medium); PGR (plant growth regulator)**.** Induction media were based on modified Murashige and Skoog (1960) for MS and Gamborg et al. (1976) B5.

3. Results and Discussion

Unfertilized ovaries of *V. turcica* have been cultured *in vitro* to obtain whole plants from the embryogenic cells.

Callus formation was observed from ovary samples of *V. turcica* in both media (MS and B5) (Table 2). In control groups cultured in the medium without PGR, neither callus nor embryos were formed. In both MS and B5 supplemented with KIN and 2,4-D, non-embryogenic calli were obtained with a high percentage (Table 2). The most efficient culture media for callus induction (92%) was recorded as MS supplemented with 2,4-D (0.5 mg L^{-1}) and KIN (0.5 mg L^{-1}) (Table 2). Also, the responding embryos values were higher in MS medium supplemented with KIN (0.5 mg L^{-1}) and 2,4-D (0.5 mg L^{-1}) (58%) than that of B5 medium (21%), which was relatively low (Table 2).

Table 2- Effects of the plant growth regulators on the production of somatic embryos in cultured unpollinated ovaries of *V. turcica*

Medium no	*Treatments*	*No. ovaries cultured*	*Percent ovaries forming a callus*	*Percent ovaries forming embryos*
	MS medium culture condition			
1	Control (PGR-free)	30	0	0
2	1 mg L^{-1} BAP + 0.2 mg L^{-1} GA_3	30	46	25
3	1 mg L^{-1}BAP + 0.1 mg L^{-1} NAA	30	21	12
4	0.5 mg L^{-1}KIN + 0.5 mg L^{-1} 2,4-D	30	92	58
	B5 medium culture condition			
1	Control (PGR-free)	30	0	0
2	1 mg L^{-1}BAP + 0.2 mg L^{-1} GA_3	30	42	0
3	1 mg L^{-1} BAP + 0.1 mg L^{-1} NAA	30	18	10
4	0.5 mg L^{-1}KIN + 0.5 mg L^{-1} 2,4-D	30	83	21

Legend: The percentages of callus or embryos per ovary were rounded to the nearest whole number.

There have been many reports on somatic embryogenesis from cultured unpollinated ovaries of many species, *Psoralea corylifolia* Linn. (Chand & Sahrawat 2007), *Azadirachta indica* (Srivastava *et al.* 2009), *Amorphophallus konjac* (Zhao *et al.* 2012). However, few reports on *in vitro* embryogenesis in *Vuralia turcica* has been reported from leaves, stems (Tekdal & Cetiner 2014a), and mature embryos (Tekdal & Cetiner 2014b). In the present study, the effects of culture conditions on the development of somatic embryos from cultured unpollinated ovaries of *V. turcica* were investigated for the first time, according to a literature review. At 15 days in culture, the primary callus formed in ovaries cultured in the medium supplemented with 0.5 mg L^{-1} KIN and 0.5 mg L^{-1} 2,4-D (Figure 2). Two types of callus, a brownish and friable type in the MS medium and a white greenish and compact type in the B5 medium, were observed (Figures 3 and 4). Callus formation was higher (92%) than all treatments. Somatic embryo formation was observed in MS medium supplemented with KIN and 2,4-D with a high frequency of 58% (Table 1).

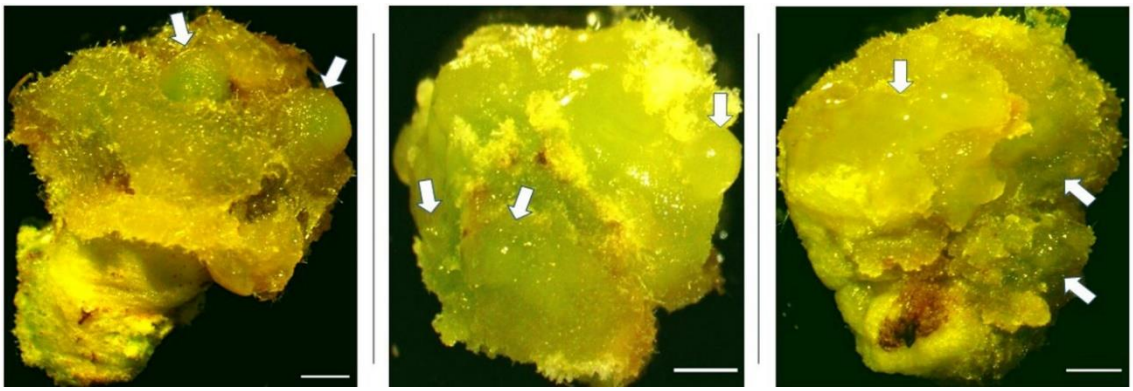

Figure 2- Embryogenic callus formation and somatic embryos (arrows) of *V. turcica* in the MS medium supplemented with 0.5 mg L^{-1} KIN and 0.5 mg L^{-1} 2,4-D; scale bar: 200 µm

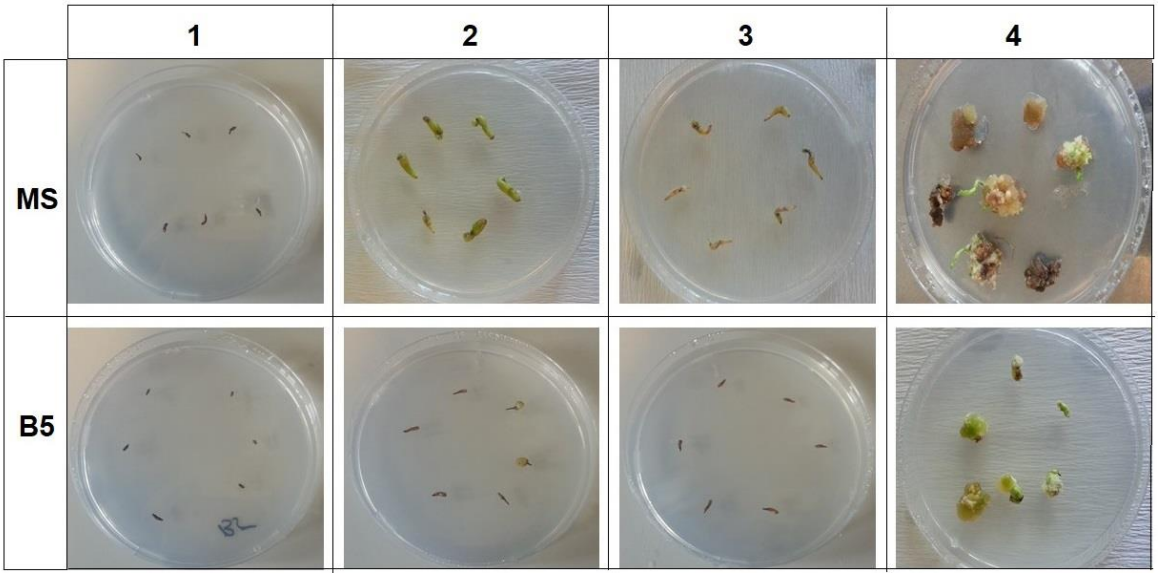

Figure 3- Calli were bursting through the ovaries of *V. turcica* after a month of *in vitro* culture (1-4 describes the medium no given in Table 2)

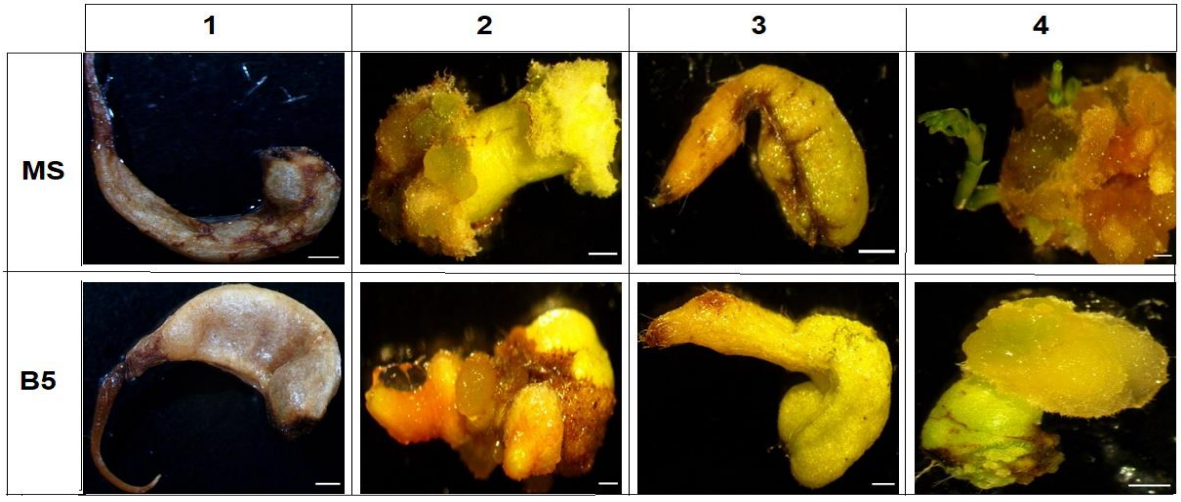

Figure 4- Regeneration of buds and callus from the ovary on MS and B5 media (1-4 describes the medium no given in Table 2) (Scale bar: 80 µm)

Callus induction and somatic embryogenesis from various explant sources can be affected by plant growth regulators, salts, and organic substances in the culture medium. The nitrate: ammonium (NO_3^-/ NH_4^+) rate and vitamin composition are the most prominent differences between MS and B5 media (Saad & Elshahed 2012) used in this study. A previous study of *Linum*

usitatissimum L. showed that callus formation was triggered when the rate of nitrate: ammonium increased. The reduction of the rate of nitrate: ammonium increased somatic embryogenesis and showed the best result of embryo development (Cunha & Fernandes-Ferreira 1999). In *V. turcica*, it was observed that callus growth was higher in the MS medium when compared to the B5 medium (Table 2). It could be possible due to a high rate of nitrate: ammonium in the MS medium.

The embryogenic calli (Figure 2) were transferred to a PGR free medium, and after transferring, the embryos were directly transformed into plantlets (Figure 5). After two months on the medium, explants developed into plantlets.

Figure 5- Plantlets regenerated from unpollinated ovary culture of *V. turcica*, at the left, regenerated plant from callus formed on MS medium with 2,4-D (0.5 mg L^{-1}) and KIN (0.5 mg L^{-1}), and at the right of a plantlet from the callus formed on B5 medium with 2,4-D (0.5 mg L^{-1}) and KN (0.5 mg L^{-1})

In the present study, plant regeneration was obtained from somatic embryos from ovary tissues of *V. turcica* successfully. This is the first report of somatic embryogenesis from unfertilized ovaries of *V. turcica*.

4. Conclusions

V. turcica is the only known species of the genus *Vuralia* and is currently in danger of extinction. For these reasons, studies on the conservation of this unusual species are of great interest. The regeneration of *V. turcica in vitro* conditions depends critically on choosing sufficient explant and culture conditions. In this study, it can be concluded that the most successful medium for plant regeneration in ovary culture was MS + KIN (0.5 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}). The *in vitro* culture of unfertilized ovaries of *V. turcica* described in this study can be applied as an alternative way to obtain somatic plants with the same characteristics as the maternal cultivar in a short time. The development of this technique will be significant not only in the conservation of this endangered plant species but also in the multiplication of this species in order to use for many agronomical purposes such as breeding and obtaining valuable compounds. Besides, for use in transformation/regeneration investigations and *in vitro* manipulation of *V. turcica*, the described regeneration method can be adapted.

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Effects of Two Different Isolates of Entomopathogen Fungus, *Beauveria bassiana* (Balsamo) Vuillemin on *Myzus persicae* Sulzer (Hemiptera: Aphididae)

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ABSTRACT
The study aimed to determine the effectivity of LD.2016 and M6-4 isolates of entomopathogen *Beauveria bassiana* on *Myzus persicae* Sulzer (Hemiptera: Aphididae). In this context, the doses of 10^6, 10^7 and 10^8 conidia mL^{-1} for isolates were used. Experiments for each dose and a control group were set up to be 50 repeats. As a result of the analysis, the r_m values of the individuals exposed to 10^6, 10^7 and 10^8 conidia mL^{-1} doses were calculated as 0.340, 0.352 and 0.337 females/female/day, respectively for the LD.2016 isolate, and 0.292, 0.263 and 0.268 females/female/day, respectively for the M6-4 isolate. The value was 0.280 females/female/day for the control group. By increased dose, the values of l_x and m_x parameters were decreased, too. Besides, the Weibull and Enkegaard models were applied on the l_x and m_x values of the populations exposed to entomopathogen isolates. Consequently, it is thought that the isolates of entomopathogen *B. bassiana*, less harmful to humans and the environment, can be used as a biological control agent in an integrated pest management program for the controlling of the pest.

Keywords: Pepper, Biological control, Integrated pest management, Aphid, Weibull

1. Introduction

The green peach aphid, *Myzus persicae* (Sulzer 1776) (Hemiptera: Aphididae), which is a polyphagous insect pest, cause damage on different plants in Turkey and can continue to do reproduce, and damage year-round under favorable conditions (Velioğlu & Toros 2002). Individuals of *M. persicae* become harmful by sucking plant saps and secrete toxic substances into the plant during the absorption. They also cause fumagine (dark-colored sooty mold) by secreting honeydew. Another form of their damage is also by transporting plant viruses. In addition, *M. persicae* is a hard insect pest to control by dint of having wide host range, multivoltine feature and short development time (Özçelik et al. 2013).

As with many insect pests, chemical pesticides are used in the management of *M. persicae*, too. Due to chemical substances have residual risks on agricultural products, possibility of adverse impact on the environment and human health and forming resistance in pests, there has been an orientation towards biological control, which is an alternative control method (Erdoğan 2015). Considering the risks of chemical control to the environment and human, biological control is one of the most reliable methods. Many biological control agents have been studied on the controlling of *M. persicae* up to the present (Özçelik et al. 2013, Andorno & López 2014, Khatri et al. 2017, Birgücü et al. 2018).

Many pathogenic organisms attack insect pests, and cause diseases upon entering their body. The use of these pathogenic organisms against insect pests for the controlling them is called microbial control. The most appropriate use of microorganisms is to create cultures in artificial media, and then to infect them sobersidedly to environment in a suitable time (Çelebi 2012). Since entomopathogenic fungi enter directly from the insect cuticle, they do not need to be ingested by the pest and taken into the body. Therefore, entomopathogenic fungi play an important role in the management of insects whose food source is plant sap. Since about 1900s, entomopathogenic fungi have been used as biological agent (Sevim et al. 2015).

At least 700 entomopathogenic fungi species belonging to 90 genera, known were identified. In many parts of world, fungal species similar to *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorok., *Isaria fumosorosea* (=*Paecilomyces fumosoroseus*) Wize and *Lecanicillium lecanii* (Zimm.) Zare & Gams against insect pests are developed for commercial purpose (Rath 2000). Entomopathogenic fungi have many remarkable benefits such as not being toxic to mammals, creating resistance against pests, providing long-term controlling against pests in nature, being effective in all stages of the insect, being able to be applied with most insecticides, being cheap and easy to apply (Sevim et al. 2015). It was determined that *B. bassiana* and *M. anisophile* isolates, which were commercially produced in the management of herbivore

insects, have no risk against bees and are applicable as biological control agents (Sevim et al. 2015, Uzuner et al. 2017). In studies on entomopathogenic fungi, it was reported that their effects increase in direct proportion with relative humidity level (Demirci et al. 2011). It is stated that, from the developed pesticides, *Lagenidium giganteum* Couch against mosquito larvae, *M. anisopliae* against cockroaches and flies, *Aschersonia aleyrodis* Webber against whiteflies, and *B. bassiana* against codling moth, Colorado potato beetle and other species from the order of Coleoptera and Lepidoptera can be applied (İnanlı et al. 2012).

Bauveria bassiana, which is abundant in agricultural lands worldwide, has a lot of hosts (Güven et al. 2014). To date, it was pointed out that *B. bassiana* has 707 different hosts, and these include 521 genera, 149 families and 15 orders (Zimmermann 2007). *B. bassiana* causes disease in species from the orders of Lepidoptera, Coleoptera, Hymenoptera, Diptera, Hemiptera, Orthoptera, Siphonaptera, Isoptera, Thysanoptera, Mantodea, Neuroptera, Dermaptera, Blattariae and Embioptera (Zimmermann 2007). It was reported that *B. bassiana*, developed specifically for China, is used in the struggle against European maize borer *Ostrinia nubilalis* Hbn. (Lepidoptera: Crambidae) and Pine lappets *Dendrolimus* spp. (Lepidoptera: Lasiocampidae). Entomopathogenic fungi *B. bassiana* was reported also in Turkey that can be used effectively in the controlling of Colorado potato beetle *Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae) (Azizoğlu et al. 2012). In the same study, it was reported that most of the entomopathogenic fungi were generally applied by the producers in the control of fruit flies and common cockchafers in ecological culture of perennial plants, and aphids and whiteflies in vegetable and greenhouse cultivation.

In this study, in order to determine the effects of 10^6, 10^7 and 10^8 conidia mL^{-1} doses of LD.2016 and M6-4 isolates of the entomopathogenic fungus *B. bassiana* on *M. persicae*, some life table parameters were calculated and the Weibull and Enkegaard mathematical models were applied to some of the obtained data. In addition, it was discussed whether it is possible to use these isolates as a biological control agent in the controlling of the pest within the scope of integrated pest management.

2. Material and Methods

2.1. Production of pepper plants and Myzus persicae

After filling half of 5 L plastic pots with a mixture of sterilized peat, soil and perlite in the ratio of 1:1:1, the process of transplanting was performed by planting pepper (*Capsicum annuum* L.) (Solanaceae) seedlings (Üç burun F1.) therein. Then, these pots which the pepper seedlings were planted in were kept in the clean climate cabinet, and maintenance and irrigation process were carried out when considered necessary. When the pepper plants reached approximately 10 cm, they were brought to the insect rearing cabinet and infected with *Myzus persicae* Sulzer (Hemiptera: Aphididae). The conditions set to 25±1 ºC temperature, 60±5% RH and 16:8 h (L:D) photoperiod in both the clean plant growing cabinet and the insect rearing cabinet. In order to ensure the continuity of insect culture, the collapsed plants were replaced with fresh those when necessary. Also diseased and damaged plants were removed from the climate cabinets. During the experiments, no pesticides and fertilizers were applied to the pepper plants.

2.2. Preparation of entomopathogenic fungus suspension

The isolates of *Beauveria bassiana* (Bals.) Vuill (BMAUM M6-4 and BMAUM LD.2016) used in this study were provided from the investigation of Baydar et al. (2016).

Entomopathogenic fungus isolates used in the experiment were cultured on Sabouraud Dextrose Agar (SDA) medium. SDA (65 g/L, Biolife®) medium was prepared with distilled water (dH_2O) and filled in 500 mL glass erlenmayer flasks and sterilized in autoclave at 121 °C for 20 minutes. Cooled to room temperature, sterilized SDA medium was poured into petri dishes (Ø 9 cm) to form a new fungal culture.

Entomopathogenic fungus spores were taken from pure cultures and spread to petri dishes containing 12-15 mL of medium, and fresh culture was established under aseptic conditions. Petri dishes were incubated at 20-25 °C and 75% rh in the dark.

Spore suspensions were prepared by adding the spores collected by gently scraping from the 14-day old fungus cultures incubated at 25±1 °C, 60±5% RH, and dark conditions in petri dishes with SDA medium into 50 mL sterile dH_2O containing 0.05% Tween 80.

The doses of the prepared spore suspensions were adjusted as 10^6, 10^7 and 10^8 conidia mL^{-1} to be applied on *M. persicae* by the help of a Thoma lam and a light microscope.

2.3. Estabilisment of the experiments

Ten 4^{th}-instar nymphs, randomly selected from *M. persicae* individuals in the insect rearing cabinet, were transferred onto a pepper leaf in a petri dish with blotter paper on the base. These aphids, which reached adulthood, were checked every day and

50 newborn aphids (1st-instar nymphs) were transferred separately to clean pepper leaves in petri dishes. Then, moults of the offsprings were checked daily and the waste skins (exuviae) were removed from the environment. Daily counts, in this way, were performed until all individuals died. When the offspring in each petri dish reached the 2nd or 3rd nymph stage, they were transferred separately to petri dishes containing pepper leaves that were immersed for five seconds in the prepared spore suspension of entomopathogenic fungus. This process was carried out separately for all three doses (10^6, 10^7 and 10^8 conidia mL^{-1}) of *B. bassiana*'s LD.2016 and M6-4 isolates.

As for the control application used in order to compare the effectiveness of the doses of both isolates, pepper leaves immersed in sterile dH_2O containing 0.05% Tween 80 for five seconds were used. Thus, 50 repetitions were created for each application.

To monitor the growth of entomopathogenic fungi, the dead aphids were taken on lams in petri dishes containing moistened blotting paper and mycelial development was examined under a binocular stereo microscope (Figure 1). The experiments were stored in climatic cabinets at 25±1 °C and 60±10% RH, and 16 hours light and 8 hours dark conditions.

Figure 1- The stages of fungus development in *Myzus persicae* individuals exposed to LD.2016 and M6-4 isolates of *Beauveria bassiana*.

2.4. Data analysis and assessments

The age-related survival rate (l_x, females/day) of all individuals and the age-related fertility rate (m_x, females/female/day) obtained by multiplying the average number of offspring by the sex factor were computed based on recorded daily data until all individuals died (Birch 1948). Net reproductive rate (R_0, females/female), that is, the average number of offspring bred by a female individual as long as her lifetime was calculated according to the formula, $R_0 = \sum l_x.m_x$ (Birch 1948). Next, by using the Euler-Lotka equation ($\sum e^{(-r_m x)} l_x m_x = 1$), Intrinsic rate of increase (r_m, females/ female/day) was calculated. Then, mean generation time (T_0, day) was found according to the formula, $T_0 = \frac{\ln R_0}{r_m}$, gross reproduction rate (GRR, females/female) according to the formula, $GRR = \sum m_x$ and finite rate of increase (λ, females/female/day) according to the formula, $\lambda = e^{r_m}$ (Birch, 1948). Meanwhile, theoretical population-doubling time (T_2, day) was also computed according to the formula, $T_2 = \frac{\ln 2}{r_m}$ (Kairo & Murphy 1995). The variable "x" in the formulas indicates the age in days of the female individuals, the coefficient "e" denotes Euler's number which is a mathematical constant (approximately equal to 2.71828) and the symbol "ln" represents the natural logarithm (logarithm based on the coefficient "e"). To use the intrinsic rate of increase (r_m) values computed on the data obtained from these populations in comparison test, the pseudo-r_m values and the standard errors thereof were calculated by the Bootstrap resampling method with the estimates 2000 times (Meyer et al. 1986; Lawo & Lawo 2011; Huang & Chi 2012; Yu et al. 2013a, b). Later, one-way analysis of variance (One-Way ANOVA) followed by Tukey multiple comparison test (Tukey 1949) was performed. Statistical analyses were done by IBM® SPSS® Statistics (Version 20.0, August 2011, SPSS Inc., Chicago, IL, USA) and MS Excel 2010 (Version 14.0) programs.

The two-parameter Weibull frequency distribution was used to define the age-related survival rate (l_x) of the populations. The parameters of this mathematical distribution model were estimated according to the formula, $S_p(x) = e^{[-(\frac{x}{b})^c]}$, $x, b, c > 0$ and in the formula, the "$S_p(x)$" value indicates the probability of the survival rate at a certain age in days; the "b" and "c" values denote scale and shape parameters of the curve, respectively (Deevey 1947; Pinder et al. 1978; Tingle & Copland 1989; Wang et al. 2000). Meanwhile, the Enkegaard regression model ($F_{(x)} = a.x.e^{(-bx)}$) was used to define the age-related fertility rate (m_x) of adult females. Where "$F_{(x)}$" is the probability of fecundity at a certain age in days, the "x" value is the female's age in days, the "a" and "b" values denote constant parameters (Enkegaard 1993; Hansen et al. 1999). The parameters of both mathematical models, the two-parameter Weibull distribution and the Enkegaard regression, were estimated by using SigmaPlot® (Version 11.0, Systat Software, Inc., San Jose California, USA) package program. The values coefficient of determination (R^2), and residual sum of squares (RSS) were used as criteria for the degree of conformity of the Weibull and Enkegaard models to the data obtained (Kontodimas et al. 2004).

3. Results and Discussion

The age-related survival rate (l_x) of *M. persicae* individuals exposed to 10^6 and 10^8 conidia mL^{-1} doses of *B. bassiana*'s LD.2016 isolate started to decrease after the 6th day and the individuals exposed to 10^7 conidia mL^{-1} dose after the 4th day. It was observed that all *M. persicae* individuals exposed to 10^6, 10^7 and 10^8 conidia mL^{-1} doses of LD.2016 isolate were died on the 32nd, 18th and 13th days, respectively. As for M6-4 isolate, the age-related survival rate (l_x) of *M. persicae* individuals exposed to 10^6, 10^7 and 10^8 conidia mL^{-1} doses began to decrease after the 8th, 7th and 1st days, respectively. It was determined that all *M. persicae* individuals exposed to 10^6 conidia mL^{-1} dose of M6-4 isolate were died on the 33rd day, and all individuals in the both groups, exposed to 10^7 and 10^8 conidia mL^{-1} doses, were died on the 19th day (Figure 2). The onset of the decrease in the age-related survival rate (l_x) of the control group was determined as the 7th day and all individuals were died on the 41st day (Figure 3).

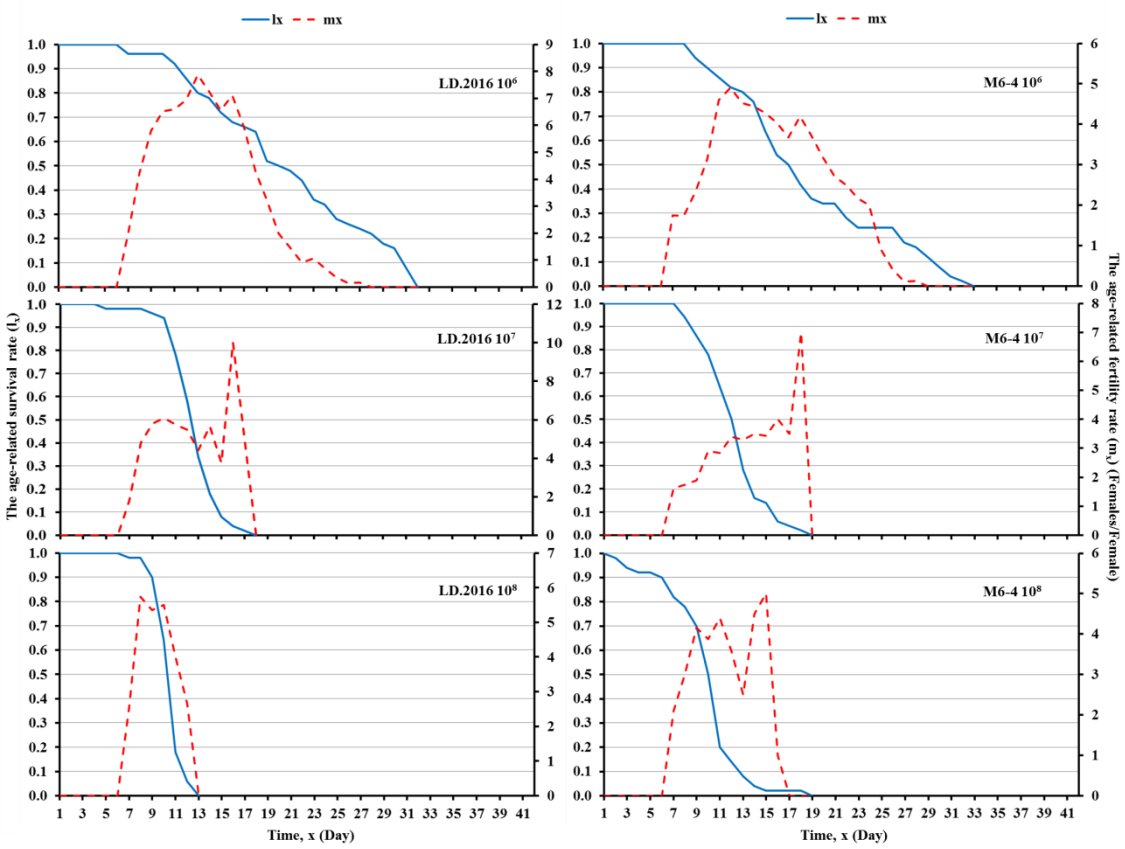

Figure 2- The age-related survival rate (l_x) and the age-related fertility rate (m_x) of *Myzus persicae* individuals exposed to different doses of *Beauveria bassiana*'s LD.2016 and M6-4 isolates

The individuals exposed to three different doses of *B. bassiana*'s LD.2016 and M6-4 isolates, and adults in the control group began to give offspring from the 7th day. *M. persicae* adults exposed to 10^6 conidia mL^{-1} dose of *B. bassiana*'s LD.2016 isolate gave the maximum offspring as 7.88 offsprings on the 13th day, the individuals exposed to 10^7 conidia mL^{-1} dose as 10 offsprings on the 16th day, and the individuals exposed to 10^8 conidia mL^{-1} dose as 5.74 offsprings on the 8th day. As for M6-4 isolate, *M. persicae* adults exposed to 10^6 conidia mL^{-1} dose gave the maximum offspring as 4.90 offsprings on the 12th day, the individuals exposed to 10^7 conidia mL^{-1} dose as 7 offsprings on the 18th day, and the individuals exposed to 10^8 conidia mL^{-1} dose as 5 offsprings on the 15th day (Figure 2). *M. persicae* adults in the control group had the most newborn as 4.69 offsprings on the 17th day (Figure 3). The curves drawn over l_x and m_x values obtained from the individuals exposed to 10^7 and 10^8 conidia mL^{-1} doses of LD.2016 and M6-4 isolates showed similar fluctuations. Again, the curves drawn over l_x and m_x values obtained from both of the individuals exposed to 10^6 conidia mL^{-1} dose of two isolates and the individuals in the control group were found to be almost similar. As the dose increased, the curves of the l_x and m_x values tended to decrease in a shorter time than those of the control group (Figures 2 and 3).

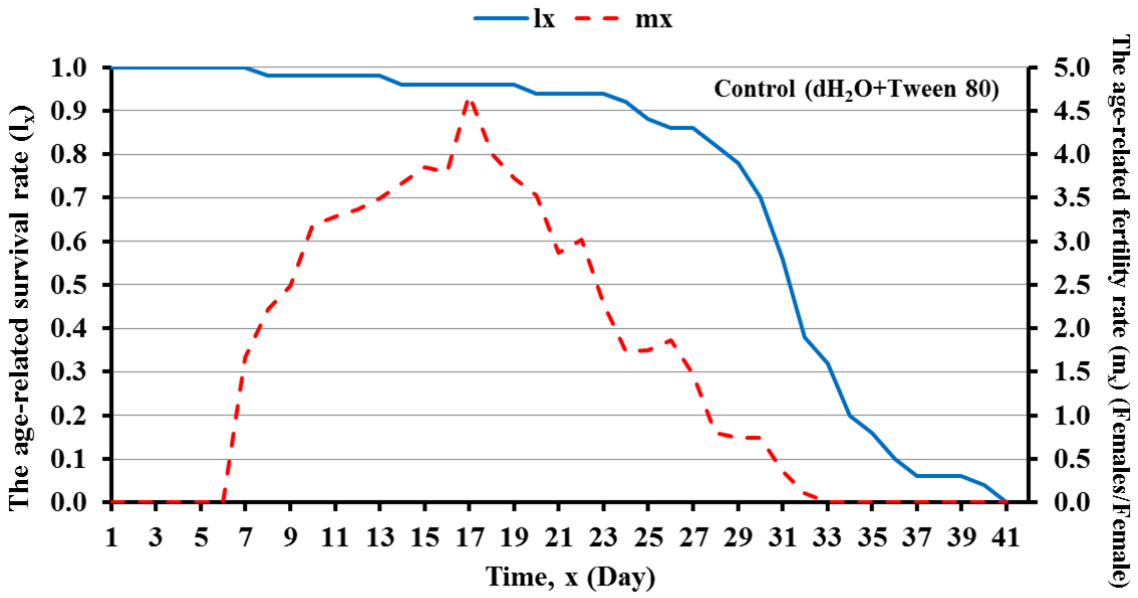

Figure 3- The age-related survival rate (l_x) and the age-related fertility rate (m_x) of *Myzus persicae* individuals used as the control group

Rashki and Shirvani (2013) found that fluctuations in the curves of l_x and m_x values obtained from *A. gossypii* individuals exposed to low concentrations (5.6 x 10^2, 10^4 and 10^5 conidia mL^{-1}) of *B. bassiana* and the individuals in the control group were nearly close. It is also reported that the l_x and m_x values of individuals exposed to 10^7 and 10^8 conidia mL^{-1} doses of that entomopathogenic fungus were close to each other. As a result, it is stated that as the conidial concentration increases, the values of l_x and m_x decrease. It was observed that the results obtained from this study are in line with the results of the present study. Table 1 shows the development and lifetimes in different biological stages of *M. persicae* individuals exposed to different doses of *B. bassiana*'s LD.2016 and M6-4 isolates.

The durations of the first nymphal stages of *M. persicae* individuals exposed to different doses of two isolates, and individuals in the control group showed variety between 1.56 and 1.82 days, and statistically all were included in the same group. Development times of the LD.2016 isolate- and M6-4 isolate-exposed *M. persicae* individuals were in the same statistical group as the control group, except those exposed to 10^8 conidia mL^{-1} dose of M6-4 isolate. The highest lifespan was in the control group with 30.16 days, and the lifespans of the LD.2016 isolate- and M6-4 isolate-exposed individuals (under the same dose conditions, 10^6 and 10^7 conidia mL^{-1}) were found as similar. The shortest lifespans were calculated as 9.74 and 9.00 days from individuals exposed to 10^8 conidia mL^{-1} doses of LD.2016 and M6-4 isolates, respectively (Table 1).

Table 1- The development times and lifespans (day) of *Myzus persicae* individuals exposed to different doses of *Beauveria bassiana*'s LD.2016 and M6-4 isolates*

Biological Stages	*Isolate*	*Dose*	*n*	*Duration*	
The 1st Nymphal Stage	Control		50	1.68 ± 0.07	a
	LD.2016	10^6	50	1.82 ± 0.10	a
		10^7	50	1.70 ± 0.11	a
		10^8	50	1.74 ± 0.12	a
	M6-4	10^6	50	1.74 ± 0.08	a
		10^7	50	1.56 ± 0.07	a
		10^8	50	1.80 ± 0.10	a
The 2nd Nymphal Stage	Control		50	1.64 ± 0.08	ab
	LD.2016	10^6	50	1.36 ± 0.08	bc
		10^7	49	1.40 ± 0.09	bc
		10^8	49	1.32 ± 0.09	bc
	M6-4	10^6	50	1.80 ± 0.06	a
		10^7	50	1.78 ± 0.07	a
		10^8	47	1.22 ± 0.10	c
The 3rd Nymphal Stage	Control		50	1.60 ± 0.08	bc
	LD.2016	10^6	49	1.24 ± 0.08	cd
		10^7	49	1.22 ± 0.07	d
		10^8	49	1.40 ± 0.08	cd
	M6-4	10^6	50	2.00 ± 0.08	a
		10^7	50	1.92 ± 0.08	ab
		10^8	44	1.50 ± 0.13	cd
The 4th Nymphal Stage	Control		49	1.90 ± 0.10	abc
	LD.2016	10^6	48	2.30 ± 0.12	a
		10^7	49	2.14 ± 0.11	ab
		10^8	49	2.02 ± 0.12	ab
	M6-4	10^6	46	1.72 ± 0.11	bc
		10^7	48	1.66 ± 0.11	bc
		10^8	38	1.44 ± 0.16	c
Development Time	Control		49	6.72 ± 0.17	a
	LD.2016	10^6	48	6.73 ± 0.16	a
		10^7	49	6.42 ± 0.16	a
		10^8	49	6.46 ± 0.16	a
	M6-4	10^6	46	6.76 ± 0.31	a
		10^7	48	6.70 ± 0.22	a
		10^8	38	5.26 ± 0.43	b
Lifespan	Control		50	30.16 ± 0.82	a
	LD.2016	10^6	50	19.96 ± 1.02	b
		10^7	50	11.84 ± 0.30	c
		10^8	50	9.74 ± 0.16	cd
	M6-4	10^6	50	18.06 ± 0.98	b
		10^7	50	11.42 ± 0.36	cd
		10^8	50	9.00 ± 0.43	d

*The means (± standard errors) with different letters within the same column, separately for each biological stage, differ significantly at the 0.05 level according to Tukey's HSD test.

Preoviposition durations of both the individuals exposed to different doses of *B. bassiana* isolates and the individuals in the control group varied between 0.00 and 0.36 days, and all of them were statistically in the same group, except those exposed to 10^6 conidia mL^{-1} dose of M6-4 isolate. Oviposition and postoviposition durations of the LD.2016 isolate- and M6-4 isolate-exposed (at the different doses) individuals were, so that within itself, statistically included in the same group. In addition, it was observed that as the dose increased, the periods of oviposition and postoviposition shorten. The longest oviposition and postoviposition durations were seen in the control group and were calculated as 18.41 and 5.37 days, respectively (Table 2).

Table 2- Daily (females/female/day) and total (females/female) numbers of offspring of *Myzus persicae* individuals exposed to different doses of *Beauveria bassiana*'s LD.2016 and M6-4 isolates*

	Dose	*n*	*Preoviposition Duration*	*Oviposition Duration*	*Postoviposition Duration*	*Daily number of offspring*	*Total number of offspring*
	Control	49	0.00 ± 0.00 b	18.41 ± 0.59 a	5.37 ± 0.48 a	2.51 ± 0.11 b	59.60 ± 2.92 a
LD.2016	10^6	48	0.00 ± 0.00 b	11.50 ± 0.66 b	2.17 ± 0.54 b	4.85 ± 0.22 a	60.84 ± 3.53 a
	10^7	49	0.10 ± 0.04 b	5.35 ± 0.26 c	0.00 ± 0.00 c	4.94 ± 0.21 a	27.68 ± 1.88 b
	10^8	49	0.00 ± 0.00 b	3.18 ± 0.15 cd	0.04 ± 0.04 c	4.85 ± 0.20 a	15.68 ± 0.88 c
M6-4	10^6	44	0.36 ± 0.10 a	10.21 ± 0.71 b	1.43 ± 0.44 bc	2.75 ± 0.21 b	34.86 ± 3.87 b
	10^7	48	0.02 ± 0.02 b	4.52 ± 0.34 cd	0.08 ± 0.05 c	2.27 ± 0.18 b	11.52 ± 1.37 c
	10^8	38	0.13 ± 0.06 b	3.16 ± 0.25 d	0.05 ± 0.05 c	2.62 ± 0.25 b	8.90 ± 1.07 c

*The means (± standard errors) with different letters within the same column differ significantly at the 0.05 level according to Tukey's HSD test.

Daily numbers of offspring of the individuals exposed to different doses of *B. bassiana*'s LD.2016 and M6-4 isolates and the individuals in the control group varied between 2.27 and 4.94 females/female/day, and the highest daily number of offspring was seen in the individuals exposed to 10^7 conidia mL^{-1} dose of LD.2016. The least total numbers of offspring was seen in the individuals exposed to 10^8 conidia mL^{-1} dose of M6-4 isolate with 8.90 females/female, and the highest in the individuals exposed to 10^6 conidia mL^{-1} dose of LD.2016 isolate with 60.84 females/female. This was followed by *M. persicae* individuals in the control group with 59.60 females/female. The difference between the minimum and maximum total numbers of offspring was found to be statistically significant (Table 2). In the study on sub-lethal effect of *M. anisopliae* of Rashki et al. (2015), it was stated that the sub-lethal concentration of the entomopathogenic fungus has no effect on fertility of *M. persicae.*

Life table parameters of *M. persicae* individuals exposed to different doses of *B. bassiana*'s LD.2016 and M6-4 isolates are seen in Table 3. The difference between the intrinsic rate of increase (r_m) and net reproductive rate (R_0) values of *M. persicae* individuals exposed to different doses of both isolates were found statistically significant.

The longest mean generation time (T_0) was determined in *M. persicae* individuals in the control group with 12.950 days, and the shortest in individuals exposed to 10^8 conidia mL^{-1} dose of LD.2016 with 8.497 days (Table 3).

The gross reproduction rate (GRR) of *M. persicae* individuals in the control group was calculated as 64.702 females/female, and this value was computed as 81.105, 58.596 and 25.827 females/female, respectively, in the individuals exposed to 10^6, 10^7 and 10^8 conidia mLl^{-1} doses of *B. bassiana*'s LD.2016 isolate. The value was also determined for the individuals exposed to 10^6, 10^7 and 10^8 conidia mL^{-1} doses of *B. bassiana*'s M6-4 isolate as 61.456, 39.097 and 34.103 females/female, respectively. The theoretical population-doubling time (T_2) was seen as the longest in the individuals exposed to 10^7 conidia mL^{-1} dose of M6-4 isolate with 2.631 days, and as the shortest in the individuals exposed to 10^6 conidia mL^{-1} dose of LD.2016 isolate with 1.869 days. The highest finite rate of increase (λ) was observed in the individuals exposed to 10^6 conidia mL^{-1} dose of LD.2016 isolate with 1.449 females/female/day, while the lowest was in the individuals exposed to 10^7 conidia mL^{-1} dose of M6-4 isolate with 1.301 females/female/day (Table 3).

Table 3- Life table parameters of *Myzus persicae* individuals exposed to different doses of *Beauveria bassiana*'s LD.2016 and M6-4 isolates

Parameters	*Control*	*LD.2016*			*M6-4*		
		10^6	10^7	10^8	10^6	10^7	10^8
Intrinsic rate of increase, r_m	0.280 **e**	0.340 **b**	0.352 **a**	0.337 **c**	0.292 **d**	0.263 **g**	0.268 **f**
Net reproductive rate, R_0	59.71 **b**	61.19 **a**	27.82 **d**	15.72 **e**	34.90 **c**	11.60 **f**	8.93 **g**
Mean generation time, T_0	12.950	11.152	9.534	8.497	11.647	9.775	8.836
Gross reproduction rate, GRR	64.702	81.105	58.596	25.827	61.456	39.097	34.103
Theoretical population-doubling time, T_2	2.182	1.869	1.967	2.064	2.229	2.631	2.575
Finite rate of increase, λ	1.374	1.449	1.422	1.399	1.365	1.301	1.309
n	40	31	17	12	32	18	18

*: The values of intrinsic rate of increase (r_m) and net reproductive rate (R_0) with different letters within the same line differ significantly at the 0.05 level according to Tukey's HSD test.

Net reproductive rate (R_0) values of both the individuals exposed to LD.2016 isolate and the individuals exposed to M6-4 isolate differed by dose. It was observed that there is an inverse proportion between dose and R_0 values. Rashki and Shirvani (2013) observed the effect of *B. bassiana* on the life table parameters of *A. gossypii* in their study, and determined that net reproductive rate (R_0) values decreased as concentration increased, as in the present study.

The present study indicated that the mean generation time (T_0), finite rate of increase (λ) and gross reproduction rate (GRR) values of both the individuals exposed to LD.2016 isolate and the individuals exposed to M6-4 isolate decreased as concentration increased, too. As for the theoretical population-doubling time (T_2), another life table parameter, increased in parallel with the concentration (Table 3). Rashki and Shirvani (2013) observed in their study that the mean generation time (T_0) and theoretical population-doubling time (T_2) values increased with concentration. However, they also stated that there was no significant difference between the individuals exposed to low concentrations ($5.6x10^2$, $1x10^4$ and $1x10^5$ conidia mL^{-1}) and the individuals in control group in terms of T_0 and T_2 values. They also reported that the finite rate of increase (λ) value was inversely proportional to the conidial concentration. The mean generation time (T_0), finite rate of increase (λ) and theoretical population-doubling time (T_2) values were similar in both studies. Bayındır et al. (2016) examined the effect of some plant extracts on the biology of *M. persicae*, found the intrinsic rate of increase (r_m), net reproductive rate (R_0), mean generation time (T_0), gross reproduction rate (GRR), theoretical population-doubling time (T_2), and finite rate of increase (λ) values as 0.37, 44.45, 10.39, 58.47, 1.89, and 1.44 females/female/day, respectively. As for the present study examining the effect of *B. bassiana* on the biology of *M. persicae*, calculated the intrinsic rate of increase (r_m), net reproductive rate (R_0), mean generation time (T_0), gross reproduction rate (GRR), theoretical population-doubling time (T_2), and finite rate of increase (λ) values as 0.280, 59.71, 12.950, 64.702, 2.182 and 1.374 females/female/day, respectively (Table 3). The intrinsic rate of increase (r_m) of *M. persicae* on pepper determined as 0.250 females/female/day in a study on demographic characteristics of the aphid carried out by Ricci et al. (2000). Another study done by La Rossa et al. (2013) calculated the intrinsic rate of increase (r_m) of *M. persicae* ranged from 0.174 to 0.281 females/female/day on nine different pepper cultivars. Also, the intrinsic rate of increase (r_m) was found as 0.319, 0.356 and 0.305 females/female/day for the offsprings of younger, middle and older aged mothers, respectively in the study of Birgücü and Bayındır-Erol (2018) about maternal age effect on biology of *M. persicae*. Again, the same study declared that the net reproductive rates (R_0) were 26.483, 38.095 and 21.474 females/female for the offsprings of younger, middle and older aged mothers, respectively.

The most fitted life curves based on the age-related survival rate (l_x) of *M. persicae* individuals exposed to different doses of *B. bassiana*'s LD.2016 and M6-4 isolates were determined by the Weibull distribution model. As the criteria for the conformity degree of the Weibull distribution models to the obtained data, determination coefficient (R^2) and residual sum of squares (RSS) values were used (Kontodimas et al. 2004). The "b (scale)" and "c (shape)" parameters in the Weibull distribution models applied on the age-related survival rate (l_x) were calculated as 22.169 and 2.676 for the individuals exposed to 10^6 conidia mL^{-1} dose of LD.2016 isolate, and as 19.539 and 2.534 for the individuals exposed to 10^6 conidia mL^{-1} dose of M6-4 isolate. Based on these results, it was seen that the *M. persicae* populations exposed to 10^6 conidia mL^{-1} doses of both isolates had an increasing population type (Figure 4 and Table 4).

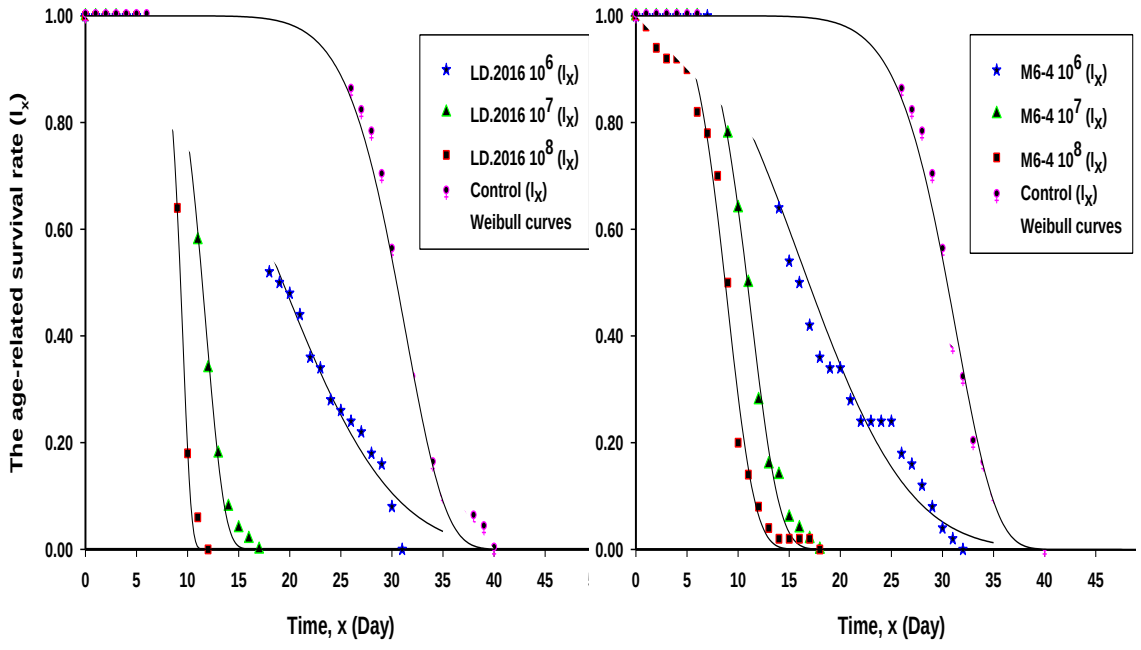

Figure 4- The Weibull distribution models fitted on the age-related survival rate (lx) of *Myzus persicae* individuals exposed to different doses of *Beauveria bassiana*'s LD.2016 and M6-4 isolates

Table 4- The parameters of the Weibull distribution models fitted on the age-related survival rate (l_x) of *Myzus persicae* individuals exposed to different doses of *Beauveria bassiana*'s LD.2016 and M6-4 isolates*

	Dose	*b (scale)*	*c (shape)*	*R²*	*RSS*
Control		31.724 ± 0.128	8.945 ± 0.403	0.992	0.041
LD.2016	10^6	22.169 ± 0.167	2.676 ± 0.079	0.994	0.022
	10^7	12.017 ± 0.059	7.428 ± 0.340	0.997	0.008
	10^8	9.594 ± 0.032	12.359 ± 0.622	0.998	0.003
M6-4	10^6	19.539 ± 0.256	2.534 ± 0.119	0.986	0.061
	10^7	11.729 ± 0.075	5.049 ± 0.212	0.997	0.010
	10^8	9.510 ± 0.139	4.351 ± 0.364	0.989	0.035

*The model parameters, b, and c, are given with their standard errors (P<0.0001)

For the individuals exposed to 10^7 conidia mL^{-1} dose of LD.2016 isolate, the "b" and "c" parameters in the Weibull distribution models were calculated as 12.017 and 7.428, and these parameters were found as 19.539 and 2.534 for the individuals exposed to 10^7 conidia mL^{-1} dose of M6-4 isolate. As for the individuals exposed to 10^8 conidia mL^{-1} doses of the isolates, the parameters "b" and "c" were calculated as 9.594 and 12.359 for LD.2016 isolate-exposed individuals and as 9.510 and 4.351 for M6-4 isolate-exposed individuals (Table 4).

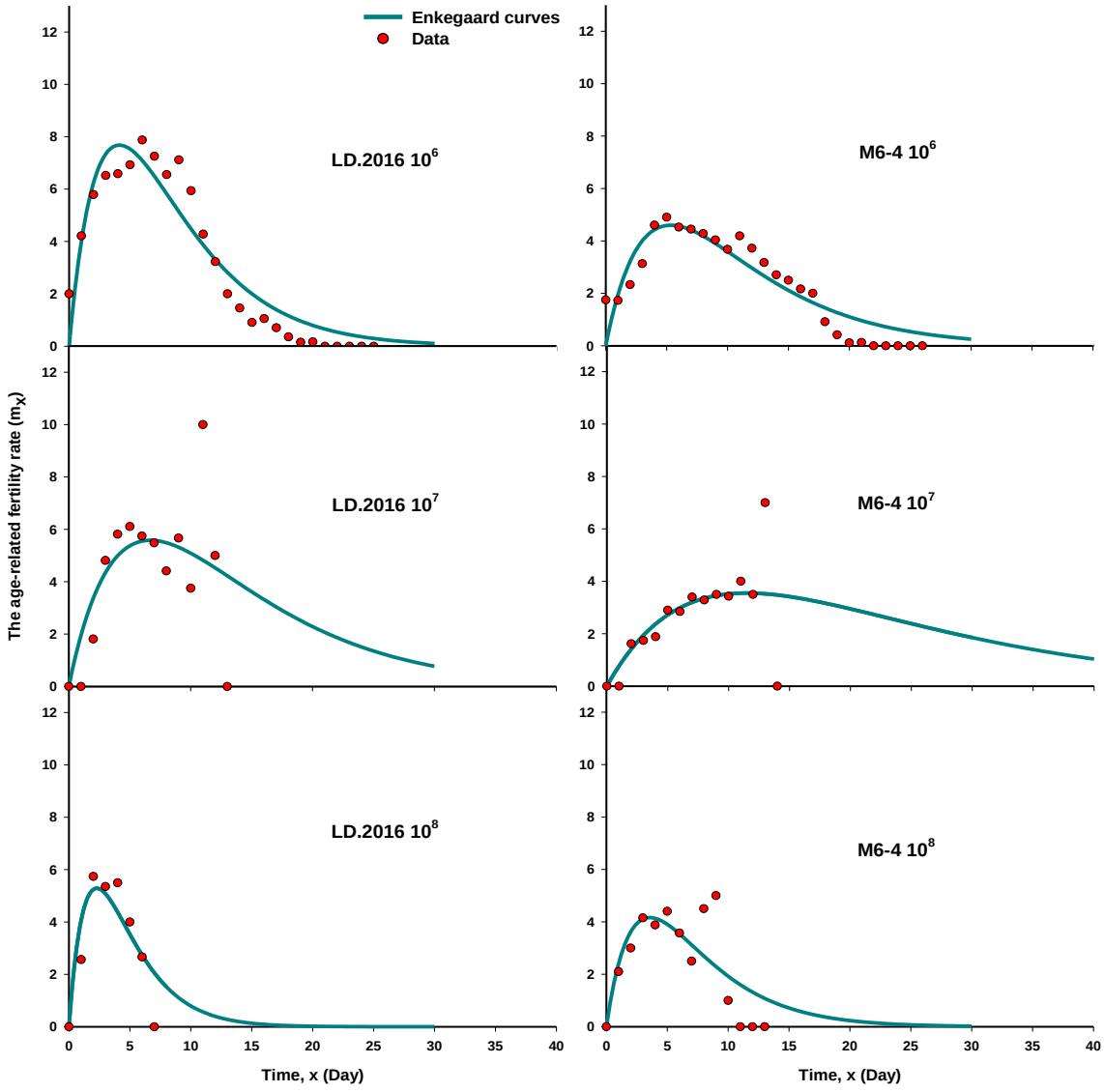

Figure 5- The Enkegaard regression models fitted on the age-related fertility rate (m_x) of *Myzus persicae* individuals exposed to different doses of *Beauveria bassiana*'s LD.2016 and M6-4 isolates

According to these results, the curves of the Weibull distribution model fitted on the age-related survival rate (l_x) of individuals exposed to 10^7 and 10^8 conidia mL^{-1} doses of both isolates tended to decrease earlier and suddenly with the effect of the entomopathogen isolates than those of others. The parameters "b" and "c" in the Weibull model for the control group were 31.724 and 8.945 (Table 4). These results point out that the control group *M. persicae* population fit the increasing type of population (Figure 4).

The most fitted models of the age-related fertility rate (m_x) of *M. persicae* individuals exposed to different doses of *B. bassiana*'s LD.2016 and M6-4 isolates were determined by the Enkegaard regression model (Figure 5). The given data in Figure 6 are the Enkegaard regression models fitted on the age-related fertility rate (m_x) of *M. persicae* individuals used as the control group. As in the Weibull distribution model, determination coefficient (R^2) and residual sum of squares (RSS) values were used as the criteria of the conformity degree in the Enkegaard regression models (Kontodimas et al. 2004).

The parameters "a" and "b" in the Enkegaard model were found as 5.058 and 0.242 for the individuals exposed to 10^6 conidia mL^{-1} dose of LD.2016 isolate, and as 2.342 as 0.187 for the individuals exposed to 10^6 conidia mL^{-1} dose of M6-4 isolate. The parameters "a" and "b" for the individuals exposed to 10^7 conidia mL^{-1} dose were determined as 2.265 and 0.149 for the LD.2016 isolate-exposed individuals and as 0.840 and 0.087 for the M6-4 isolate-exposed individuals. As for the individuals exposed to 10^8 conidia mL^{-1} doses of the isolates, the parameters "a" and "b" were computed as 6.280 and 0.437 for LD.2016 isolate-exposed individuals and as 3.172 and 0.280 for M6-4 isolate-exposed individuals. As for in the model belonging to the control group, the parameters "a" and "b" were found as 1.813 and 0.163, respectively (Table 5).

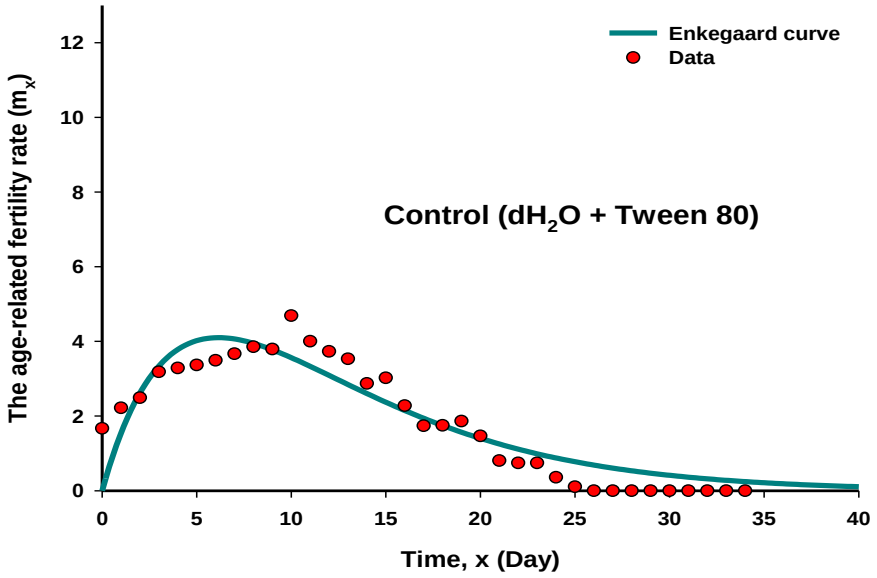

Figure 6- The Enkegaard regression models fitted on the age-related fertility rate (m_x) of *Myzus persicae* individuals used as the control group

Table 5- The parameters of The Enkegaard regression models fitted on the age-related fertility rate (m_x) of *Myzus persicae* individuals exposed to different doses of *Beauveria bassiana*'s LD.2016 and M6-4 isolates*

	Dose	*a*	*b*	*R²*	*RSS*
Control		1.813 ± 0.151	0.163 ± 0.008	0.869	10.727
LD.2016	10^6	5.058 ± 0.446	0.242 ± 0.012	0.904	20.798
	10^7	2.265 ± 0.735	0.149 ± 0.040	0.474	55.687
	10^8	6.280 ± 1.546	0.437 ± 0.069	0.783	8.299
M6-4	10^6	2.342 ± 0.227	0.187 ± 0.011	0.853	11.761
	10^7	0.840 ± 0.315	0.087 ± 0.038	0.464	25.412
	10^8	3.172 ± 0.784	0.280 ± 0.042	0.617	18.076

*: The model parameters, a, and b, are given with their standard errors (P<0.0001)

4. Conclusions

The statistical analysis applied to the obtained data demonstrated that net reproductive rate (R_0), the mean generation time (T_0), finite rate of increase (λ) and gross reproduction rate (GRR) values decreased with increasing concentration. In addition, the Weibull distribution and Enkegaard regression models applied on the age-related survival rate (l_x) and the age-related fertility rate (m_x) of *B. bassiana*'s LD.2016 and M6-4 isolates-exposed *M. persicae* individuals showed compatibility to obtained data. According to these results, it is seen that these entomopathogenic fungus isolates may be used within the scope of the integrated control method to be applied against *M. persicae*. Entomopathogenic fungi may be a suitable alternative instead of chemicals, since they do not have any toxic effects on mammals (Sevim et al. 2015). Also, a study performed on sub-lethal effect of combination of *M. anisopliae* and imidacloprid on life table of *M. persicae* asserted that the applications made did not have a significant effect on the values of mean generation time (T_0). Although most sub-lethal dose applications do not have a significant effect on the life table characteristics of *M. persicae*, the method would be a suitable procedure for controlling aphids by increasing the fungal concentration (Rashki et al. 2015). In future studies, it would be beneficial to investigate the effectiveness of these isolates of *B. bassiana* against *M. persicae* under field and greenhouse conditions. In addition to this, another point to be considered is that the determination of the effects of these isolates on wildlife and natural enemies are essential. What has been explained so far showed that *B. bassiana*'s LD.2016 and M6-4 isolates can likely be considered to be involved in biological control practices within the scope of an Integrated Pest Management (IPM) program to be prepared against *M. persicae*.

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Five new records of eriophyid mites (Acari: Eriophyoidea) from herbaceous plants and fruit trees in Van province, Turkey

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ABSTRACT

Five species of eriophyoid mites were detected as new records for the mite fauna of Turkey. The samples were collected on herbaceous plants and fruit trees in Bahçesaray, Edremit, İskele and Akdamar island of Van province, Turkey between 2014 and 2016. The identified eriophyid species are *Aceria camdeboo* (Meyer, 1981) on *Celtis* sp. (Cannabaceae); *A. trifolii* (Nalepa, 1892) on *Vicia biennis* L. (Fabaceae), *Aculus parakarensis* (Bagdasarian, 1972) on *Amygdalus communis* L. (Rosaceae), *Leipothrix moraceus* (Castagnoli, 1980) on *Morus alba* L. (Moraceae) and *Phyllocoptes obtusus* (Nalepa, 1891) on *Salvia* sp. (Lamiaceae). The species are depicted, measured and information on their hosts, damage symptoms and geographical distribution are given.

Keywords: Eriophyoidea, New record, *Aceria*, Van, Turkey

1. Introduction

Eriophyoid mites are very small, obligatory phytophagous invertebrates and the majority of these mites are host specific (Lindquist et al. 1996). Weed-associated eriophyoids are considered to have high potential as biological control agents because of their host specificity (Smith et al. 2010). Turkey has a rich eriophyoid biodiversity because of the geographical position and botanical history of the country (Ekim & Güner 2000; Karagöz 2003). Many studies were carried out to determine the eriophyoid fauna of Turkey (Denizhan et al. 2006, 2008; Denizhan & Çobanoğlu 2010). Denizhan et al. (2015) listed eriophyoid mites in a catalogue along with remarks and information on their current distribution in Turkey and they reported that a total of 130 eriophyoid species for the Turkish fauna up to March 2013. Then, eight eriophyoid species were determined as new records for Turkey: *Aceria tenuis* (Nalepa 1891), *Epitrimerus gibbosus* (Nalepa 1892) and *Quadracus urticae* (Keifer 1944) on weed plants in Samsun (Diler & Ozman-Sullivan 2011), *Aceria sobhiani* Sukhareva, 2001 on *Acroptilon repens* L., *Aceria carduui* Petanovic, Boczek and Shi 2002 on *Carduus pycnocephalus* L. in Ankara (Diler & Ozman-Sullivan 2016), *Aceria stefanii* (Nalepa 1898) on pistachio trees in the South-Eastern Anatolia (Usanmaz et al. 2018), *Aceria diospyri* (Keifer 1944) on *Diospyros kaki* L. in Yalova (Denizhan 2018) and *Rhyncaphytoptus castaneae* (Farkas 1960) on *Castanea sativa* Mill. in Aydın (Gokce et al. 2020).

Although only 138 eriophyoid mite species have been recorded in Turkey to date, there is still many more to be discovered. Therefore, the aim of this study is to add five new species from Van region in Turkey. These new records, increased the total number of eriophyoid species to 143 in Turkey.

2. Material and Methods

The plant samples were collected from herbaceous plants and fruit trees in Bahçesaray, Edremit, İskele and Akdamar island of Van province, located in the Eastern part of Turkey, between 2014 and 2016. Eriophyid mites collected from the plants were directly examined under a dissecting stereo-microscope (Leica ES2) and mounted on microscope slides in F-medium according to Keifer (1975). The identifications were made with the help of a phase-contrast microscope (Leica DM 1000). The morphological nomenclature follows Lindquist et al. (1996), all the measurements were made according to Amrine & Manson (1996) and De Lillo et al. (2010). The systematic classification follows Amrine et al. (2003). Information on the hosts and damage symptoms, geographical distribution of these species and GPS coordinates of the location of each sample are provided. The Prodorsal shield, empodium and genital area are figured. The voucher specimens of the species are kept in the mite collection of the Faculty of Science, University of Trakya, Edirne, Turkey.

3. Results and Discussion

Five eriophyoid mites were identified as new records for the mite fauna of Turkey from herbaceous plants and fruit trees in Van province: *Aceria camdeboo* (Meyer, 1981), *A. trifolii* (Nalepa, 1892), *Aculus parakarensis* (Bagdasarian, 1972), *Leipothrix moraceus* (Castagnoli, 1980) and *Phyllocoptes obtusus* (Nalepa, 1891). Information on the measurements, drawings, hosts, damage symptoms and geographical distribution of these species are given below.

Family: Eriophyidae Nalepa, 1898
Subfamily: Eriophyinae Nalepa, 1898
Genus: *Aceria* Keifer, 1944
***Aceria camdeboo* (Meyer, 1981)**

Female: 188–217 µm long, 43–54 µm wide; gnathosoma 9–12 µm long; gnathosomal setae 3–4; chelicerae 10–11 µm; Prodorsal shield 20–22 µm long, 30–31 µm wide; dorsal setae 11-13 µm long (Figure 1a).

Leg: Foreleg 18 µm long; tibia 4 µm; tarsus 5 µm; tarsal solenidion 7 µm; empodium 5 µm long; empodium 3 rayed (Figure 1c). Hindleg 19 µm long; tibia 4 µm; tarsus 5 µm; tarsal solenidion 7 µm; empodium 5 µm long; empodium 3 rayed.

Genitalia 10 µm long; 13 µm wide; female genital cover flap smooth; genital setae 10 µm (Figure 1b).
Material examined: Bahçesaray, Van (38° 19' 49 N; 42° 10' 22 E; 1557m); 15.08.2015 (3♀♀), 27.07.2016 (5♀♀, 1♂)
Host Plant: *Celtis* sp. (Cannabaceae)
Geographical distribution: South Africa (Amrine & Stasny, 1994) and Turkey (present study)

Remarks: We observed that the mite produces bead-like galls. Twelve species of eriophyoid were found on the *Celtis*. Since *Aceria* species are not detected on *Celtis* spp. in the Palearctic region (Amrine & Stasny, 1994), *A. camdeboo* is also a new record for the Palearctic region.

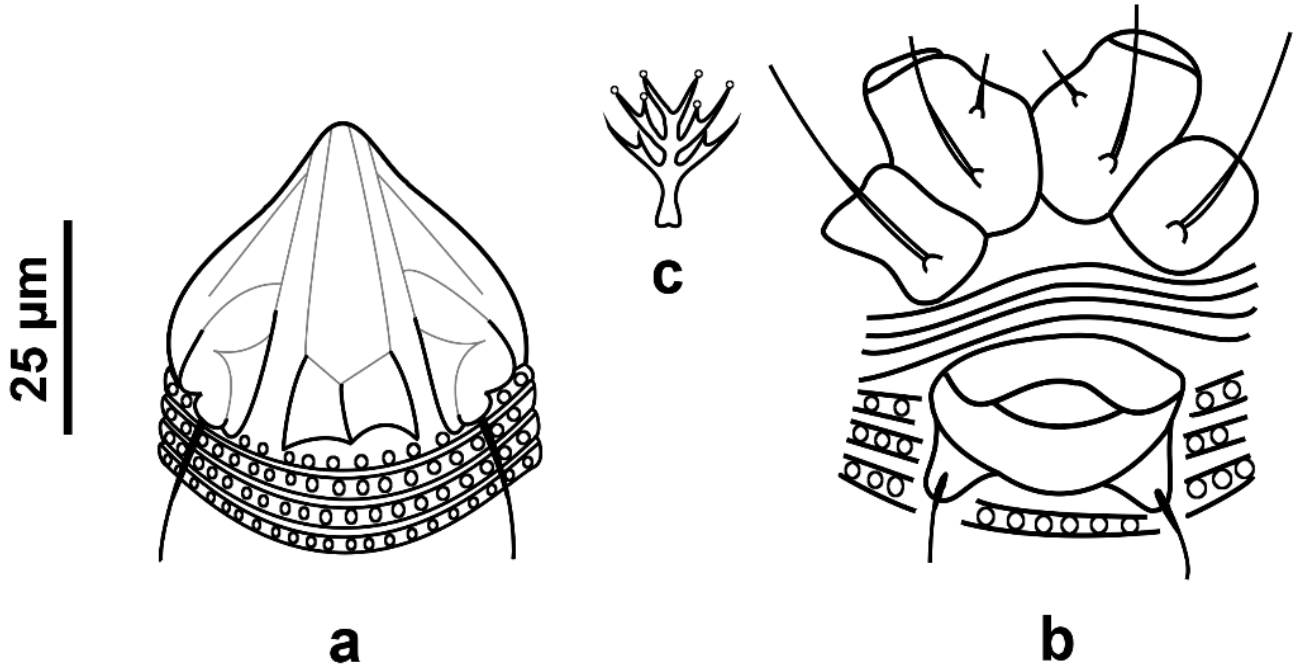

Figure 1- *Aceria camdeboo* a- Prodorsal shield, b- Genitalia, c- Empodium

***Aceria trifolii* (Nalepa, 1892)**

Synonym: *Eriophyes plicator* var. *trifolii* (Nalepa, 1892)
Female: 149–200 µm long, 56–60 µm wide; gnathosoma 9–11 µm long; gnathosomal setae 3–5; chelicerae 6–10 µm; Prodorsal shield 33–35 µm long, 32–37 µm wide; dorsal setae 10–17 µm long (Figure 2a).

Leg I. 26–35 µm long; tibia 5–6 µm; Tarsus 5–7 µm; tarsal solenidion 7–8 µm; empodium 5–6 µm long; empodium 5 rayed. Leg II. 27–32 µm long; tibia 5–7 µm; tarsus 5–7 µm; tarsal solenidion 7–8 µm; empodium 5–6 µm long; empodium 5 rayed.

Genitalia 10–13 µm long; 17–28 µm wide; female genital cover flap (8 ridges); genital setae 12–19 µm (Figure 2b).

Material examined: Bahçesaray, Van (38° 19' 49 N: 42° 10' 22 E; 1557m); 14.08.2014 (10♀♀).
Host Plant: *Vicia biennis* L. (Fabaceae)
Geographical distribution: Bosnia, France, Germany, Hungary, Italy and Turkey (present study).
Remarks: We observed that the mite caused leaf curling and bleaching. The mite was originally reported on *Trifolium arvense* L. and then *Medicago falcata* L., *M. lupulina* L., *Ononis minutissima* L., *Trifolium dubium* Sibth., *T. pratense* Sibth., *P. repens* L., *Vicia hirsuta* L. in the Palearctic region, and caused proliferation of flowers and deformation of leaves. *Vicia biennis* is recorded as a new host plant for *A. trifolii* in this study. The lengths of opisthosoma, gnathosoma and prodorsal shield, which are important features for species identification, of the Turkish specimens of *Aceria trifolii* differs slightly in the Turkish specimens from that of the type specimens. The lengths of opisthosoma and gnathosoma are longer in the Turkish specimens, 200 µm and 11 µm but 190 µm and 9 µm in the type specimens. The length of prodorsal shield in the Turkish specimens (35 µm) is narrower than in the type specimens (38 µm).

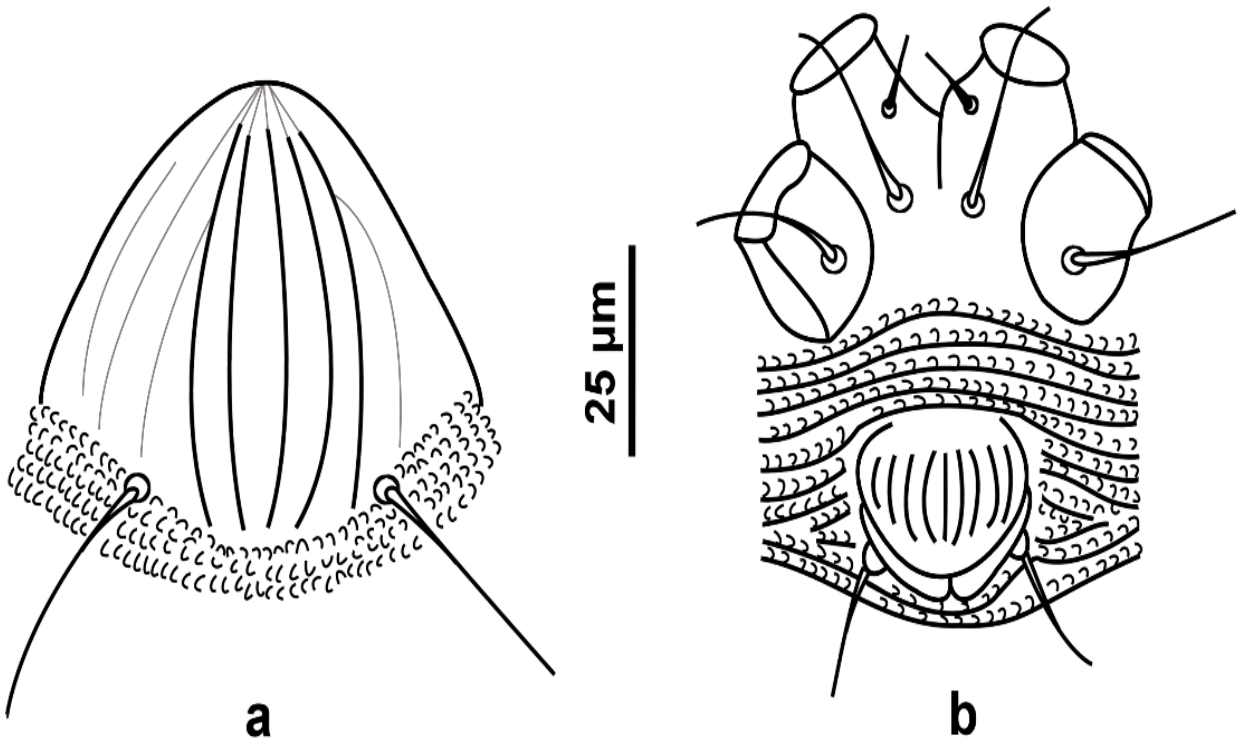

Figure 2- *Aceria trifolii* (Nalepa, 1892) a. Prodorsal shield, b. Genitalia

Subfamily: Phyllocoptinae
Tribus: Anthocoptini
Genus: *Aculus* Keifer, 1959
Aculus parakarensis (Bagdasarian, 1972)

Female: 149–158 µm long, 45-51 µm wide; gnathosoma 17-21 µm long; gnathosomal setae 2-3; Prodorsal shield smooth, 23-26 µm long, 29-33 µm wide, dorsal setae 10-14 µm long (Figure 3a).

Leg I. 24 -26 µm long; tibia 5-6 µm; tarsus 6-7 µm; empodium 8-9 µm long; empodium 5 rayed. Leg II. 22-24 µm long; tibia 4-5 µm; tarsus 5-7 µm; empodium 8–9 µm long; empodium 5 rayed (Figure 3c). Genitalia 11–14 µm long; 18–20 µm wide; female genital flap smooth; genital setae11–14 µm (Figure 3b).

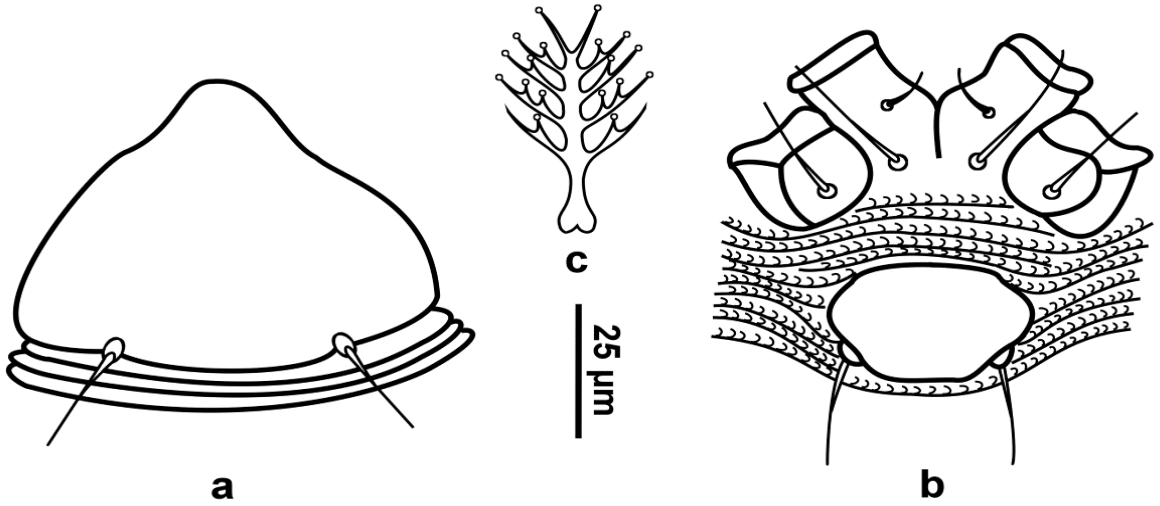

Figure 3- *Aculus parakarensis* (Bagdasarian, 1972) a- Prodorsal shield, b- Genitalia, c- Empodium

Material examined: Akdamar island, Van (38° 24' 04 N; 43° 14' 12 E; 1802m); 21.08.2014 (7 ♀♀).
Host plants: *Amygalus communis* L. (Rosaceae)
Geographical distribution: Armenia, Bulgaria, Hungary (Amrine et al. 2003) and Turkey (present study).
Remarks: This species is a vagrant on the undersurface of leaves without causing apparent damage. *Aculus parakarensis* has only been found in the Palearctic region so far, and its widespread host is *Amygalus communis* (Ripka, 2007).

Genus: *Leipothrix* Keifer, 1966
***Leipothrix moraceus* (Castagnoli, 1980)**
Female: 160–230 µm long, 59-63 µm wide; gnathosoma 7-8 µm long; gnathosomal setae 6-7; chelicerae 6-11 µm; Prodorsal shield 48-50 µm long; 53-55 µm wide; dorsal setae 11–18 µm long (Figure 4a).

Leg I. 33-36 µm long; tibia 5–6 µm; tarsus 4-5 µm; tarsal solenidion 4-5 µm; empodium 5–6 µm long; empodium 4 rayed. Leg II. 41-43 µm long; tibia 5–6 µm; tarsus 4-5 µm; tarsal solenidion 5-6 µm; empodium 5–6 µm long; empodium 4 rayed.
Genitalia 11–12 µm long; 20–22 µm wide; female genital flap smooth; genital setae 14–16 µm (Figure 4b).

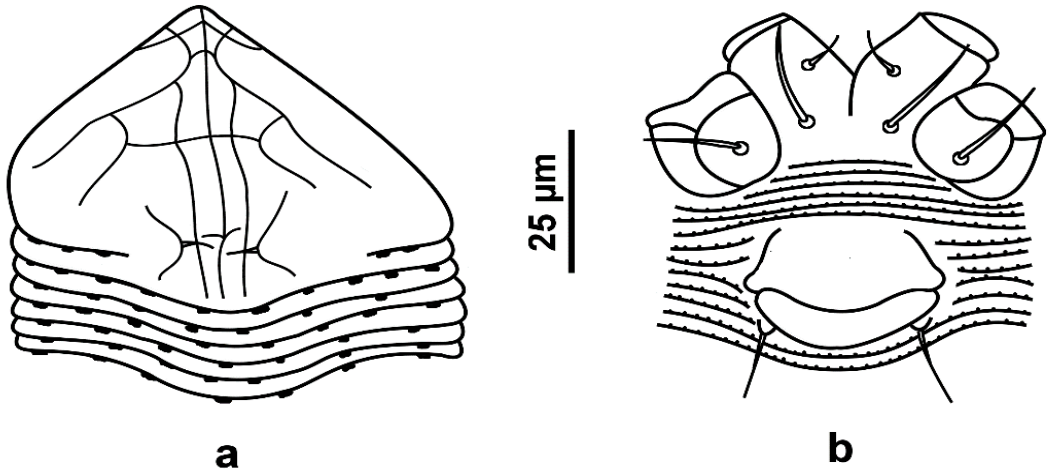

Figure 4- *Leipothrix moraceus* (Castagnoli, 1980) a- Prodorsal shield, b- Genitalia

Material examined: Edremit, Van (38° 20' 29 N: 43° 02' 09 E; 1669m); 07.07.2015 (11♀♀, 1♂).
Host Plant: *Morus alba* L. (Moraceae)
Geographical distribution: Italy (Castagnoli, 1980), China (Wang et al. 2017) and Turkey (present study).
Remarks: This species was previously found in Palearctic and Oriental regions (Amrine & Stasny, 1994). We observed that this species caused rust of leaves. The lengths of the body, prodorsal shield and tibia of the Turkish specimens of *Leipothrix moraceus* differs from that of the type specimens as follows: body length in the Turkish specimens (230 µm) is longer than in the Italian specimens (215 µm); length of prodorsal shield is also longer in the Turkish specimens (53 µm) than Italian specimens (50 µm) and the tibia in the Turkish specimens (6 µm) is slightly shorter than the that of the Italian specimens (8 µm).

Genus: *Phyllocoptes* Nalepa, 1887
Phyllocoptes obtusus (Nalepa, 1891)

Female: 150 µm long, 45 µm wide; gnathosoma 20-22 µm long; gnathosomal setae 2-3; chelicerae 12-18 µm; Prodorsal shield 38-40 µm long;11-13 µm wide; dorsal setae14-17 µm long (Figure 5a);

Leg I. 28-33 µm long; tibia 5-6 µm; Tarsus 5-6 µm; empodium 7 µm long; empodium 4 rayed. Leg II. 35-36 µm long; tibia 5-7 µm; tarsus 6-7 µm; empodium 6-7 µm long; empodium 4 rayed.

Genitalia 11-15 µm long; 22-29 µm wide; genital seta 18-20 µm (Figure 5b); female genital cover flap (9 ridges).

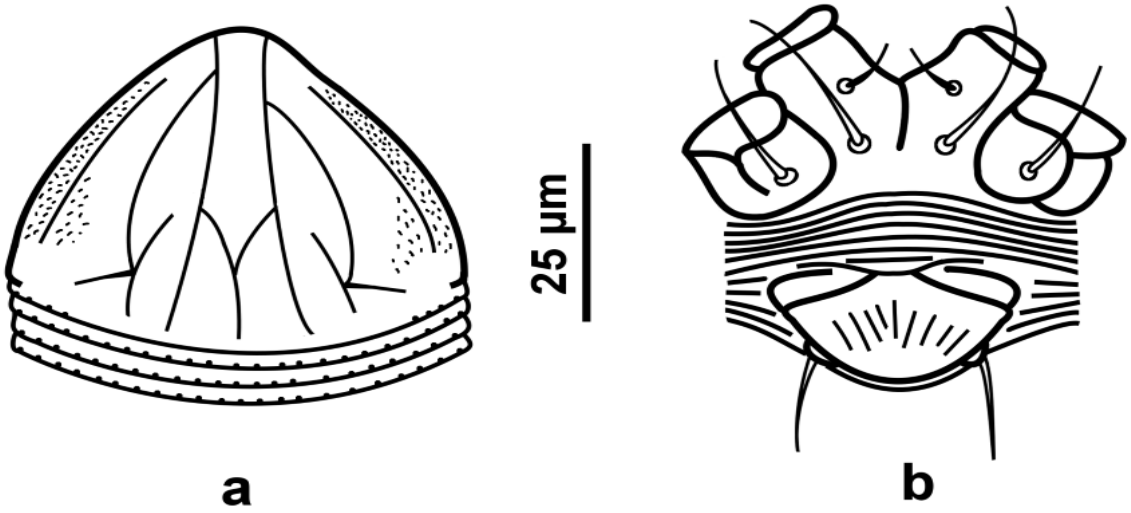

Figure 5- *Phyllocoptes obtusus* (Nalepa, 1891) a- Prodorsal shield, b- Genitalia

Material examined: İskele, Van (38° 31' 53 N; 43° 19' 40 E; 1658m); 28.06.2015 (5 ♀♀).

Host Plant: *Salvia* sp. (Lamiaceae)

Geographical distribution: Austria, Hungary, Russia, Yugoslavia (Amrine et al. 2003; Ripka, 2007) and Turkey (present study).

Remarks: This species is a vagrant on the undersurface of the leaves. There are approximately 900 species of *Salvia* genus in the world and 97 species exist in Turkey. Approximately 51 species of *Salvia* genus are endemic in Turkey (İpek & Gürbüz, 2010). It is known that eriophyoid mites are host specific and show different symptoms according to the host species (Lindquist et al. 1996).

4. Conclusions

Eriophyoids are very important phytophagous pests on crops, herbaceous plants, grasses and shrubs, and they are mostly host-specific or associated with a few hosts within a single genus or family. These mites have great potential for use as biological control agents of weeds because of their host specificity and their ability to significantly reduce the target weed's fitness (Smith et al. 2010). Knowledge of host plant specificity is necessary to develop effective control strategies and is fundamental in the application of the mites as biological control agents (Smith et al. 2010). To determine the host specificity of herbivores and to understand the role of each host species in the biology of the herbivore, information on the level of infestation of a pest on a particular host species is required (Skoracka & Dabert, 2010; Skoracka & Kuczyński, 2012).

In this study showed that, *Aceria camdeboo*, *A. trifolii*, *Phyllocoptes obtusus*, *Aculus parakarensis* and *Leipothrix moraceus* are recorded for the first time in Turkey. The influence these mites may have on the Turkish flora or their potential as weed control agents must still be determined. Future studies should continue focusing on eriophyoid mites as control agents of weeds, not ignoring their pest status.

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Pit-1 Gene Polymorphisms in Anatolian Black, Holstein Friesian, Brown Swiss and Simmental Cattle Reared in Turkey

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ABSTRACT
The aim of this study is to determine the genetic variation of the Pit 1 gene and comparison polymorphisms between four cattle breeds (Anatolian Black, Holstein Friesian, Brown Swiss, and Simmental). A total of two hundred animals were used for this purpose in four different cattle breeds, each with 50 heads. Genetic variations between breeds were identified via RFLP method by PCR. The allele frequency A and B for Anatolian Black, Holstein Friesian, Brown Swiss, and Simmental was 0.260, 0.740; 0.320, 0.680; 0.100, 0.900; 0.230, 0.770 respectively. While the genotype frequency AA, AB and BB for Anatolian Black, Holstein Friesian, Brown Swiss, and Simmental was 0.10, 0.32 and 0.62; 0.10, 0.44 and 0.46; 0.00, 0.20 and 0.80; 0.10, 0.26 and 0.64 respectively. According to the chi-square test, all breeds were found to be in Hardy–Weinberg equilibrium (P>0.05). As a result, it can be said that with a more comprehensive study that will include economic traits in these breeds, revealing association analyses would be more informative in the future.

Keywords: Pit-1, Genetic variations, Cattle breeds, Allele frequency, Genotype frequency, Hardy–Weinberg equilibrium

1. Introduction

Improvement of livestock has focused on the selective breeding of individuals with superior phenotypes. Most economic traits generally controlled by a large number of genes in animals which are largely influenced from the genotype and environment (Williams 2005). It seems to be difficult to determine the best genotypes carrying alleles by taking into account the phenotypic values of animals in quantitative characters (Aytekin & Boztepe 2013). More recently, new technologies and methods such as QTL and candidate gene approach have emerged to determining the characteristics of these genes. One of the candidate genes that affect the production of milk, protein quality, protein content, body fat percentages, immunity, growth and development traits in cattle is Pit-1 gene.

Pit-1 gene, known as POU1F1, is a pituitary-specific transcription factor responsible for pituitary development and hormone expression in mammals (Cohen et al. 1996). It is an essential part of the body development process. It activates growth hormone gene, prolactin activator, Thyroid-stimulating hormone and receptor of hormone which is releasing growth hormone (Pytlewski et al. 2018). The bovine Pit-1 gene is located in chromosome 1 (BTA1) and consists 6 exons and 5 introns. Pit-1 gene coding for a protein consisting of 129 amino acids (33 kDa) with DNA-binding POU domain (Moody et al. 1995; Thuy et al. 2018). The pit-1 gene is one of the strong candidate genes which associated with body weight, average daily gains, milk production and reproduction traits in cattle (Chauhan et al. 2015; Moravcíková et al. 2013; Thuy et al. 2018). Woollard et al. (1994) firstly identified *Hinf*I polymorphism of bovine Pit-1 gene by RFLP method. Molecular basis of this polymorphism was the silent mutation (G→A) located within the exon 6 of the Pit-1 gene (Moravcíková et al. 2013).

The aim of this study is to determine the genetic variation of the Pit 1 gene and to compare polymorphism between Anatolian Black, Holstein Friesian, Brown Swiss, and Simmental cattle breeds.

2. Material and Methods

Whole blood samples were collected from Konya city for Holstein Friesian and Brown Swiss, Kütahya for Simmental cattle and Ankara city for Anatolian Black cattle. Ethical approval was given by the Faculty of Veterinary Medicine ethical committee (No:2021/125). The genetic analyses were performed in Animal Science Biotechnology Laboratory, Faculty of Agriculture, Selcuk University. A total of 200 heads of animals, 50 heads of each breed were used in the study. EDTA-containing tubes were

used to prevent blood clotting during sample collection. Blood samples were stored at 20 °C. Blood samples were taken from the tail vein of cattle. Genomic DNA was extracted from whole blood using the Quick Gene DNA whole blood kit S (DB-S) (KURABO, Japan). The primer sequences and PCR conditions are given in Table 1. The PCR was achieved in a reaction volume of 10 µL containing 1 µL DNA, 5 µL of 2X Dream *Taq* Green PCR Master Mix (Thermo Scientific, USA), 0.30µL for each primer (10 µ mol) (Macrogen, Turkey) and 3.4 µL distilled water. PCR products were digested with fast digest enzyme (Thermo Scientific) which containing 5 µL PCR product, 8.5 µL distilled water, 1 µL 10X buffer and 0.5 µL restriction enzyme (total of 15 µL). Digestion products were separated on 3% agarose gel at 85 V for 50 min, in 0.5X TBE buffer stained by ethidium bromide with used 100bp plus DNA marker (Vivantis, Malaysia). The results were checked under ultraviolet lights.

The Chi-square test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium was carried out by using PopGene32 ver. 1.32 (Yeh et al. 1997).

Table 1- The primer sequence, fragment sizes, PCR condition and restriction enzyme for Pit-1 gene

Gene	*Primer sequence*	*Base pair*	*Reference*	*PCR conditions*	*Restriction Enzyme*
Pit-1	5′- AAACCATCATCTCCCTTCTT-3′ 5′- AATGTACAATGTGCCTTCTGAG-3′	451	Woollard et al. (1994)	95 °C 10m, 95 °C 30s, 57.1 °C 1m, 72 °C 2 m, 35 cycles 72 °C 10m	*Hinf*I

3. Results

A 451 bp region of intron 5-exon 6 of the Pit-1 gene was amplified. The polymorphism was observed after products were digested with *Hinf*I enzyme. Digestion of the PCR fragment of Pit-1 with *Hinf*I resulted in fragment lengths of 451 bp for AA; 451, 244, 207 bp for AB and 244, 207 for BB (Figure 1). The allele and genotype frequency for four cattle breeds are given in Table 2.

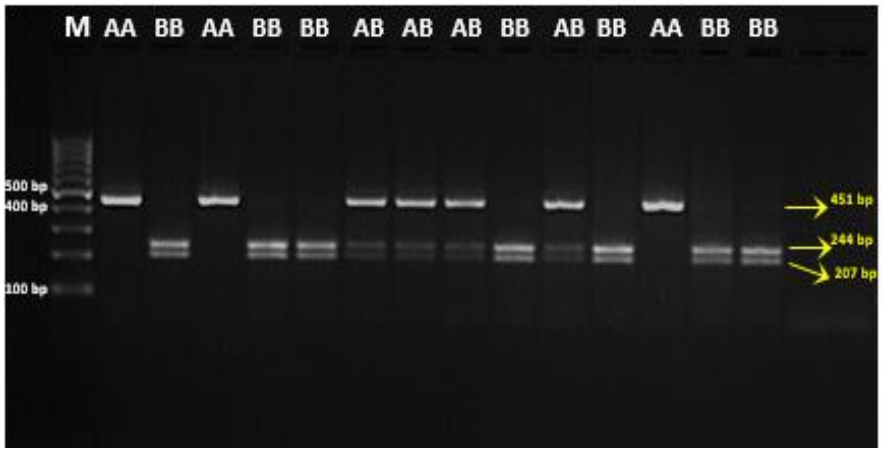

Figure 1- Agarose gel electrophoresis of digested products of Pit-1 gene with *Hinf*I restriction enzyme in cattle; M: 100bp Plus DNA Ladder (Vivantis Technologies), AA: 451 bp, BB: 244 and 207 bp and AB: 451, 244 and 207 bp

Table 2- The genotype and allele frequencies of Pit-1/HinfI polymorphism in four cattle breeds

Breeds	*N*	*Genotype frequencies*			*Allele frequencies*		
		AA	*AB*	*BB*	*A*	*B*	*χ2*
AB	50	0.100	0.320	0.620	0.260	0.740	1.800 (P>0.05)
HF	50	0.100	0.440	0.460	0.320	0.680	0.006 (P>0.05)
BS	50	0.000	0.200	0.800	0.100	0.900	0.617 (P>0.05)
SIM	50	0.100	0.260	0.640	0.230	0.770	3.536 (P>0.05)

AB: Anatolian Black; HF: Holstein Friesian; BS: Brown Swiss; SIM: Simmental; P>0.05: in Hardy-Weinberg equilibrium

It is found that AA genotype frequency in Anatolian Black, Holstein-Friesian and Simmental is similar. In contrast AA genotype frequency of Brown Swiss was showed a difference from other cattle breeds. The highest A allele frequency was found in Holstein-Friesian breed as 0.320, while the highest B allele frequency was found as 0.900 in Brown Swiss breed. According to allele frequency, the most variation (heterozygosity) is genetically in HF (0.44), but the least BS (0.18).

4. Discussion

Pit-1 is an important candidate gene that associated with milk yield and growth traits. Pit-1 includes the two most frequent alleles A and B. It assumed that the gene that carrying the A allele is associated with milk yield and that carrying the B allele with growth traits and fat percentage. In this study, the A allele frequency in Anatolian Black, Holstein Friesian, Brown Swiss and Simmental determined as 0.260, 0.320, 0.100 and 0.230 respectively. On other hand the B allele frequency determined as 0.740, 0.680, 0.900 and 0.770 respectively. The results revealed that the B allele frequency is higher than A frequency in four cattle breeds. These results are in agreement with most of the demonstrated previous studies as detailed below. B allele frequency in various cattle breeds was as follows; 0.81 in Italian Holstein bulls (Renaville et al. 1997), 0.84 in Holstein (Hori-Oshima & Barreras-Serrano 2003), 0.75 Poland Black cattle (Dybus et al. 2004), 0.76 in Qinchuan and 0.868 in China Holstein (Yan et al. 2006), 0.72 in Sarabi (Zakizadeh et al. 2007), 0.78 in Simmental (Coşier et al. 2007), 0.87 in Charolais (Carrijo et al. 2008), 0.81 in Limousin and 0.82 in Angus (Zhang et al. 2009), 0.91 in Jordan native cattle (Jawasreh et al. 2009), 0.90 in Romanian Black cattle (Carsai et al. 2012), 0.98 in Bali (Jakaria and Noor 2015), 0.99 in Grati-Ongole Grade (Hartati et al. 2018). A allele frequency in various cattle breeds was as follows: in Italian Holstein-Friesian Bulls 0.188 (Renaville et al. 1997), in Holstein cows 0.170 (Heidari et al. 2012), in East Anatolian Red 0.41 (Özdemir 2012), in Romanian Black and White and Romanian Grey Steppe 0.100 and 0.250 (Carsai et al. 2012), in Slovak Spotted cattle 0.29 (Moravcíková et al. 2013), in Bali Cattle 0.018 (Jakaria and Noor 2015), in Sahiwal cattle 0.194 (Chauhan et al. 2015), in Holstein 0.253 (Yasemin et al. 2017), in Holstein 0.32 (Bayram et al. 2017), in Holstein Frisian dairy cows bred in Vietnam 0.216 (Thuy et al. 2018).

Briefly, it can be seen in the Table 3, the A allele frequency was generally found to be less than the B allele in previous studies in different breeds for Pit-1 gene. A similar tendency has also been expressed by Aytekin and Boztepe (2013) as a result of the relationship between Pit1-*Hinf*I polymorphism and milk production traits, it can be suggested that A allele and AA genotype are exploited for selection of dairy traits. The present investigation may provide additional base data for future genetic assessments of these breeds.

Table 3- Statements of the literature on the Pit-1 polymorphisms

References	*bp*	*Breeds*	*N*	*Genotype frequencies*			*Allel frequencies*	
				AA	*AB*	*BB*	*A*	*B*
Renaville et al. (1997)	451	Italian Holstein-Friesian bulls	89	0.022	0.315	0.553	0.188	0.812
	451	Belgian Blue	350	0.200	0.445	0.355	0.53 0.423*	0.47 0.577*
Hori-Oshima and Barreras-Serrano (2003)	451	Holstein	196	0.026	0.257	0.717	0.155	0.845
Oprządek and Flisikowski (2003)	451	Black-and- White bulls	144	0.063*	0.368*	0.569*	0.247	0.753
Zhao et al. (2004)	451	Angus beef cattle	416	0.111	0.440	0.450	0.331*	0.669*
Mattos et al. (2004)	1.355	Gry bulls	40	0.900	0.100	0.000	0.95	0.05
Dybus et al. (2004)	451	Poland Black-and-White cows	900	0.052	0.382	0.566	0.243	0.757
Vargas et al. (2004)	451	Holstein-Friesian	46	0.10	0.35	0.55	0.283*	0.717*
Javanmard et al. (2005)	600	Sarabi	82	0.451	0.341	0.207	0.622	0.378
		Golpayegani	57	0.614	0.263	0.123	0.746+	0.254
		Sistani	38	0.842	0.158	0.000	0.921	0.079+
		Taleshi	70	0.614	0.314	0.071	0.771	0.229
		Manzadrani	26	0.692	0.269	0.038	0.827	0.173
		Dashtiyari	8	0.625	0.000	0.375	0.625	0.375
		Golpayegani x Brown Swiss F_1	13	0.000	0.769	0.231	0.385	0.615
Kai et al. (2006)	451	Nanyang	100	0.210	0.510	0.280	0.465	0.535
Zakizadeh et al. (2007)	451	Manzadrani	96	0.167*	0.406*	0.427*	0.370	0.630
		Sarabi	84	0.083*	0.381*	0.536*	0.274	0.726
		Golpayegani	110	0.109*	0.455*	0.436*	0.336	0.664
		Holstein	111	0.059*	0.297*	0.644*	0.208	0.792
Coşier et al. (2007)	1350	Simmental	76	0.118	0.197	0.685	0.217	0.783
Carrijo et al. (2008)	1301	Charolais	232	-	-	-	0.13	0.87
		Nelore	277	-	-	-	0.27	0.73

Table 3 (Continued) - Statements of the literature on the Pit-1 polymorphisms

References	*bp*	*Breeds*	*N*	*Genotype frequencies*			*Allel frequencies*	
				AA	*AB*	*BB*	*A*	*B*
Mukesh et al. (2008)	1350	Indian native cattle (*Bos indicus*)	723	0.002	0.119	0.881	0.063	0.937
Edriss et al. (2008)	451	Holstein cows (four herds)	262	0.031	0.450	0.519	0.256	0.744
Zhang et al. (2009)	451	Qinchuan	67	0.030	0.403	0.537	0.232	0.768
		Limousin x Qinchuan	47	0.043	0.277	0.681	0.181	0.819
		Angus x Qinchuan	36	0.111	0.444	0.444	0.333	0.667
		Germany Yellow x Qinchuan	42	0.071	0.214	0.714	0.178	0.822
Jawasreh et al. (2009)	422	Jordan native cattle	36	0.000	0.176	0.8235	0.088	0.912
		Holstein-Friesian	45	0.046	0.255	0.697	0.174	0.826
Misrianti et al. (2010)	611	Holstein-Friesian	45	0.022	0.444	0.533	0.244^{+}	0756^{+}
Biranvand et al. (2010)	451	Najdi	84	0.0357	0.2976	0.6666	0.1845	0.8155
Özdemir (2012)	260	Eastern Anatolian Red	71	0.14	0.54	0.32	0.41	0.59
		Holstein	181	0.04	0.31	0.65	0.20	0.80
Carsai et al. (2012)	451	Romanian Black and White cattle/ high milk production individuals	60	0	0.182	0.818	0.091	0.909
		Romanian Black and White cattle/ low milk production individuals	60	0	0.200	0.800	0.100	0.900
		Romanian Grey Steppe	60	0	0.500	0.500	0.250	0.750
Heidari et al. (2012)	1355	Holstein	100	-	-	-	0.170	0.830
Aytekin and Boztepe (2013)	451	Brown Swiss	301	0.12	0.51	0.37	0.374	0.626
Moravcíková et al. (2013)	260	Slovak Spotted cattle	110	0.087	0.417	0.496	0.2955	0.7045
Jakaria and Noor (2015)	451	Bali	245	0.00	0.04	0.96	0.018	0.982
		Madura	68	0.00	0.07	0.93	0.037	0.963
		Pesirir	100	0.01	0.13	0.86	0.075	0.925
		Aceh	25	0.00	0.08	0.92	0.040	0.960
		Katingah	50	0.00	0.10	0.90	0.050	0.950
Chauhan et al. (2015)	600	Sahiwal cattle	77	0.0389	0.3116	0.6493	0.1948	0.8051
Ahmadi et al. (2015)	611	Holstein	57	0.35	0.36	0.59	0.22	0.78
Ebrahimi Hoseinzadeh et al. (2015)	451	Holstein	100	0.06	0.40	0.54	0.26	0.74
Trakovická et al. (2015)	260	Slovak Simmental	288	0.052	0.347	0.600	0.226	0.774
Yasemin et al. (2017)	447	Holstein	146	0.548	0.3973	0.5479	0.2534	0.7466
Bayram et al. (2017)	600	Holstein	350	0.176	0.286	0.536	0.32	0.68
Hartati et al. (2018)	1301	Grati-Ongole Grade	107	0.000	0.009	0.991	0.005	0.995
Thuy et al. (2018)	451	Holstein	125	0.080	0.272	0.648	0.216	0.784
Gökcan (2019)	451	Holstein	52	0.019	0.231	0.750	0.135	0.865

N: observed number; *: Values calculated from allele frequencies and $^{+}$corrected values

5. Conclusions

The present investigation can be used as an indication for improvement of economically traits in the dairy cattle and for determination of the status of these four breeds reared in Turkey in addition studies are needed to perform an association researches with economically traits.

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Cooling Potential of Bin Stored Wheat by Summer and Autumn Aeration

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ABSTRACT

A one-dimensional mathematical model based on the formulation of mass and energy balance in stored grain was used to simulate grains storage conditions. The objective of such simulations was to produce grain ventilation strategies. The model was validated using data obtained from the monitoring of wheat stored in a galvanized steel cylindrical tank with corrugated conical bottom ventilated by perforated distribution pipes. A control strategy based on night time aeration from July to November followed by day time aeration for December to January was applied. Good agreement between the predicted and measured storage conditions has been observed (R^2= 0.9698, S.E.= 1.479 °C in average temperature and R^2= 0.99, S.E.= 0.00079 kg $kg^{-1}$for moisture content). Night time grain aeration provided sufficient cooling (temperature near 10 °C in November). However, an 18% grain humidification process was induced. Day time aeration started at the end of November corrected this humidification effect for a grain temperature of 15 °C and a grain moisture content of 15% on dry basis.

Keywords: Heat and mass transfer, Computer simulation, Grain storage, Mathematical modelling, Warm climate, Tunisia

1. Introduction

Cereal harvesting in Tunisia usually begins by mid-June, when grain temperature exceeds 25 °C. Bin storage exposes cereals to a range of complex ecological factors which, if improperly managed, can lead to important economic losses for the Tunisian grain industry. The main management factors are storage temperature and moisture content. Storage temperature is important because it directly affects grain storage quality through pest development and matter losses (Muir 1970; Sun & Woods 1994; Maier et al. 1996). The moisture content controls the development of fungi, and the growth of insects and mites populations (Longstaff 1994). Proper grain storage requires preventive management strategies rather than the application of solutions as problems occur. Since aeration is capable of controlling grain temperature and moisture, it is the most common preventive technique against grain deterioration agents. Aeration consists in forcing ambient air through the grain mass to remove respiration heat and moisture, when the ambient air enthalpy and moisture content is appropriate. Aeration is challenging in tropical and subtropical climates, where ambient air temperature and/or relative humidity is too high to achieve proper grain curing, especially during the critical storage period such as from July to December in Tunisia. Actually, aeration is not a common management practice for grain storage in Tunisia, simply because of generally high ambient temperatures during the early storage grain period. The main method for grain preservation relies on pesticide applications to control insect growth and bin-to-bin transfer to reduce grain temperature. The potential use of aeration to cool stored grains for their preservation has not been thoroughly evaluated in Tunisia. Usually, during the Tunisian summer and fall, the ambient air is characterized by a high daytime temperature and low relative humidity, and a lower night temperature with a higher relative humidity. The potential use of ambient air aeration to protect stored grain masses against deterioration warrants investigation.

Accurate prediction of stored grain ecosystem helps to develop and evaluate ventilation strategies adapted to the climatic characteristics of the storage region. Methods of predicting temperature and grain moisture content can be used to evaluate the effectiveness of aeration and estimate if chemical treatments are required to fight insects, mites and fungi. However, the testing of various aeration strategies to establish best practices to preserve stored grain masses is expensive since it requires the monitoring of temperature and humidity at several points in a large number of grain bins, and for several seasons.

Simulation models can economically be used to predict the temperature and moisture of stored grain, under different climatic conditions and aeration strategies. Generally, these models are used to evaluate the efficacy of ambient air aeration, to estimate the maximum safe storage period for grain and to predict the required aeration time. The outputs of simulation models

allow for the analysis of stored grain aeration viability for a specific region. Many mathematical models are available to simulate the heat and the mass transfers in aerated bulk stored grains. (Jia et al. 2001; Thorpe 2001; Andrade et al. 2002 ; Iguaz et al. 2004)

Generally, these models are based on energy and mass balances. In some cases, it is also possible to estimate the temperature distribution, the moisture content and the time required to cool a grain mass.

Some authors have developed control strategies for grain aeration systems. Using a software package called AERO (de Carvalho Lopes et al. 2008). de Carvalho Lopes et al. (2008) implemented a control strategy that relates four conditions (C1, C2, C3 and C4): C1 pertains to the dew point; C2 is evaluated by AERO 1 and AERO 2 to maintain a safe grain moisture content (de Carvalho Lopes et al. 2008), and on recommendations presented by Navarro and Noyes (Navarro & Noyes 2001). The control strategy aimed at providing low and uniform grain mass temperatures and to maintain a safe grain moisture content. The simulation process based on the ambient data (temperature and humidity) and the measured storage conditions of the grain predict the aerated grain temperature and moisture content. By comparing the simulated and measured stored grain conditions, the fan air flow rate was either turned on, turned off or maintained in its previous state.

The four conditions set by the AERO control strategy (de Carvalho Lopes et al. 2008) to limit the fan operation time could be applied to Tunisian climatic conditions. In fact, during night time, the aeration will usually be limited by condition C2 because of the high air relative humidity and during daytime, the 4 conditions (C3 or C4 and C1 and C2) could be met to turn on the aeration then a grain heating effect can be observed.

Other authors have developed control strategies based on historical weather data to determine the number of hours during which ambient air temperatures are below a reference level. Based on initial grain mass storage conditions, this temperature is closely related to the storage period and to the lower limits for insect development (Frank & Arthur et al. 2003; Arthur & Casada 2005) . In Tunisia, this control strategy is challenged by the low cooling potential of daytime air temperatures and its negative effect on grain humidity because of higher night time relative humidity.

Most of the modelling work was specifically developed to predict the temperature evolution of grain masses. Subtropical climates as that of Tunisia provide a limited time for the cooling of grain masses by aeration, which directs the investigation toward night time ventilation performance. In Tunisia, aeration can cool the grain mass during lower night temperatures and limit microorganism growth, but under close monitoring of grain moisture content. Aeration with air with high relative humidity can lead to a gain in grain moisture promoting microorganism growth. Usually, microorganism development is strongly linked to both grain temperature and moisture content.

Accordingly, this general study aims at developing strategies for the aeration of grains in storage under Tunisian climatic conditions, mainly cooling under grain humidification control. The objective of this study was to evaluate the effectiveness of aeration during summer and fall, to cool bin stored wheat under the typical Tunisian conditions. This objective was achieved by 1) Using a model, predicting grain temperature and moisture under bin storage; 2) Validating the simulation results with experimental data; 3) Identifying aeration conditions which can preserve wheat in storage from July to December.

2. Material and Methods

2.1. Heat and mass transfer model

To describe the evolution of wheat temperature and moisture, aerated with ambient air, a model was developed based on the mathematical formulation of heat and mass balance transfer for a control cylindrical volume.

To develop balance equations, the following assumptions were adopted to simplify the simulation:

1. Heat and mass transfer through the walls of the storage bin are neglected;
2. The heat flux produced by the storage insects and microorganisms is neglected;
3. The ventilation air flow is assumed unidirectional and parallel to the vertical wall of the bin;
4. The air distribution is assumed uniform and isotropic, and;
5. Heat and mass transfer are assumed to occur between the grain and the interstitial air.

The control volume (Figure 1) has a height of Δz and a circular cross section area A.

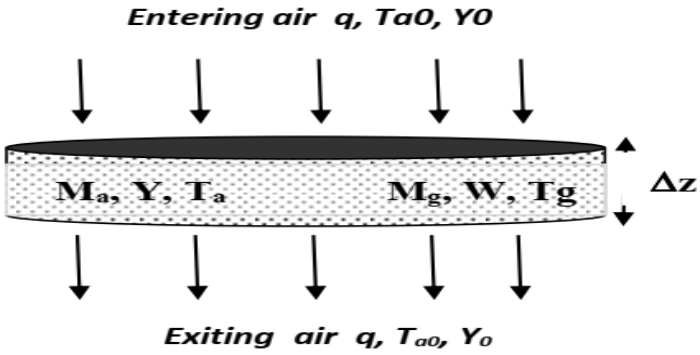

Figure 1- Modelled aeration air flow through the control volume

The mass balance for the moisture of the grain in each control volume (Figure 1) on a dry basis is described by Equation (1).

$$\frac{\partial Y}{\partial t} = \frac{v}{\varepsilon \Delta z}(Y_0 - Y) - \frac{(1-\varepsilon)\rho_g}{\varepsilon \rho_a}\frac{\partial W}{\partial t} \quad (1)$$

Where; Y, is the moisture content of the air leaving the control volume (kg kg^{-1})on a dry basis; Y_0, is the moisture content of the ambient air entering the control volume(kg kg^{-1}) on a dry basis;v , the aeration air velocity (m s^{-1}); ε , the grain porosity (decimal); Δz is the section height (m);ρ_a, the density of interstitial air (kg m^{-3});ρ_g,the bulk density of the grain (kg m^{-3}) and w is the grain moisture content (%) on a dry basis.

The heat balance in the grain mass control volume is described by Equation (2).

$$\frac{\partial[M_g c_g T_g]}{\partial t} = h_V V(T_a - T_g) + \frac{\partial w}{\partial t} M_g h_{vap} - \frac{\partial w}{\partial t} M_g c_v (T_a - T_g) \quad (2)$$

Where;T_g, is the grain temperature (°C);M_g, is the grain mass in the control volume (kg) on a dry basis; Ta, is the temperature of the aeration air in the control volume (°C); c_g, the specific heat of dry grain (J $kg^{-1}K^{-1}$); c_v, is the specific heat of water (J $kg^{-1}K^{-1}$);h_v, is the grain volume convective heat transfer coefficient (W $m^{-3}K^{-1}$) and h_{vap} is the latent heat of water vaporization (J kg^{-1}).

The left-hand side of Equation (2) was determined as a numerical derivation over time as described by Equation (3).

$$\frac{\partial[M_g c_g T_g]}{\partial t} = M_g\left[c_g \frac{\partial T_g}{\partial t} + T_g \frac{\partial c_g}{\partial t} + c_g \frac{\partial T_g}{\partial t} + c_g T_g \frac{\partial w}{\partial t}\right] \quad (3)$$

The grain mass in the control volume is described by Equation (4).

$$M_g = (1-\varepsilon)\rho_g A \Delta z \quad (4)$$

Combining Equations (2), (3) and (4), the following Equation (5) represents the time partial derivative expression of grain temperature as described by Equation (5).

$$\frac{\partial T_g}{\partial t} = \frac{h_v(T_a - T_g)}{(1-\varepsilon)\rho_g c_g} + \frac{1}{c_g}\frac{\partial W}{\partial t} h_{vap} - \frac{c_v}{c_g}(T_a - T_g)\frac{\partial W}{\partial t} - \frac{T_g}{c_g}\frac{\partial c_g}{\partial t} - T_g \frac{\partial W}{\partial t} \quad (5)$$

The heat balance in the interstitial air of the control volume is described byEquation (6).

$$\frac{\partial[M_a c_a T_a]}{\partial t} = G_a(T_{a0} c_{a0} - T_a c_a) + M_g \frac{\partial W}{\partial t} h_{vap} - h_V V(T_a - T_g) + c_v(T_a - T_g) M_g \frac{\partial W}{\partial t} \quad (6)$$

Where; T_{a0}, is the temperature of the aeration air entering the control volume (°C); M_a, is the air mass in the control volume; c_a, is the specific heat of air in the control volume (J $kg^{-1}K^{-1}$);c_{a0}, is the specific heat of air entering the control volume (J $kg^{-1}K^{-1}$) and G_a is the air mass flow rate (kg s^{-1}).

The left-hand side of Equation (6) was determined as a numerical derivation over time in the form of Equation (7).

$$\frac{\partial[M_a c_a T_a]}{\partial t} = M_a\left(c_a \frac{\partial T_a}{\partial t} + T_a \frac{\partial C_a}{\partial t} + c_a T_a \frac{\partial y}{\partial t}\right) \quad (7)$$

The air mass in the control volume is described by Equation (8).

$$M_a = \varepsilon \rho_a A \Delta z \quad (8)$$

The air mass flow rate, Ga, through the interstitial space of the control volume is described by Equation (9).

$$G_a = \rho_a A\, v \quad (9)$$

Combining Equations (7), (8) and (9), the time partial derivative expression of grain temperature is described by Equation (10).

$$\left(\frac{\partial T_a}{\partial t}\right) = \frac{1}{\varepsilon \rho_a \Delta z C_a}\left[\rho_a v \left(T_{a0} C_{a\,0} - C_a\, T_a\right) + (1-\varepsilon)\rho_g \Delta z \left[\frac{\partial W}{\partial t} h_{vap} + C_v\left(T_a - T_g\right)\frac{\partial W}{\partial t}\right] - h_v A \Delta z \left(T_a - T_g\right)\right] - T_a\left(\frac{1}{C_a}\frac{\partial C_a}{\partial t} + \frac{\partial Y}{\partial t}\right) \quad (10)$$

The heat transfer between the aeration air and grain is calculated based on the volume or surface heat transfer coefficient. Bala& Woods (1984)proposed the following expression for the global volumetric heat transfer coefficient estimated by Equation(11).

$$h_v = 49{,}32\ q^{\mathbf{0.6}} \quad (11)$$

Where; q, is the surface air mass flow rate ($kgm^{-2}\ s^{-1}$).

With a maximum error of 0.02% in the temperature range from 0 to 50 °C, the latent heat of vaporization of water is presented by Cengel and Boles, according to Thorpe (2001):

$$h_{vap} = 2501330 - 2363Ta \quad (12)$$

Brookeret al. (1992) reported that the specific heat of grain changes according to the grain moisture content variation:

$$C_g = 1000(a + b\ w) \quad (13)$$

The density of intergranular air was calculated by using Equation (14), presented by (Chung & Pfost, 1967), in order to consider the altitude effects on this parameter.

$$\rho_a = \frac{325.8 P_a}{101.325(T_a + 273.15)} \quad (14)$$

Where; Ta, is the aeration air temperature (°C) and Pa is the barometric pressure (kPa).

The grain drying rate, $\frac{\partial W}{\partial t}$, described by Equation (15) (Iguaz et al. 2003) represents the time variation of grain moisture content and was found to satisfactory predict the drying kinetic of grain.

$$\frac{\partial W}{\partial t} = -K(w - W_e) \quad (15)$$

Related to air temperature by Equation(16), K is the drying constant in s^{-1} (Menzies & O'Callaghan, 1971).

$$K = 2000 e^{-\frac{5094}{273.15 + T_a}} \quad (16)$$

W_e, is the grain equilibrium moisture content in kg kg^{-1} [dry basis] calculated using the Chung&Pfost (1967) equation (Equation17), which was approved by the ASAE (Brooker et al. 1992).

$$W_e = -\frac{1}{B}\ln\left(-\frac{T_a + C}{A}\log r\right) \quad (17)$$

Where; A, B and C are constant values that depend on the stored product (Table 1) and r is the relative humidity of the aeration air, %.

The partial differential equations (1), (5) and (10) that describe the heat and mass transfer in the bulk stored grains of the control volume were programmed using MATLAB (R2013b). The ODE solver 15 s was used to solve the differential equations with an absolute tolerance of 10^{-6} because the temperature and the relative humidity of the aeration air vary arbitrarily with time.

2.2.Experimental device

To validate the simulation model, an aeration experiment was conducted on wheat stored in a bin at the Medjezel Bab station in central Tunisia (Ecole Supérieure des Ingénieurs de l'Equipement Rural at the University of Jendouba, Tunisia). The circular storage bin was of corrugated galvanized steel with a conical bottom, equipped with perforate air distribution ducts. The bin dimensions and the location of the grain temperature sensors are shown in Figure 2. Three temperature sensors were connected to a Duoline Manager Data acquisition system.

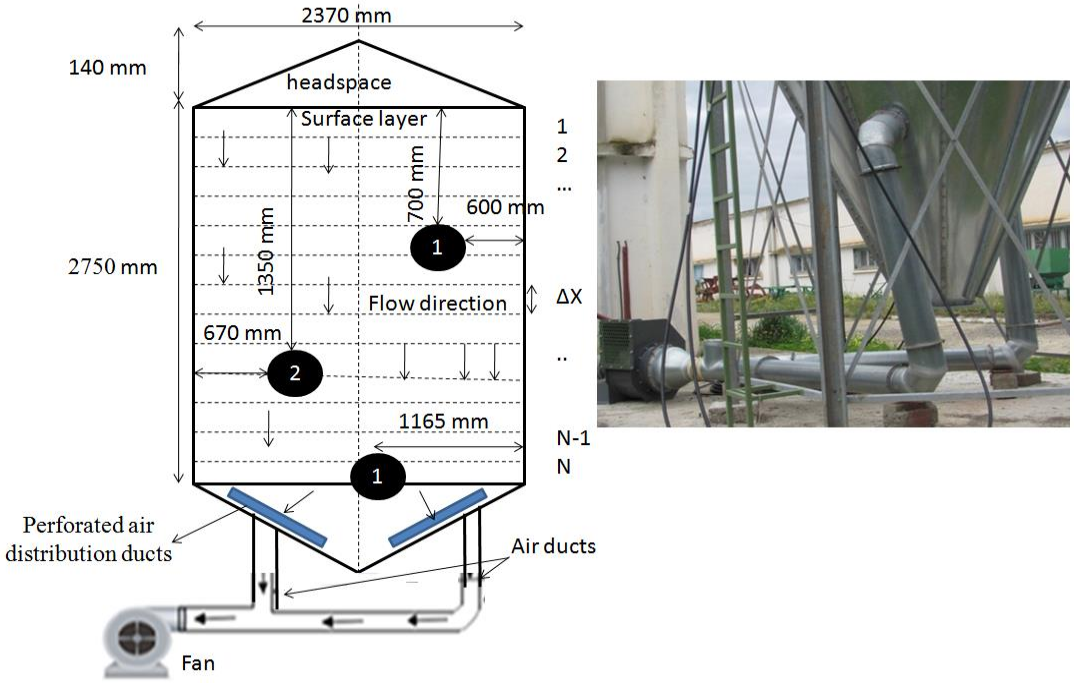

Figure 2- Pictures and diagram of the experimental bin

Bin temperatures data were recorded every 60 min using probes with an accuracy of ± 0.1 °C. Aeration was achieved by mechanically sucking ambient air through the grain mass (downward airflow) when the cooling process was possible.

The main objective of this study was to verify the cooling effectiveness of summer and fall aeration for stored wheat under typical Tunisian climatic conditions. Thus, the weather data was obtained from the closest weather recording station, located in Beja. The daily favourable aeration period was selected from a monthly calculation of hourly averages of air psychometrics parameters (temperature and relative humidity). The selection was based on the analysis of historical available data for period with no missing values of hourly temperature and relative humidity for 2010, 2011 and 2012 years, from August 1 to January 10.A monthly hourly average of psychometric parameters of the air (temperature and relative humidity) was calculated using equation (18).

$$.X_{ij} = \frac{1}{n}\sum_{j=1}^{n} x_{ij} \quad 0 \leq i \leq 23 \tag{18}$$

Where;X_{ij} , is the monthly hourly average of psychometrics parameters for ambient air;x_{ij}, is the hourly parameters of the air (temperature and relative humidity); n, is the number of days of the month and i is the hour time indicator of the day.

Figure 3-4 shows the evolution of hourly monthly average air temperature and relative humidity. For 2010 to 2012, air temperature and air relative humidity evolve in a sinusoidal wave but in opposite phase.

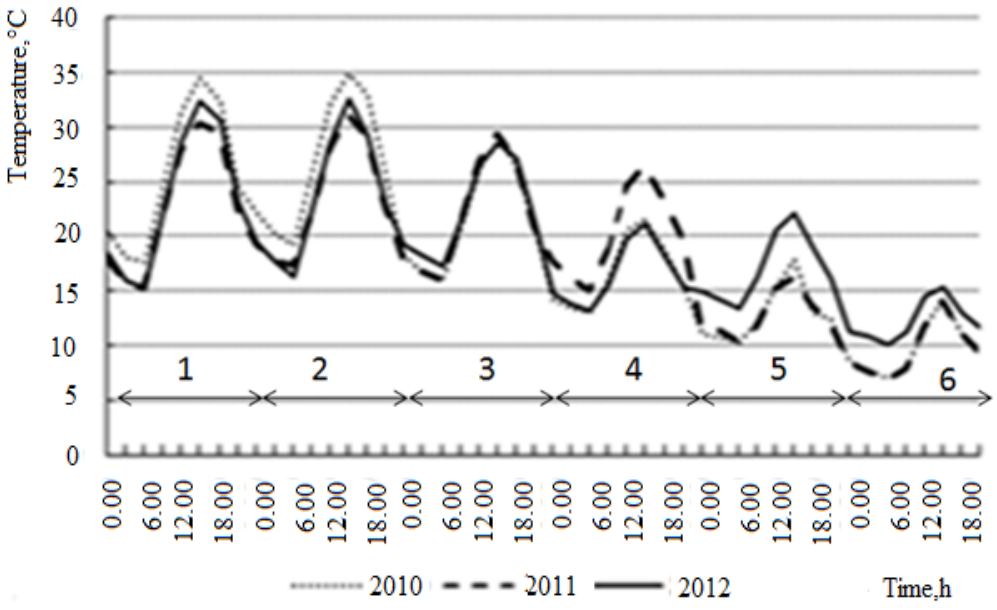

Figure 3-Average monthly hours of ambient temperature at the Beja weather station during the period of 1st August to 10th January (1: August, 2: September, 3: October, 4: November, 5: December, 6: December 31 to January 10)

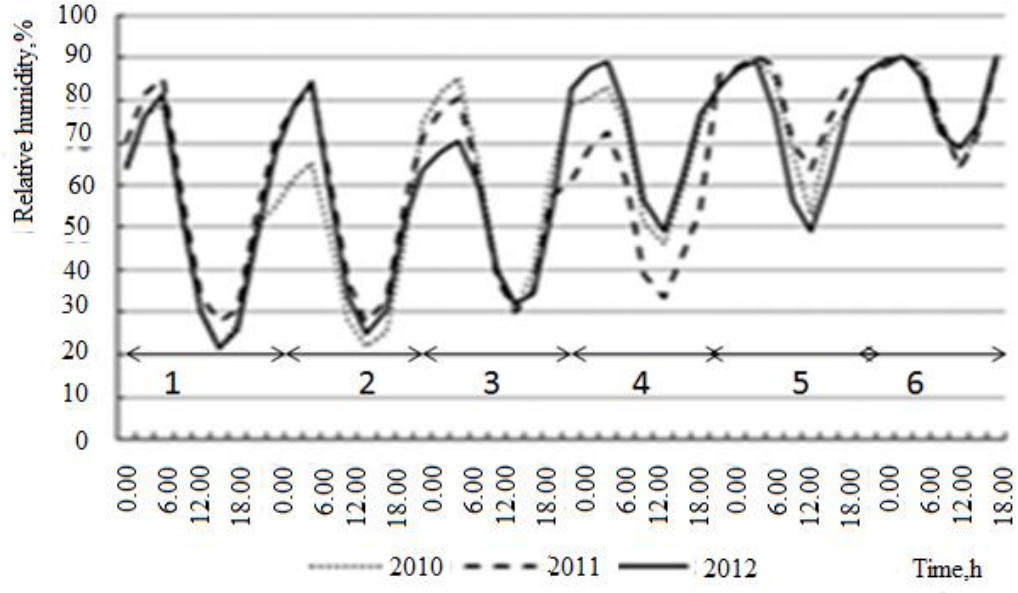

Figure 4 -Average monthly hours of ambient relative humidity of the Beja station during the period of 1st August to 10th January (1: August, 2: September, 3:October, 4: November, 5: December, 6:December 31 to January 10)

During the summer period the hours available for cooling aeration are usually between midnight and 6:00 am where air temperature and humidity are below 20 °C and 80% respectively. Nocturnal aeration especially during the fall still offers air temperatures under the upper limit for insect development of < 15 °C (Figure 4). However, towards the end of November, the air relative humidity exceeds 85% at night time, which can induce grain humidification.

To achieve the main objective, the experiment and the analysis were done for the period of July 9th, 2018 to January 10th, 2019. A such period corresponds to the storage beginning until the hypothetical cooling achievement.

For the experiment, the fan was operated from 12:00 am until 6:00 from July 9th to November 29th2018 and from 10:00 am to16:00 pm from November 30th2018 to January 10th2019. During the period of July 9th to November 29th2018, the night aeration was activated for 144 days at a rate of 6 hours per day (from 12 p.m. to 6 a.m.) or 864 hours of night aeration. However, during the period of November 30th2018 to January 10th2019, daytime aeration was activated for 42 days

corresponding to252 hours of aeration. The initial moisture content of the stored wheat was 13.7% (dry basis). Initially, the temperature recorded by sensors 1, 2 and 3 (Figure 2) was 26.1, 27.2 and 26.5 °C, respectively. Table 1 shows the ambient aeration air and stored wheat thermo physical properties, where the grain bulk density and porosity were determined experimentally (Table1). The temperature and relative humidity of the ambient air were measured continuously by two sensors (Pt-100, Testo, Germany with a measuring accuracy Δ1.55% for relative humidity and Δ0.58 °C for temperature), installed in a shaded area close to the experimental bin.

Table 1 - Aeration air and stored wheat thermo physical properties

Term	*Value or equation*	*Reference*
Grain porosity (decimal)	0.49	measured
Bulk density of the grain (kg m^{-3})	863	measured
Specific heat of water (J kg^{-1} K^{-1})	4186	(Navarro and Noyes 2001)
Specific heat of humid air (J kg^{-1} K^{-1})	Ca = 1 + 1.805Y	(Ojer 1995)
Chung- Pfost constants	A=921.65	(ASAE D245.4.1994b.)

To evaluate the moisture content of the grain (dry basis), samples were taken every 15 days till the end of October 2018and twice a week for the remainder of the experimental period (from October 31st2018 to January 10th2019) under higher risk of stored grain humidification. During aeration, the hourly ambient air temperature and relative humidity were taken as model inputs to predict the evolution of the moisture content and temperatures of grain at the level of the three sensors (Figure 2).

Typical airflow rates recommended for stored wheat are 0.08 $m^3min^{-1}m^{-3}$. Because the grain cooling rate can't be improved with higher air velocity after a specific relative short time of aeration, the influence of airflow velocity was low on cooling rate even for aerated stored fruits with higher moisture content (Behaeen et al.2018). Thus, the consumption of energy could be reduced by selecting an acceptable low airflow velocity in aerated grain storage. Furthermore, to minimize the grain moistening effects during night time aerations, the airflow rate used in the experiment is 0.49 $m^3h^{-1}m^{-3}$ (0.008 $m^3min^{-1}m^{-3}$).

The standard error of residuals (S. E.), average of absolute values of the residuals (AAVR), the correlation coefficient (R^2) and index of agreement (d) were used to evaluate the accuracy of the model. The parameters: AAVR, S.E. and d are described by Equations (19), (20) and (21) respectively (Mayer & Butler 1993; Willmott 1981; Yang and Huffman 2004).

$$AAVR = \frac{\sum_{i=1}^{n}|P_i - P_i^*|}{n} \quad (19)$$

$$S.E. = \sqrt{\frac{\sum_{i=1}^{n}(P_i - P_i^*)^2}{n-1}} \quad (20)$$

$$d = 1 - \frac{\sum_{i=1}^{n}(P_i - P_i^*)^2}{\sum_{i=1}^{n}(|P_i - \overline{P^*}| + |P_i^* - \overline{P^*}|)^2} \quad (21)$$

Where;P_i, is the experimental parameter of the model (moisture content, grain temperature);P_i^*, is the predicted parameter of the model and n is the number of testing data. The index of agreement is dimensionless. The units of AAVR and S. E. depend on the evaluated parameter of the model. Thus, in this study, to assess the temperature parameter P_i and P_i^* represent hourly experimental and predicted temperatures. However, to assess the moisture content P_i and P_i^* represent experimental and predicted moisture contents at the moment of sampling.

3. Results and Discussion

3.1 Evolution of the grain moisture contents

3.1.1 Measured grain moisture content

During the first 510 hours of night aeration of the grain from July 9th to October 1st, 2018 (period A1 in Figure 5), the grain moisture content did not change significantly. After this period, the stored grain started to pick up humidity increasing risks of losses (Figure 5).

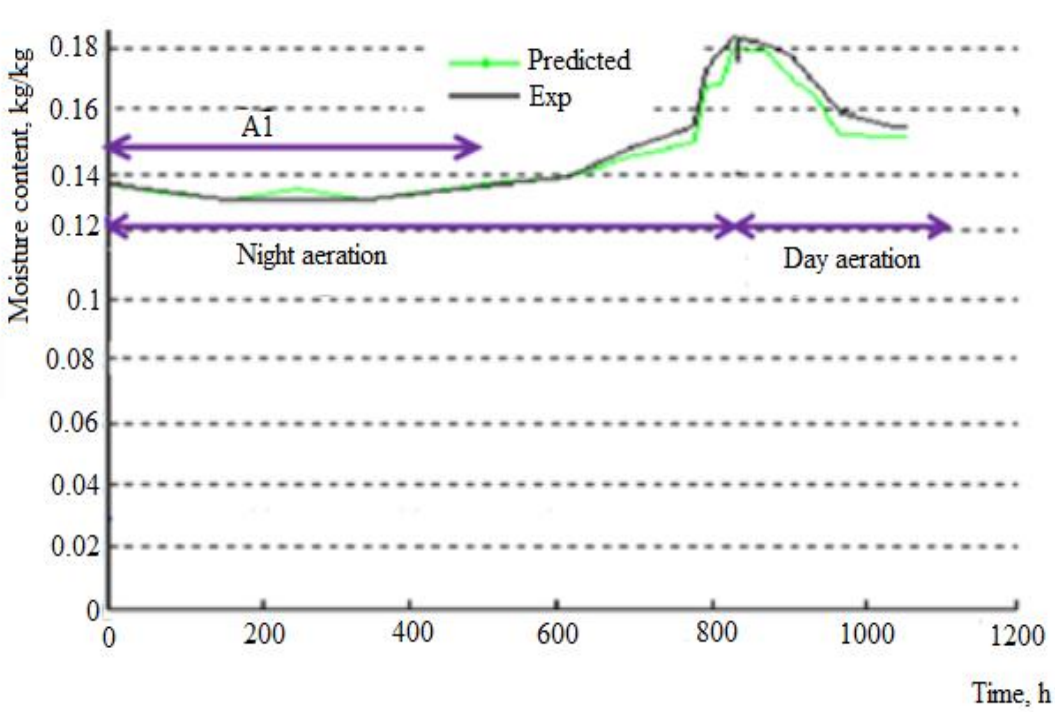

Figure 5 - Predicted and measured grain moisture content during the period of July 09th2018 to January 10th, 2019

In fact, night time ambient air in Northern Tunisia usually offers a higher relative humidity than the day time ambient air, often reaching saturation. During the 864 hours of night aeration from July 9th to November 29th, the stored grain gained from 13.7 to 18.36% (dry weight basis) in humidity (Figure 5). To avoid grain deterioration from such a high grain moisture content, aeration was switched to a daytime schedule from November 30th to January 10th for a period of 252 hours of daytime aeration (daily from 10:00 am to16:00 pm) to take advantage from the lower day time air temperature and relative humidity. The low grain temperature and moisture could thus be maintained.

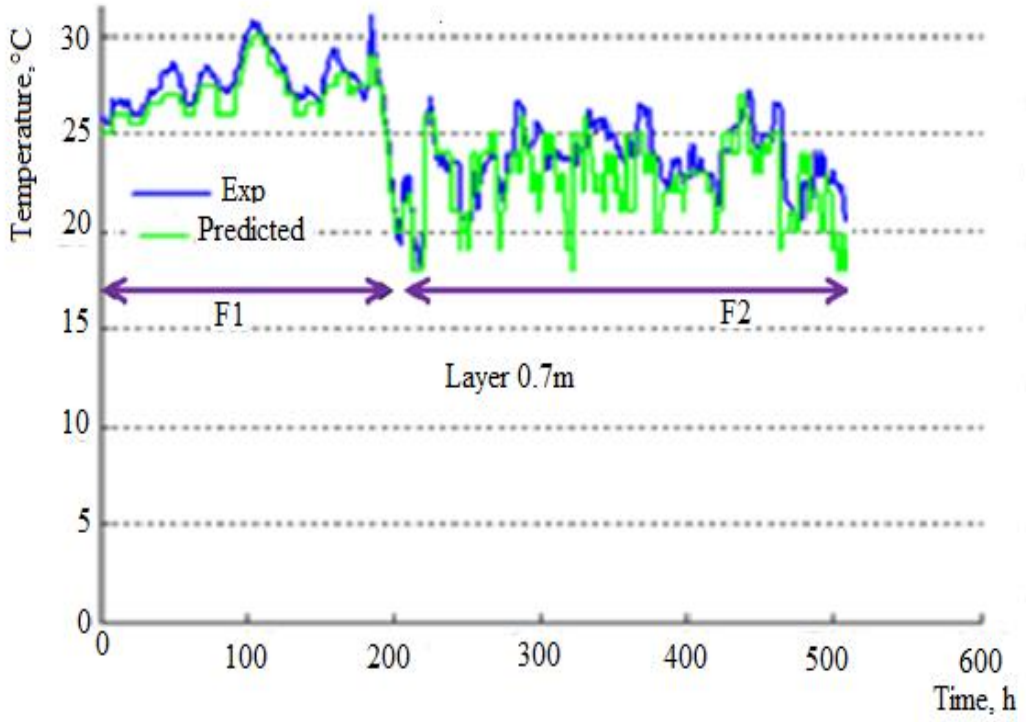

Figure 6 - Predicted and measured grain temperature from July 09th to September 30th 2018 for a grain layer height of 0.7 m

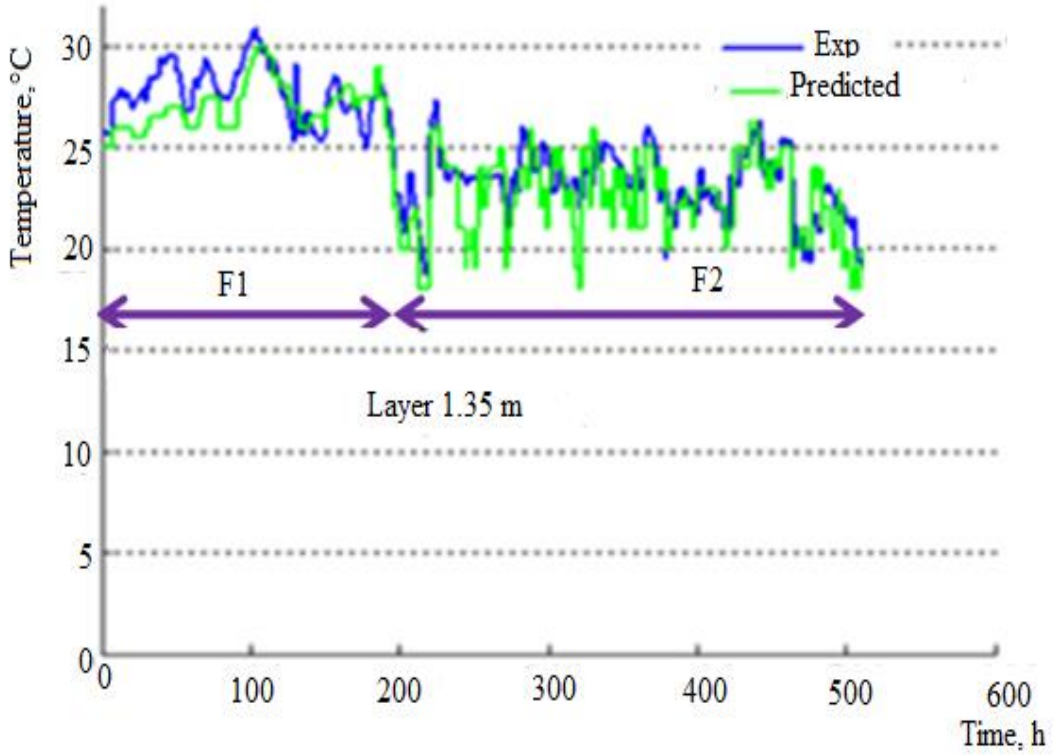

Figure 7- Predicted and measured grain temperature from July 09th to September 30th 2018 for a grain layer height of 1.35 m

The adjustment in aeration program as off November 29th2018 produced the required drying effect predicted by simulation (Day aeration Figure 5). Furthermore, no microorganism activity was observed during the whole experimental period which is an improvement as compared to the usual insect's infestation, generally observed by the end of September for bulk grain with no chemical treatments. When harvested at the end of June, the grain is relatively free of insects. But, insects will there of migrate into the stored grain mass and multiply (Hagstrum, 2000). Generally, insects appear in the upper layers of the grain mass (0 to 1.2 m) because of its aeration, and theirs growths begins in September (Flinn et al. 2010). Insect growth in surface layers is also encouraged by the thermal front of September where the night temperatures are below 20 °C (Figure 5 to 7).

3.1.2 Simulated grain moisture content

Figure 5 shows the evolution of measured and simulated moisture content (dry basis) of the stored grain in response to the aeration scheme. It is observed that the simulated and measured moisture contents are very similar, representing a good fit between simulated and measured values (Table 2).

Table 2 - Aeration air and stored wheat thermo physical properties

Parameter	***Layer***	R^2	***AAVR***	***S.E***	***d (%)***
Temperature	1	0.9545	1.1032°C	1.6160°C	97.43
	2	0.9765	1.1447°C	1.5923°C	97.54
	3	0.9784	0.7967°C	1.2289°C	82.96
Moisture content		0.99	0.0032 kg kg^{-1}	0.0007 kg kg^{-1}	99.9

AAVR and S.E provide a term-by-term comparison of the predicted and observed values. The model also produced small AAVR and SE (AAVR=0,00326 kg $kg^{-1}$and S.E.= 0,00079 kg kg^{-1}), with a strong correlation R^2=0.99.The high index of agreement (99.9%) indicates the simulation accurately estimated the observed variations. This index varies from 0 to 100% where the 100% reflects perfect agreement between observed and predicted data. According to Willmott (1981), this important index measures the degree of error free model predictions.

The validation of the model allowed for its use in predicting the moisture content of aerated stored grain for various aeration schemes to avoid or delay its deterioration.

3.2. Temperature evolution of the aerated bin stored grain

3.2.1 Measured temperature

Measured wheat temperatures at different levels of the grain mass surface are shown in Figures. 6 - 11. Figures 6, 7 and 8 show the evolution of grain temperature for the period of July 09th to September 30th with a cumulative of 504 hours of night aeration. However, Figures 9, 10 and 11 show the evolution of grain temperature during the period of October 01st 2018 to January 10th 2019, with a cumulative of 612 hours of aeration. The measured temperatures at the end of the summer aeration period were around 24 °C for the three sensors (Figures 6, 7 and 8). During this period of summer aeration, the grain bulk was protected from self and solar radiation heating. Indeed, the first 200 hours of night aeration were used to attenuate and cushion the impact of heat exchanges through the roof and walls of silo during off-peak hours of days when the ambient air temperature generally exceeds 35°C (Figures 6, 7 and 8, phase F1).

The continuation of night aeration during the 304 hours of the phase F2 was marked by an oscillation of the temperature of the grain layers around 24 °C with a cooling effect at the end of this aeration phase, F2 (September 30th) (Figures 6-8).

A significant grain cooling was achieved by the night aeration program during the months of October and November (phase F3). The reached measured temperatures after 360 hours (phase F3, October 01st 2018 to November 29th 2018) of aeration was 10 °C for the three grain layers (Figures 9-11). By the end of October, the measured temperatures for the three sensors were close to 17 °C and no insect's development was observed. Field research confirms the benefits of an initial cooling cycle from the mid 30 °C to about 24 °C, followed by further cooling to 17 °C when outside ambient temperatures are consistently below this threshold (Arthur & Casada 2005).

Figures 6 to 10 illustrate the obvious cooling trend measured by the three bin temperature sensors located in Figure 2 during the night aeration scheme. By the end of October, the grain temperature reached 17 °C for the three sensors. Applied as off the end of November, (phase F4 in Figures 9, 10 and 11), the daytime aeration scheme maintained acceptable low grain temperatures recorded by the three sensors, and dried the grain to a safe level (Figure 3).

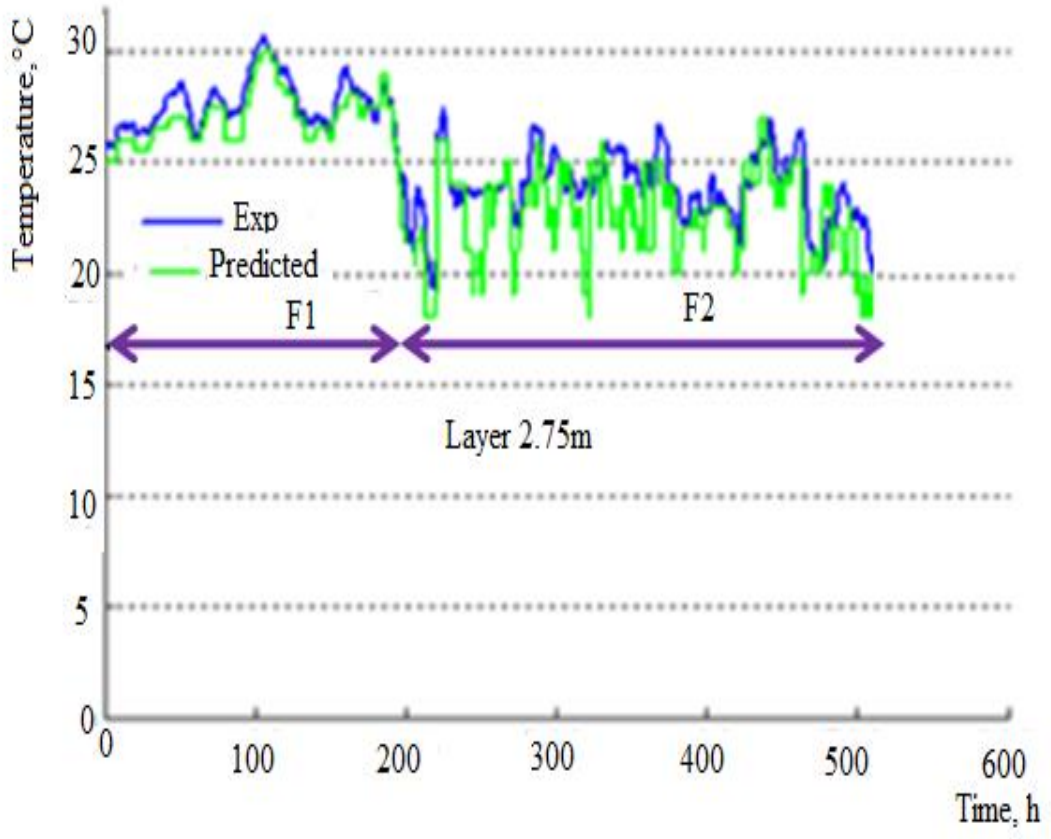

Figure 8- Predicted and measured grain temperature from July 09th to September 30th 2018 for a grain layer height of 2.75m

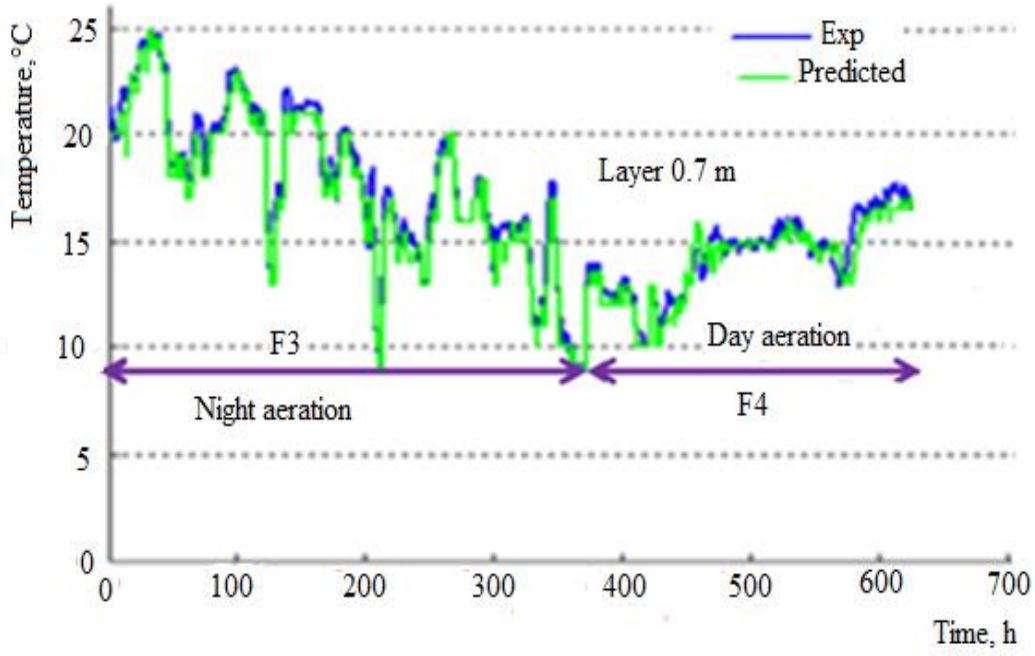

Figure 9- Predicted and measured grain temperature from October 01st, 2018 to January 10th, 2019 for a grain layer height of 0.7 m

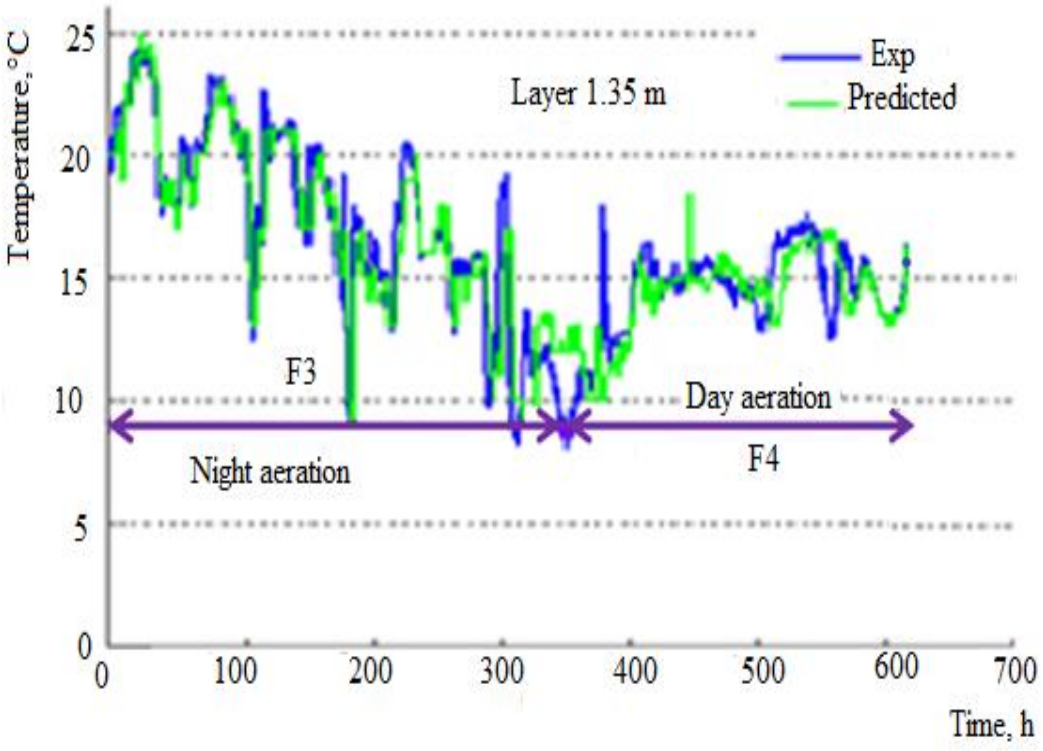

Figure10- Predicted and measured grain temperature from October 01st, 2018 to January 10th, 2019 for a grain layer height of 1.35 m

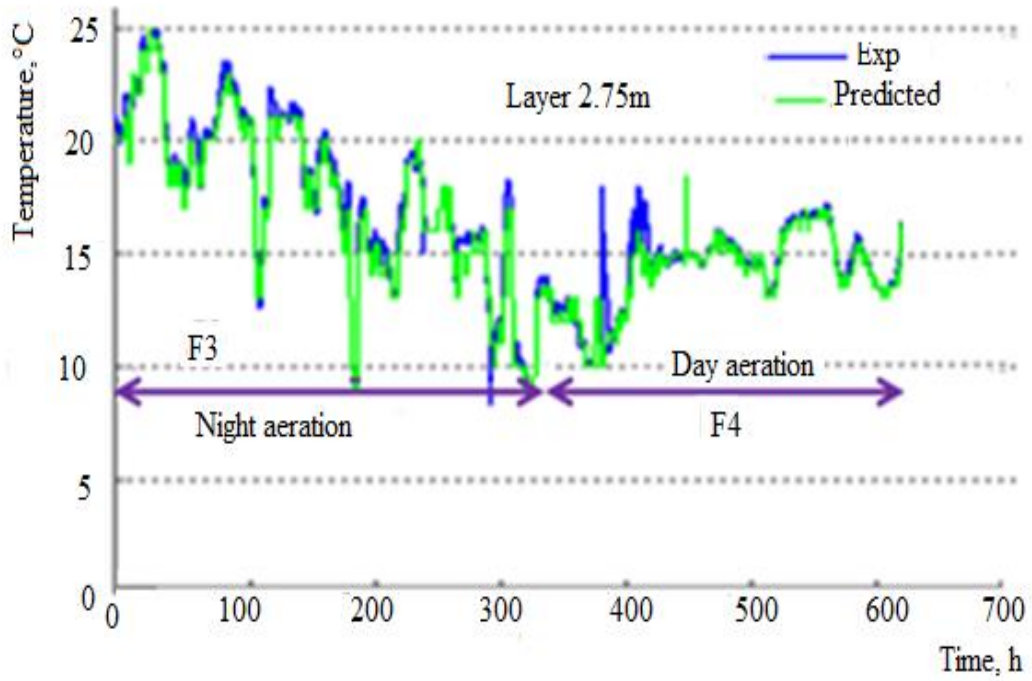

Figure 11- Predicted and measured grain temperature from October 01st, 2018 to January 10th, 2019 for a grain layer height of 2.75 m

3.2.2 Simulated temperature

The model simulation produced results similar to those observed. Figures 6 to 11 illustrate the similar predicted and measured temperatures for the three layers of grains (S.E. below 1.7 °C, AAVR below 1.2 °C and a correlation exceeding 0.95) (Table 1). However, the predicted values were always lower than these measured mainly during the summer aeration scheme because:

- The predicted temperatures of the upper layer (Sensor N° 1) at the beginning of the period were significantly lower than that observed. The model did not take into account the silo head space warming effect of about 8 °C (depending on temperature sensor in the headspace). The discrepancy was higher early in the aeration scheme because of radiation effects. Differences for sensor 2 were explained by a longer sunlight exposure of the bin left side, quantified by AAVR (1.10 °C for Sensor N°1and 1.14 °C for Sensor N° 2). During the rest of the cooling period, the predicted temperature values follow those measured.

- The predicted temperature was the average of the simulated layers. Because of delays in layer cooling, a logic discrepancy occurred. The discrepancy tended towards a minimum after the entire layer cooling. A larger discrepancy was observed for thicker simulated layers. The discrepancy is explained in Table 2 by the index of agreement (d); indeed, the delay importance for layer 3 provides an agreement between observed and predicted value of 82.96%; however, the agreement increases as the thickness decreases, being greater than 97% for thicknesses of 0.7 m and 1.35 m.

4. Conclusions

The objective of the project was to validate a simulation model to recommend aeration schemes for grain stored under Tunisian climatic conditions.

According to the measured and simulated stored grain mass temperature and moisture content, it can be concluded that:

- Simulation using the appropriate mathematical model gives a good prediction of the aerated stored grain system (R^2=0.99 for moisture content with a range of 0.95 to 0.98 for the 3 layers). The reliable simulation outputs can be used to provide the appropriate aeration scheme to safely store grain masses.

- Night aeration seems to be a good alternative to avoid overheating of the stored wheat from July to August, in Tunisia and subsequently cool the grain to safe temperatures preventing the development of insects.

- Night aeration has a moistening effect of the grains which was simulated and observed. However, critical values were reached in late November when daytime ventilation can be used to maintain low temperatures while maintaining safe grain moisture levels. Lower winter daytime air relative humidity allowed for the observed and simulated grain drying effect.

For continental climatic conditions such as those of Northern Tunisia, night and day time grain aeration schemes can be combined to prevent the development of insects and microorganisms in stored grain masses. In fact, no microorganism activity was observed during the whole experimental period where stored wheat was properly aerated. This is a major advantage for grain storage facilities in Tunisia, where a general lack of proper storage schemes produces insect and mold infestations by September, yearly.

The mathematical model validated for grain bin storage conditions can be further used to develop appropriate aeration schemes for subtropical climatic conditions such as those of Tunisia where the differences between the ambient aeration air and the grain mass temperatures are small.

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