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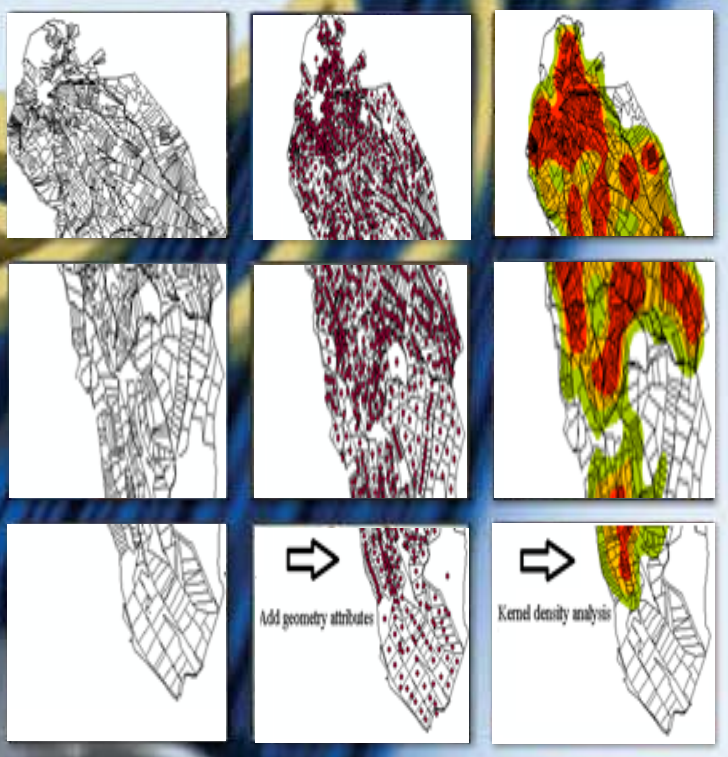

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Effect of Soil Organic Matter and Humates on Adsorption and Desorption Chemistry of Iodide in an Aridisol

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ABSTRACT
The translocation of iodine (I) from soil to food chain is largely determined by its adsorption/desorption reaction in soils. In this study, the effects of commercial humates (HA) applied on an Aridisol and indigenous soil organic matter (SOM) on the adsorption and desorption of iodide were investigated. For this reason, 1% and 3% HA (w/w) were incorporated into the whole soil (WS) and organic matter free (OMF) soil samples. Then soil samples were equilibrated with 0, 2, 4, 6, 8 and 10 mg L^{-1} iodide solution prepared in 0.01 molar $CaCl_2$ for 40 h. The sorption data were better described by Langmuir isotherm (R^2= 0.938) than Freundlich isotherm (R^2= 0.763). The Langmuir sorption maximum of WS was 19.8 mg kg^{-1}. Freundlich isotherm parameters were n= 0.89 and K_f= 2.165. Sorption maximum of OMF soil significantly increased up to 35.5 mg kg^{-1}. HA applications reduced iodide sorption maximum of both WS and organic OMF soil samples. Desorption rate of the WS ranged between 0-15.3% whereas it decreased 0-0.65% upon removal OM. HA treatments, in general, reduced the desorption rates. However, increasing HA application resulted in higher desorption ratio in both WS and OMF soils. Consequently, either SOM or HA has preeminent role in the adsorption-desorption chemistry in soils.

Keywords: Humates; Organic matter; Iodide; Adsorption; Desorption

1. Introduction

Iodine (I) is an essential nutrient element for mankind and animals, despite a limited number of researches about its essentiality for plants, animal and plant-origin foods should contain sufficient amounts of I. Approximately one-third of the world's population is suffering inadequate I intake (Andersson et al 2012). Iodine is an important micro element for all mammals' health, and an adult human being is required to have 100-150 μg day^{-1} in the diet (Johnson 2003). Despite average I concentration in soils (2.8 mg kg^{-1}) are generally higher than those in parent materials (0.5 mg kg^{-1}) (Kabata-Pendias 2011), plants suffer very low I concentration (0.005-10.4 mg kg^{-1}) (Shacklette & Cuthbert 1967) leading I deficiency-induced diseases such as goiter, cretinism, low IQ, abortions, birth anomalies and higher neonatal death (Laurberg et al 2010) in animal and/or human being. Traditionally extracts of kelp and seaweeds had long been used to treat goitre (Alejandro et al 2017) because of their very high I concentration; currently cabbage (leaves) and onion (bulb) are of terrestrial plants contain relatively higher I (Shacklette & Cuthbert 1967) can alternatively be included in dietary intake at larger portions. Therefore, it is essential to understand the ability of a soil, which is the main source of the food chain, to bind I and meet the requirement of plants. Since supplemental intake of I salts to correct its deficiency can be

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problematic, the occurrence of sufficient soil-I should be main goal to maintain well-being ecology of the soil-plant-animal-mankind food chain. On the other hand, the mechanisms controlling I transfer to the food chain in terrestrial environments and the components of the global I cycles have not yet been clearly elucidated (Johnson 2003). For this reason, there is a need to understand the I-soil interactions, plant uptake and metabolisms. The researchers have proved that OM is critical in the partition of I in the soil environment. The investigations revealed the relation between SOM content and I concentrations and humus is the primary reservoir of I in the soil (Dai et al 2009; Smyth & Johnson 2011; Xu et al 2011).

Humates (HA) is a colloidal fraction of humus containing both aliphatic and aromatic compounds. It has been shown that HA has a large surface area and is highly effective in determining I dynamics in the soil due to its occurrence in the soil organic matter, SOM (Francois 1987; Hansen et al 2011; Xu et al 2011). Although there are some structural differences between HA used in agriculture and indigenous soil humic substances, it is clear that the existing functional groups are similar. For this reason, the relationship between HA and I is critical to understand the dynamics of the I-HA complexes in the soil. Best of our knowledge, there are limited number of works in the literature elucidating I and HA relation in the soil environment (Christiansen & Carlsen 1991; Reiller et al 2006; Choung et al 2013). In general, the reaction between I and HA has not been clearly demonstrated since most of the studies had used complex aerosols-like environments consisting of other I-reactive components. These complexities can be explained as follows: the HA-I complex is mainly reduced towards reactive intermediates such as I_2 or HOI; followed by electrophilic substitution reactions with electron donor groups on HA (Francois 1987). Whitehead (1974) reported that phenolic and amino acid groups with a weak acid character on HA are the potential binding sites for I.

In this study, the effects of SOM and HA, both of which can regulate to some extent I cycle in soil environment, on adsorption/desorption properties of iodide on an Aridisol were investigated. For this purpose, adsorption isotherms and desorption ratio of iodide by WS and OMF soil treated with two doses of HA were obtained.

2. Material and Methods

2.1. Sampling location and treatments

Soil sampling site, Polatlı Agricultural Estate Farms, situates in Polatlı, Ankara (39° N 32° E). The soil, Yüzükbaşı soil series, is classified as a typical Aridisol. The composite surface soil samples (0-30 cm) were sieved through 2 mm for physical and chemical soil analyses. SOM was oxidised by analytical reagent grade 30% H_2O_2 (Hartge 1971) and dried at 40 °C till constant weight. Then both whole soil (WS) and organic matter free (OMF) soils were incorporated in 1% and 3% HA on weight bases and left incubation at 28 °C constant temperature and 70% of field capacity in dark for 60 days. Then the soil samples were subjected to the adsorption/desorption experiments.

2.2. Soil analyses

The descriptive soil properties were analysed according to common methods given by Sparks et al (1996). Analysed parameters were: both soil pH and electrical conductivity in saturation paste, cation exchange capacity (CEC) by Na acetate saturation, calcium carbonate equivalent by Scheibler calcimeter, OM by $K_2Cr_2O_7$ wet oxidation, available potassium (K) extracted by neutral ammonium acetate, and available phosphorus extracted by 0.5 M $NaHCO_3$ at pH 8.5. Soil texture was measured by Bouyoucous hydrometer method (Gee & Bauder 1986).

2.3. Descriptive analysis of commercial humate

The HA was kindly supplied by "Biyotar Corporation". The following properties of HA were determined: total humic acid + fulvic acid (TS 5869 ISO 5073), total nitrogen (Kjeldahl method), pH and EC (in 1:10 HA: distilled water) and total concentrations of P, K, Fe, Cu, Zn, Mn, Cr, Ni, Pb, and Cd in wet digests (HNO_3/$HClO_4$) were determined by ICP-OES. The properties of HA were given in Table 1.

Table 1- Chemical properties of the commercial humate used in the experiment

Parameters	*Results*	*Parameters*	*Results*
Total humic acid+fulvic acid (%)	66.4	Total copper (mg kg^{-1})	24.6
Total N (%)	0.73	Total zinc (mg kg^{-1})	92.7
Moisture (%)	7.94	Total manganese (mg kg^{-1})	131.2
pH_{H2O} (1/10)	8.65	Total nickel (mg kg^{-1})	89.3
EC (1/10, mS cm^{-1})	6.38	Total chromium (mg kg^{-1})	30.2
Total phosphorus (%)	0.54	Total lead (mg kg^{-1})	23.0
Total potassium (%)	9.89	Total cadmium (mg kg^{-1})	3.00
Total iron (%)	1.14		

2.4. Adsorption-desorption experiments

2.4.1. Adsorption

Prior to sorption experiment, soil samples were passed through 0.5 mm sieve. Then 2.5 g of WS, OMF soil, and HA-treated WS and OMF soil samples were put into 50 mL polypropylene centrifuge tubes with three-fold. Soils were then equilibrated with 25 mL of 0, 2, 4, 6, 8, and 10 mg I L^{-1} (KI) solution prepared in the 0.01 M $CaCl_2$ background solution at 25 °C for 40 h. The supernatant solutions were obtained by centrifugation at 10000 rpm for 5 min and subsequently filtered through 0.22 mm filter paper. Then iodide equilibrium concentrations of the filtrates were determined by an ion meter (Proline Plus, Iodine Comb. ISE/BNC). The deviation (RSD) was always less than 5% between the replicates.

2.4.2. Desorption

In order to desorb the adsorbed iodide 25 mL of background solution was added and the suspensions were then shaken on a reciprocal shaker at 25 °C for 40 h. The supernatants were separated as described in the adsorption study.

2.4.3. Adsorption isotherms

The amount of adsorbed iodide concentration was calculated from the difference between the initial and equilibrium concentrations. The obtained sorption data were subjected to the Langmuir and Freundlich adsorption models given below:

Linear Langmuir Isotherm: $Ce/Si = Ce/b+1/kb$
Freundlich isotherm, $Si= K_f\ Ce^n$, can be linearized as $Log\ (Si) = Log\ K_f + n\ log\ C_e$

Where; Si, the amount of iodide adsorbed (mg kg^{-1}); Ce equilibrium concentration (mg L^{-1}); k and b are coefficients related to bounding energy and maximum adsorption; K_f and n are coefficients.

2.5. Statistical analysis

The confirmation of the sorption data to the adsorption isotherms was tested by regression analysis. The isotherm adsorption parameters were subjected to one-way ANOVA to reveal the effect of SOM and the added HA. Mean separation of the treatments was performed by Duncan test in MSTAT-C environment.

3. Results and Discussion

The experimental soil was clay textured, slightly alkaline reaction, non-saline, very rich calcium carbonate equivalent; poor in OM, total nitrogen content and available P concentration and sufficient available K concentration (Table 2).

Table 2- Descriptive physicochemical properties of the experimental soil

Soil Properties	*Unit*	*Results*
Textural class (C)	% Clay	39.81
	% Silt	38.25
	% Sand	21.94
pH (Saturation paste)	-	7.86
Electrical conductivity (EC)	dS m^{-1}	0.252
Calcium carbonate equivalent ($CaCO_3$)	g kg^{-1}	169
Organic matter	g kg^{-1}	7.00
Aggregate stability	%	51.40
Total nitrogen	%	0.035
Available potassium	mg kg^{-1}	347
Available phosphorus	mg kg^{-1}	2.40
Iodine	mg kg^{-1}	0.0047

3.1. Isotherm parameters

3.1.1. Adsorption parameters of WS

Iodide sorption parameters of WS and OMF were given in Table 3. Sorption data were better described by Langmuir isotherm because the determination coefficient of Langmuir isotherm (R^2= 0.9376) was higher than the one belonging to Freundlich isotherm (R^2= 0.7626). However, determination coefficients of Langmuir isotherm were even improved upon HA treatment to 0.9991 and 0.9999 for 1% and 3% HA-treated WS (HATWS) respectively. In contrast, Freundlich isotherm described sorption data progressively poorer for HATWS. Calculated Langmuir maximum sorptions of HATWS were 19.8, 17.1 and 15.0 mg kg^{-1} for 0, 1 and 3% HA treatments, respectively. Whereas bounding energy coefficient significantly increased upon HA treatment as 0.753, 6.95, and 37.0 mL g^{-1} for 0, 1, and 3% HA treatments, respectively.

Table 3- Multiple comparison of sorption isotherm parameters

Soil	*Treatments*	*Langmuir isotherm*			*Freundlich isotherm*		
		b *mg kg^{-1}*	*k* *mL g^{-1}*	R^2	*n*	K_f	R^2
WS	WS	19.8 b	0.753 e	0.9376	0.89b	2.10 d	0.7626
	1% HATWS	17.1 cd	6.95 d	0.9991	1.193ab	3.71 c	0.4557
	3% HATWS	15.0 e	37.0 a	0.9999	1.06ab	3.30 c	0.4556
OMF	OMF	35.5 a	0.58 e	0.8831	0.74ab	2.69 a	0.9109
	1% HATOMF	17.7 c	28.4 b	0.9999	1.27a	4.30 b	0.3935
	3% HATOMF	15.8 de	15.4 c	0.9998	1.22ab	3.61 c	0.4489

WS, whole soil; OMF, organic matter free soil; HAT, humic acid treatment; b, adsorption maximum; k, bonding energy coefficients of Langmuir isotherm; n, a measure of intensity of adsorption and; K_f, indicator of maximum adsorption of Freundlich isotherm. Different letters in the same column indicate significant differences among the treatments

HA-induced Freundlich adsorption intensity coefficients (n) were 0.89, 1.93 and 1.06 for 0, 1, and 3% HA treatments, respectively (Table 3). The respective K_f coefficients were calculated as 2.10, 3.71, and 3.30 mg kg^{-1}. HA incorporation increased the K_f constant when compared with the original soil.

3.1.2. Adsorption isotherm parameters of organic matter free soil

Freundlich isotherm (R^2= 0.9109) slightly better described sorption data of OMF soil whereas sorption data of HA-treated organic matter free (HATOMF) soils clearly conformed Langmuir isotherm to a determination coefficient above 0.9998. Freundlich isotherm, on the other hand, failed to describe sorption data showing a determination coefficient below 0.4489. Langmuir sorption maximum progressively decreased by HA application in the OMF soil as 35.5, 17.7 and 15.8 mg kg^{-1}

for OMF, 1 and 3% HA, respectively. As small as 1% HA addition to soil resulted in 50.1% reduction in the sorption maximum. The calculated bonding energy coefficients did not show consistent increases that were 0.58, 28.4, and 15.4 for 0, 1, and 3% HA treatments.

The adsorption intensity of OMF soil (0.74) was significantly smaller than those obtained for HATOMF (Table 3). There were no differences between the HA treatments. K_f coefficients which are indicators of adsorption capacity ranged between 2.69 and 4.30. The highest K_f value was obtained for 1% HA treatment. Typically slope of Freundlich isotherm is smaller than "1" as observed in the OMF soil. However, HA application increased slope above "1" suggesting a different mechanism.

As shown in Table 3, the highest adsorption maximum value was found in OMF. HA treatments had an apparent decrease in OMF soil samples comparing to the WS. This could be an indication of interaction between the indigenous and commercial HA which significantly decreases sorption sites of HA for I. Commercial humates are salts of humic substances, which are more soluble and thus more reactive in soil (Lobartini et al 1992; Lyons & Genc 2016). Humic substances can construct random manner linkages (Garcia et al 2016) with even SOM or clay minerals resulting in extraordinarily complex materials. Newly formed organo-mineral complexes in the soil, in fact, block a significant portion of I sorption sites in OMF soil sample. On the other hand, anionic nature of commercial HA which generally preferentially adsorbed on the clay surfaces over anions such as phosphates (Uygur & Karabatak 2009). Dai et al (2009) pointed out the significance of the OM in soil environments in controlling I geochemistry. Adsorption studies with dissolved humic acids indicated that the contribution of clay fraction was over 90% (Pan et al 2010) which suggest clay fraction in this study can be responsible for very high sorption maximum in the OMF.

3.2. Effects of treatments on desorption ratio

3.2.1. Desorption in whole soil

The desorption ratio (DR) dependent on the initial concentration and treatments of the soil are summarized in Table 4. The highest value for WS without HA was found to be 15.08% at 4 mg L^{-1} initial concentration. Further increase in the initial concentration did not improve the desorbability of iodide. Despite DRs in 1% HATWS were smaller than the ones obtained from WS there was a gradual continuous increase in DR upon 1% HA treatment. The maximum value (6.28%) was therefore recorded for 10 mg I L^{-1} (Table 4). The higher HA treatment (3% HA) resulted in the higher DR for each respective initial concentration. The maximum desorption was obtained at maximum initial concentration. The equilibrium pH at desorption remained relatively very narrow range between 7.76-7.91.

Table 4- The effects of initial iodine concentration on the desorbability ratio of iodine-adsorbed and equilibrium pH

IC (mg kg^{-1})	*Whole soil*						*Organic matter-free soil*					
	0% HAT		*1% HAT*		*3% HAT*		*0% HAT*		*1% HAT*		*3% HAT*	
	DR (%)	*pH**	*DR (%)*	*pH*	*DR (%)*	*pH*	*DR (%)*	*pH*	*DR (%)*	*pH*	*DR (%)*	*pH*
0	0.00	7.77	0.00	7.76	0.00	7.78	0.00	7.52	0.00	7.75	0.00	7.76
2	8.35	7.79	1.08	7.77	4.14	7.79	0.44	7.65	0.90	7.76	2.60	7.77
4	15.08	7.80	2.21	7.80	5.02	7.82	0.51	7.77	1.64	7.81	4.56	7.78
6	13.16	7.81	3.08	7.83	5.98	7.83	0.48	7.77	2.36	7.81	4.80	7.81
8	13.57	7.83	4.27	7.88	8.88	7.85	0.51	7.78	3.57	7.86	6.48	7.84
10	15.31	7.83	6.28	7.91	9.81	7.92	0.65	7.79	4.78	7.87	7.94	7.90

IC, initial concentration; HAT, humic acid treatment; DR, desorption ratio of the adsorbed-iodine; *, equilibrium pH for desorption study

3.2.2. Desorption in organic matter-free soil

The DRs of iodide drastically reduced down 0.44-0.65% range upon removal of SOM (Table 4). The equilibrium pH varied relatively larger range between 7.52-7.79. The higher rate of HA addition-induced increases in the desorption rate

that 3% HA treatment was more effective below initial concentrations of 4 mg L^{-1}. The maximum desorption ratios for both HA treatments were obtained at 10 mg I L^{-1} initial concentration that was 4.78% for 1% HA and 7.94% for 3% HA. Neither initial concentrations of iodide nor HA application rate affected equilibrium pH (7.76-7.90) of desorption supernatants.

3.3. Comparison of adsorption/desorption isotherms

Very low desorption ratios of iodide indicated that there is likely to be apparent hysteresis between adsorption-desorption isotherms which can be caused by strong binding energy of iodide to surfaces that can be attributed to chemisorption reactions. Commercial humate incorporation into either WS or OMF soils caused an increase in DR. This suggests that humate can be preferentially adsorbed on the surface which would inhibit specific adsorption of iodide. As a result, desorption ratios increased as higher amounts of HA incorporation. Despite some reduction in the specific sorption sites they are not fully occupied by HA. On the other hand, HA may have some non-specific sorption sites as indicated by rate-induced increases in the desorption ratios.

An apparent increase in the sorption maximum from 19.8 to 35.5 mg kg^{-1} upon removal of SOM put forward the significance of the OM in sorption mechanism of iodide. SOM is to form organo-mineral complexes with clay minerals which could be critical for partitioning of external iodide. Substantial decreases in the desorbability of iodide in OMF soil and increases after HA incorporation indicated that well-humified organic substances are to block specific sorption sites on the colloidal mineral surfaces. Dai et al (2004) also found that SOM had a significant negative effect on iodate adsorption and that they found iodate adsorption capacities of 9-34 mg kg^{-1} in the soil studied.

A variety of soil minerals such as calcite, chlorite, epidote, goethite, gypsum, hematite, kaolinite, bentonite, muscovite, and quartz showed little iodide or iodate adsorption at alkaline pH range of 7.5 and 8 due to the existence of little pH dependent positive charges (Ticknor & Cho 1990) and negative charges on clay minerals repel iodide anion. Similarly, alluvial soils retained as little as 1.4-4% of the I added whereas only 1.35-4.1% of the I-adsorbed was desorbable (Nath et al 2010).

The OM present in the soil appears to have an adverse effect on iodide adsorption. In such situation, the reaction of iodide with soluble HA should also be regarded. In contrast, Merzweiler et al (1987) reported I contents of 220 soil samples were highly correlated with the SOM. In a similar manner, the sorption of I^- and IO_3 on humic acid was found substantially higher than those of clay minerals indicating a specific role of SOM in I retention in soils (Muramatsu et al 1990). This controversial situation may be explained by the preferences of soil positive charges for OM over I in time, and added I can only react with positive charges of OM. At this point, the affinity of humate surfaces and soluble humate to iodide is a matter of fact in our case. The data suggested that HA used in this study can have relatively smaller amounts of physisorption sites than the indigenous soil organic matter as indicated lower desorbability in HATWS. On the other hand, the added HA can be a structural component of SOM during the incubation period of the soil as indicated similar desorption ratio in HA treated both WS and OMF soil samples (Table 4). HA can fix significant amounts of iodide by inner-sphere complexes as well. Dai et al (2004) reported that SOM is an adverse and significant effect on I adsorption and that they found iodate adsorption capacities in soil between 9-34 mg kg^{-1}. In contrast, McNally (2011) reported that clay minerals and iron oxides were more effective than SOM in I adsorption. Whitehead (1978) related very high sorption capacity of soils to clay and OM forming under weathering conditions especially in a very high precipitation induced environment. This strong binding, in turn, could be the reason for geochemical I deficiency and goiter disease. There are however many reports pointing out a specific role in I adsorption in soils (Whitehead 1973; Lieser & Steinkopff 1989; Akca et al 2014).

4. Conclusions

The results of this study indicated that the effect of SOM on the adsorption and desorption of I was critical. Either indigenous SOM or incorporated HA reduced the sorption sites on the sesquioxides and clay minerals whereas the formation such organo-mineral complexes are to increase the bioavailability of I in soils. Despite HA applications increased the bioavailability of added I in the studied soil, different soil properties can have different impacts on the adsorption processes. Therefore further researches may be needed by using soils with different physicochemical properties and even in the model soil component systems.

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Kernel Density Analysis of Parcel Size and Shapes Before and After Land Consolidation: A Case Study from Aşağısümenli Village in Malatya, Turkey

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ABSTRACT
Land consolidation (LC) projects are a set of applications that improve the economics of enterprises by assembling fragmented, dispersed, and irregular parcels. As the parcel densities coalesce around the village centre, the operation becomes easier, and fuel costs are reduced. Besides, the size of the parcel is one of the most important factors that increase the income of the enterprises, as well as the plant pattern, agricultural production form, soil quality, talents, labour force and technology features. The aim of the current study conducted within Aşağısümenli LC project in Malatya, Turkey, was to assess the density of small parcels around the village centre by using kernel density analysis as one of the geospatial analyses and to investigate the spatial distribution of irregular parcels with shape index. To identify the smallest parcels spatial distribution, 50%, 75% and 90% bandwidths were determined. Before LC, the average parcel area within 50%, 75% and 90% bandwidth was 0.69, 0.93 and 1.07 ha; after LC was 0.89, 1.45 and 1.63 ha, respectively. The area averages of parcels between 50% and 75% bandwidths before LC were 1.79 between 75% and 90% bandwidths and 4.77 ha out of 90% bandwidth; after LC, 1.60, 2.47 and 3.13 ha, respectively. As a result, the small parcels after LC were more concentrated around the village centre than before LC. Moreover, it can be said that the density of the small rectangular shaped parcels around the centre of the village is a positive result in terms of reducing the operation cost.

Keywords: GIS; Kernel density analysis; Geospatial analysis; Land consolidation

1. Introduction

Optimum enterprise size is one of the most controversial topics in agriculture. In general terms, optimum enterprise size means agricultural enterprise size with the lowest cost (Gökçe & Adanacıoğlu 2002). Land consolidation (LC) applications help to decrease land fragmentation and hence increase enterprise size (Demirtaş & Sarı 2003). Land fragmentation increases transportation distance which leads to more labour and time losses (Kakwagh et al 2011). The aim of LC is to eliminate these problems and try to achieve maximum benefit with minimum input (Boztoprak et al 2015). Also, while reallocation is being made, it is stated that the enterprises that would decrease land fragmentation should be encouraged and given priority in the location of the parcel which is close to the settlement and the area which is larger than the agreement.

Some significant effects of the LC works include the following: reduction of the distance from the enterprise centre to the village centre, making new and shorter roads, decreasing the winding roads with rectangle parcel shapes, decreasing the number of the parcels and increasing their sizes. However, in some cases (Değirmenci et al 2017) the number of parcels increased after LC due to many shares. This situation originates the social structure of the LC area. Generally, the

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number of parcels is increasing after LC projects in regions with a high number of family members. In addition to LC, expropriation (Boztoprak et al 2015) also affects the size of the parcel. In the study conducted in DSI (State Hydraulic

Works) 12. District region, the effects of expropriation on road construction and parcel size was investigated. Results showed that the size of the parcel decreased by 15.70%. This situation leads to a decrease in per capita income (Kumbasaroğlu & Dağdemir 2007; Looga et al 2018). Although the owners of the parcels appear single after the LC, they are divided because of some problems (Kirmikil et al 2017). Therefore, LC studies should be done in a multifaceted way, and the social-cultural characteristics of the region should be determined in a good idea. For LC projects to be successful, many factors should be taken into consideration, and monitoring studies should be done carefully.

Kernel density analysis is used in the following ways: to reduce traffic accidents on cross-roads (Xie & Yan 2008); determination of movement and habitat use by fish in rivers (Alp et al 2018); assessment of accumulation of environmental pollution (Sirirwardane et al 2015); landscape change (Carmona et al 2010), road density as well as its impact on fragmentation (Cai et al 2013). Along with these studies, kernel density analysis can be used to determine the frequency of water table level, salinity content rate, plant density, etc. in agriculture. The studies on kernel density show that the importance of determining density using GIS allow researchers evaluate change.

Parcel size has one of the most significant effects on production (Değirmenci et al 2017). The complexity of shapes can be measured with various shape indices used commonly by researchers (Aslan et al 2007; Jiao et al 2012; Demetriou et al 2013; Kwinta & Gniadek 2017). Unshaped parcels and parcel sizes should be taken into consideration together due to the connection they have on the effect on production. Parcel shape and size have long been a question of great interest in a wide range of fields. Previous published studies are limited and there is no considerable amounts of studies suggest an association between parcel size and shape. In land consolidation projects, parcel size and shape are of significant importance and surprisingly, the spatial distribution of parcel size considering shape have not been closely examined.

This study aims to analyse the distribution of small scale parcel sizes around the village centre and the spatial distribution of complexity of parcels before and after the LC project. In the province of Malatya, LC projects' data of Aşağısümenli Village was selected as the material, and kernel density analysis was used to determine the density of the parcels by using geographic information system programmes. The parcel sizes found before and after the LC were examined in the 50%, 75% and 90% band segments (core range contours) within the total parcel number.

2. Material and Methods

Aşağısümenli Village is located in the Arguvan district of Malatya province between 38°52″12′ North parallels and 38°13″38′ Eastern meridians. Figure 1 shows the LC project before and after including the location.

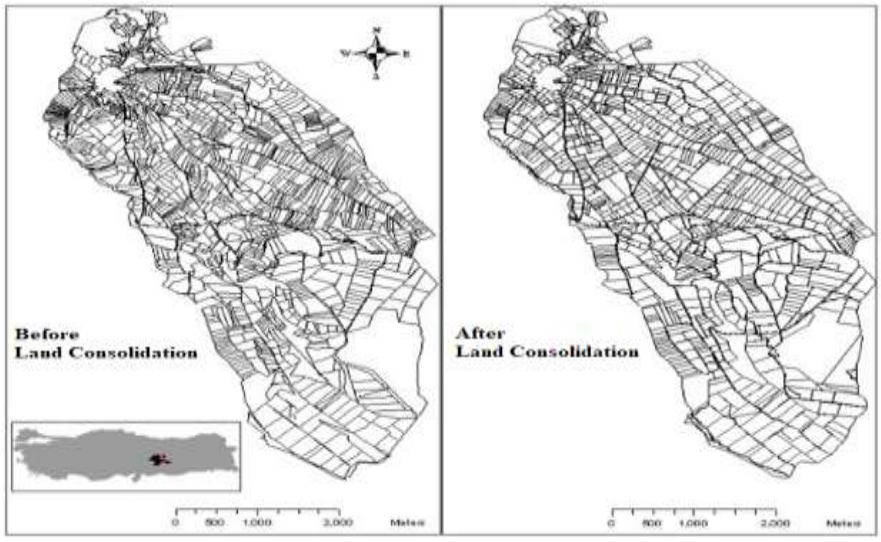

Figure 1- Aşağısümenli land consolidation project before and after land consolidation

The region is under the influence of the continental climate. Winters are cold and snowy, and summers are usually hot and dry. The average annual rainfall is 374.9 mm; the average temperature is 13.6 °C (Meteorological Service 2018). The number of parcels was 1357; the total area was 1963.94 ha (average 1.45 ha) before LC; after LC, the number of parcels decreased to 1082, and the total area decreased to 1936.74 ha (mean 1.79 ha). Data were obtained from the company which designed the project.

The kernel density analysis calculates the density of features in a neighbourhood around those features. It can be estimated for both point and line features (ArcGIS 2018). In this context, Kernel density analysis of ArcGIS (ArcMap 10.5) was used to show districts where small parcels were located. Parcels which have polygon features were changed into point features due to kernel density analysis works with the point or line features. In this context, each point shows centroid of the parcel presents a polygon (Figure 2). ArcMap calculates the kernel density analysis following the algorithm:

1. Calculate the mean centre of the input points.
2. Calculate the distance from the (weighted) mean centre for all points.
3. Calculate the (weighted) median of these distances, *Dm*.
4. Calculate the (weighted) Standard Distance, *SD*.
5. Apply the following formula (Equation 1) to calculate the bandwidth:

$$SR = 0.9 \times min \left(SD , \sqrt{\frac{1}{\ln(2)}} \times Dm \right) \times n^{-22} \quad (1)$$

Where; *SR* is search radius, *SD* is the standard distance, *Dm* is the median distance and *n* is the number of points if no population field is used, or if a population field is supplied, *n* is the sum of the population field values (ESRI 2019).

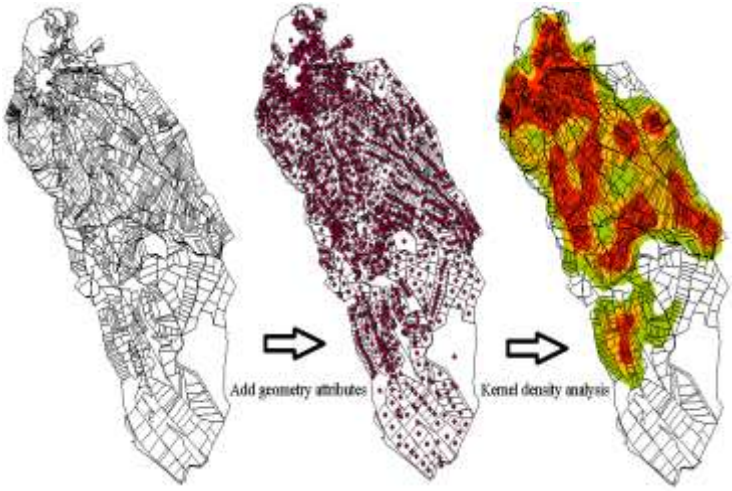

Figure 2- A methodology of kernel density analysis of parcels

Buffer analysis was used to show parcel location change clearly. We consider buffer analysis is tools which may make readers see the change of it with core range contour around village centre. Core range contours were divided into 3 groups which are 50%, %75 and 90% which are considered representative range. These classifications indicate the smallest parcels. For example the core range contours 50% represent the smallest parcels according to the sort by size. These ranges can be considered with different percentages of core range contours according to researcher aims as well.

Fractal dimension is an index of many shape indices that measure shape complexity and is used in many studies (Aslan et al 2007; Demetriou et al 2013; Bayram & Değirmenci 2018). We chose the index due to the precision level measuring agricultural parcels' complexity (Bayram & Değirmenci 2018). It is calculated by using the following Equation (2);

$$FD = \frac{2\ln(P_i)}{\ln(A_i)} \quad (2)$$

Where; *FD* is the fractal dimension, P_i is the perimeter of *i* parcel, A_i is the area of *i* parcel. This ratio can take values from 1 to 2, and 1 shows optimum shaped parcels.

3. Results and Discussion

Figure 3 shows the core ranges of contours and distance from the village centre within 2000 metres buffers before and after LC. The size of parcels within the core range contours shows the smallest parcels before or after LC. 50% of all parcels which have the smallest size within the project are shown in red core contour, 75% in yellow core contour and 90% in green core contour.

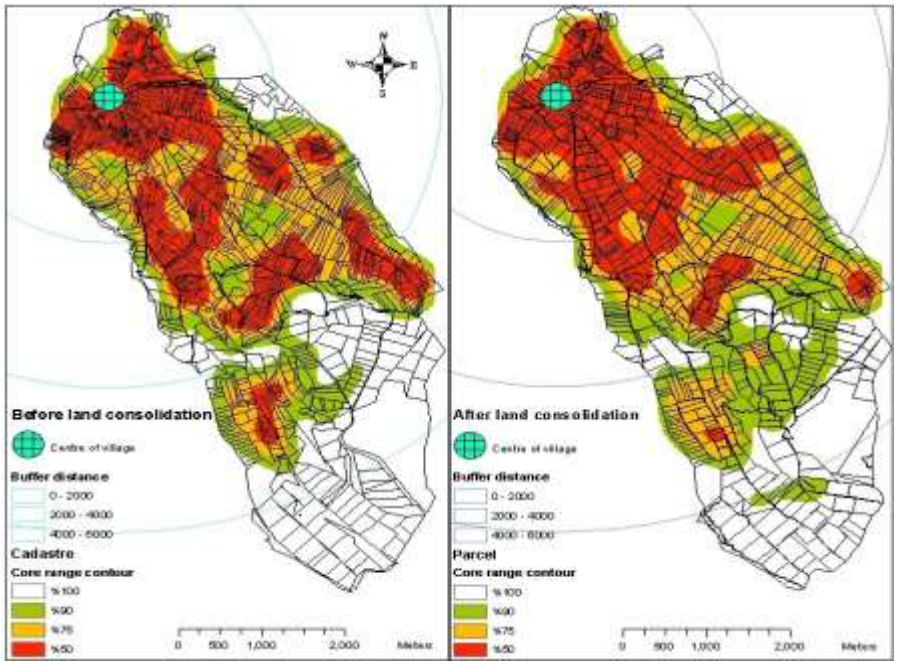

Figure 3- Kernel density analysis according to parcel size before and after land consolidation project

Table 1 illustrates total, average area and the number of parcels as well as average fractal dimension before and after LC within core range contours. Red represents 50% of the number of parcels present within core range contour 50% and an average parcel size of 0.7 ha for 687 parcels which covered 469.7 ha before LC. Yellow represents 75% of the number of parcels within the 75% core range contour which includes 937.9 ha with 1017 number of parcels; the average area was 0.9 ha before LC. Green represents 90% of the number of parcels which cover 1310.1 ha with 1221 parcels, and the average area was 1.1 ha before LC. After LC, total area, average area and number of parcels for 50% core range contour, were 729.6 ha, 0.9 ha and 541 respectively; for 75% core range contour were 1168.1 ha, 1.5 ha and 811 respectively; for 90% core range contour were 1582.8 ha, 1.6 ha and 973, respectively. The average fractal dimension value (1.38) after LC increased from the optimum value (1.37) which was not considerable change. In the present study, fractal dimension values increased with increase in parcel area. It was determined that the parcels where the density of small size parcels was previously located (red contour) had more regular shapes before and after LC and vice versa.

Correlation analysis showed a significant negative relation between area and fractal dimension ($P<0.01$) before LC. Results of the present study showed parcel area decreases of 1 metre, fractal dimension decrease of 0.24 and closing to 1 which is the optimum value. The Pearson correlation of area and fractal dimension in this study was-0.301, and the P-value is smaller than 0.001; in other words, there was a significant negative correlation between these parameters after LC.

Table 1- Descriptive statistics of kernel density before and after land consolidation

Core range contour (bandwidth)	*Before land consolidation*				*After land consolidation*			
	Total area (ha)	*Average area (ha)*	*Number of parcels*	*Average FD*	*Total area (ha)*	*Average area (ha)*	*Number of parcels*	*Average FD*
50%*	469.7	0.7	687	1.35	729.6	0.9	541	1.29
75%	937.9	0.9	1017	1.36	1168.1	1.5	811	1.32
90%	1310.1	1.1	1221	1.37	1582.8	1.6	973	1.35
100%	1963.9	1.4	1357	1.37	1936.7	1.8	1082	1.38
50-75%	468.3	1.4	331	1.38	438.4	1.6	274	1.38
75-90%	372.3	1.8	207	1.40	414.7	2.5	168	1.48
90-100%	103.9	4.8	137	1.41	353.9	3.1	113	1.62

*Core range contours: 50%, 75% and 90% indicate the first smallest parcels by sort, 100% represent all parcels

Figure 3 shows the small size parcels located around the village centre after LC. Farmers who had small size parcels consumed less fuel and earned more comparing due to the shorter road before LC. Farm size was not a matter of this study; however, the size of parcels and the size of the farm are closely related (Assunçao & Ghatak 2003; Barrett et al 2010). In this context, parcel size was interpreted as a factor decreasing agricultural production.

In this case, it can be said that enterprises with small parcels could benefit. Lu et al (2018) mentioned that the economic examination owned by all enterprises have to be taken into consideration. In their study, when the Simpson index one of the fragmentation indices decreases by 1 unit, the average cost decreases by 39%. Their results indicated that enterprises are negatively affected by fragmentation. Lu et al (2018) also mentioned that 1 unit increase in parcel size ensures an 8% decrease in the average cost of an enterprise. In this study, the parcel size, which was 0.7 ha before LC, increased to 0.9 ha when the smallest parcel was examined for 50% core range contour. For 75% core range contour, the parcel size increased from 0.9 to 1.5 ha and within 90% core range contour, parcel size increased from 1.1 ha to 1.5 ha.

Moreover, the number of parcels decreased from 1357 to 1082. In this case, it was concluded that the fragmentation decreased and had an effect on the average parcel size. According to the previous study by Lu et al (2018), it can be said an increase of 0.4 ha leads to a 3.2% decrease in the average cost of the farmers in the village.

Small parcels may be shown with simple classification, but the density of small size parcels cannot be seen clearly in areas where they mostly gather (Figure 4). Kernel density analysis shows a good performance in clearly understanding the density of small size parcels and how they changed after LC contrary to the classification. Shortly, the figure is a strong evidence of kernel density analysis to show the density of parcels.

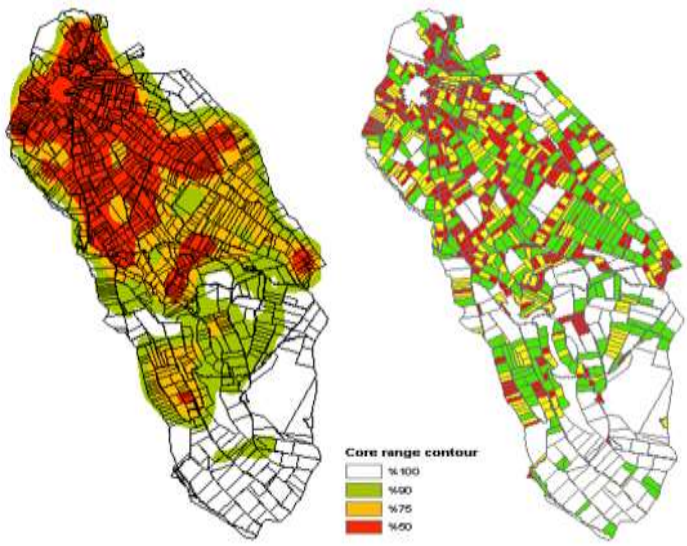

Figure 4- General view of kernel density and classification analysis

4. Conclusions

If small size parcels are reallocated around the village centre, it may help farmers with low-income. Sufficient agricultural land can be arranged according to the distance to the village centre. Engineers who are in charge of LC projects can take into consideration the distances of these small size parcels when they hold interviews with farmers. Farmers should be encouraged to farm together. These precautions may prevent migration to cities, thanks to increased production. Kernel density could be used to determine the frequency of parcels according to size. It is found that parcels have a high density within first bandwidth with a less complicated shape according to spatial distribution. After LC projects, it may be essential to reallocate small size parcels nearer to the village centre and to reshape the big size parcels to increase production. In this study, land fragmentation was not taken into consideration. As a conclusion, kernel density analysis can help managers to see change parcels location with size by sort in land consolidation projects. The analysis may assist engineers to do better projects with regard to spatial distribution of parcels before and after land consolidation. Future studies on this topic may be carried out on land fragmentation and conduct comparisons with sufficient agricultural land size and shape of enterprises.

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The Use of an Alternative Differential Set for Determination of *Pyrenophora teres* f. *maculata* Pathotypes

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ABSTRACT
Pyrenophora teres f. *maculata* incites spot form of barley net blotch disease. For determination of *Pyrenophora teres* f. *maculata* pathotypes, a differential set consisted of 22 international cultivars and genotypes and a susceptible Turkish barley variety Bülbül 89 were tested using 45 isolates obtained from different regions of Turkey. Nineteen pathotypes were determined out of 45 isolates used. It appears that this differential set could be useful for determination of *P. teres* f. *maculata* pathotypes.

Keywords: Spot form of net blotch; Barley differential set; *Drechslera teres* f. *maculata*

1. Introduction

Barley net blotch disease caused by the fungus *Pyrenophora teres* (anamorph: *Drechslera teres*) is a common and important disease which lowers the yield and quality of the barley in the world (Mathre 1982; McLean et al 2009; Karakaya et al 2014). *Pyrenophora teres* has two biotypes. *P. teres* f. *maculata* and *P. teres* f. *teres* incite spot and net forms of net blotch disease, respectively (Liu et al 2011). Resistant cultivars are preferred in disease control. However, pathotypes of the fungus complicate the resistance studies. In order to control the pathogen, information about the pathotypes of the fungus is necessary. For pathotype determination studies, different researchers used different cultivars and genotypes. However, most of the time, comparison of these pathotypes were difficult (Wu et al 2003; Grewal et al 2008; Boungab et al 2012; McLean et al 2014a; McLean et al 2014b). This study aimed at contributing to development of an international set for determination of *Pyrenophora teres* f. *maculata* (*Ptm*) pathotypes.

2. Material and Methods

This study was carried out in laboratory and greenhouse of Plant Protection Department of Faculty of Agriculture, Ankara University, Turkey.

Between 2015-2017 surveys were conducted in various provinces of Turkey and 1, 6, 5, 3, 1, 6, 2, 5, 2, 1, 2, 1, 1, 2, 1, 1, 2, 1, 1 and 1 *P. teres* f. *maculata* samples were obtained from Niğde, Diyarbakır, Ankara, Eskişehir, Adıyaman, Konya, Kırşehir, Şanlıurfa, Kayseri, Afyonkarahisar, Kahramanmaraş, Kırıkkale, Aksaray, Çankırı, Sivas, Yozgat, Mardin, Kilis, Edirne and Gaziantep provinces of Turkey, respectively. For obtaining samples, barley planting areas in

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each location were considered. In surveys, systematic sampling method was used (Aktaş 2001). Samples were obtained from a diverse set of provinces. Leaves showing characteristic spot form of net blotch symptoms were selected. These leaves were subjected to surface sterilization using 1% NaOCl for 1 minute and they were kept in blotter for 4-5 days. Under a stereomicroscope, single spores were taken and transferred to Potato Dextrose Agar plates. From diseased barley and wild barley (*Hordeum spontaneum*) plants 45 *Ptm* single spore isolates were obtained. Typical *Pyrenophora teres* f. *maculata* conidia were observed in a light microscope. Symptom morphologies of these isolates were verified using the susceptible barley cultivar Bülbül 89 (Mathre 1982; Çelik Oğuz & Karakaya 2017).

Barley differential cultivars and genotypes Chebec, Haruna Nijo, CI3576, Torrens, Keel, TR250, CI9214, Galleon, CI9819, CI11458, CI5286, CI5791, CI7584, CI9776, CI16150, Skiff, Steptoe, Kombar, Cape, Stirling, Summitt and Arimont were obtained from Mark S. McLean (Agriculture Victoria, Horsham, Australia). In addition, susceptible Turkish cultivar Bülbül 89 (Çelik Oğuz & Karakaya 2017) was added to the set.

Under greenhouse conditions, differential set genotypes were planted in plastic pots, 7 cm in diameter, containing topsoil. Each pot contained 5-10 seeds. There were three replications arranged in a completely randomized fashion. Ten days old cultures grown on Potato Dextrose Agar were used as inoculum. Fungal cultures were scraped using a paintbrush and washed through cheesecloth with water. Inoculum which consisted of mycelium pieces, was adjusted to $1.5\text{-}2.0 \times 10^5$ mycelium parts per mL. For each 100 mL of inoculum suspension, one drop of Tween 20 was added (Aktaş 1995). Inoculation of the barley seedlings were performed at the two to three leaf stages (Z12-13; Zadoks et al 1974). Fungal suspensions were sprayed onto barley differential set seedlings. Inoculated plants were kept in closed transparent lid boxes covered by transparent nylon covers for 72 h in a greenhouse at high humidity. The nylons were then removed and ventilation lids were opened for another 24 h. The temperature of the greenhouse was 18±1-23±1 ºC during night and day with a 14h/10h light/dark period. Following this period, box lids were opened. Seven days after inoculation, barley seedlings were assessed for disease severity using the spot form scale described by Tekauz (1985).

For pathotype determination, methods outlined in Wu et al (2003) and Çelik Oğuz & Karakaya (2017) were used. Seven days later following inoculation, plants were evaluated using a 1-9 scale developed by Tekauz (1985). For evaluation, second leaves were used. Scale values between 1-5 and 6-9 were considered as resistant and susceptible, respectively. Differential test genotypes were numbered 1 through 23 and pathotypes were determined according to their responses to these differential set genotypes. For example, isolate PTM 42 from Yozgat province showed susceptible reactions (>5) on genotypes 13 (Galleon), 18 (Steptoe) and 19 (Stirling) and showed resistant reactions (≤5) on the other differential set genotypes. Therefore, this pathotype was named as 13-18-19. Isolates exhibiting resistant reactions (≤5) to all differential test genotypes were termed as pathotype 0.

3. Results and Discussion

The scale values of 45 isolates ranged between 1-8 (Figure 1). Nineteen pathotypes were determined using 45 *Ptm* isolates based on their differential reactions to 23 barley genotypes (Tables 1 and 2). No genotype was either resistant or susceptible to all isolates. Genotypes Chebec, CI5286, CI7584, CI9819 and CI16150 exhibited resistant reactions to 43 isolates (95.5%). These genotypes were susceptible to only 2 isolates. Genotypes Arimont, CI5791, Skiff and TR250 showed resistant reactions to 42 isolates (93%). Genotypes CI3576, CI9214, CI9776 and Torrens exhibited resistant reactions to 91% of the isolates (41 isolates). Genotypes Cape, Keel, Galleon, Haruna Nijo, Kombar, Summitt, CI11458 and Stirling showed resistant reactions to 88%, 86%, 84%, 84%, 82%, 82%, 80 and 80% of the isolates, respectively. Cultivar Steptoe exhibited susceptible reaction to 18 isolates (40%) and cultivar Bülbül 89 showed susceptible reaction to 19 isolates (42%).

In previous studies, different differential test genotypes were used by different researchers. McLean et al (2014b) performed a study between 2008-2013 in Australia, South Africa, Finland and Canada and developed a new *Ptm* differential set. This set consisted of Arimont, Baudin, Beecher, Cape, Chebec, CI11458, CI3576, CI5286, CI5791, CI7584, CI9214, CI9776, CI9819, CI9831, CI16150, Galleon, Haruna Nijo, Keel, Kombar, Skiff, Steptoe, Stirling, Summitt, Torrens, TR250 and Yagan cultivars and genotypes. In this study, virulence diversity among the isolates was observed. Among these barley genotypes Arimont, Cape, Chebec, CI11458, CI5286, CI5791, CI7584, CI9214, CI9776, CI9819, CI16150, Galleon, Haruna Nijo, Keel, Kombar, Skiff, Steptoe, Stirling, Summitt, Torrens and TR250 were also used in our current study and it is concluded that these genotypes could be used as *Ptm* differential test genotypes.

Figure 1- Reactions of barley differential set genotypes to *Pyrenophora teres* f. *maculata* isolates according to Tekauz (1985) scale; R, resistant; R-MR, resistant-moderately resistant; MR, moderately resistant; MR-MS, moderately resistant-moderately susceptible; MS, moderately susceptible; MS-S, moderately susceptible-susceptible; S, susceptible

Using 11 differential set genotypes, Akhavan et al (2016) identified 13 pathotype groups out of 27 isolates used. Two groups contained 52% of the isolates. Wu et al (2003) used a differential set containing 25 barley genotypes. In their study, 4 pathotypes were distinguished among the 8 isolates. Tekauz (1990) used 11 barley differential set genotypes. From 42 isolates 20 pathotypes were distinguished. Using 16 differential set genotypes and 60 *Ptm* isolates, McLean et al (2014a) determined 33 pathotypes.

In Turkey, Çelik Oğuz & Karakaya (2017) used 25 differential set genotypes. From a total of 50 isolates, 26 *Ptm* pathotypes were determined. In our current study, we used 23 differential set genotypes and from a total of 45 isolates, 19 pathotypes were distinguished. Karki & Sharp (1986) used a differential set which consisted of 20 genotypes. In their study, 6 groups were evident among the 14 isolates used. Gupta et al (2012) used a differential set which consisted of 26 genotypes. In their study, 7 groups were found among the 49 isolates used.

In our current study, differential genotypes Chebec, CI5286, CI7584, CI9819 and CI16150 exhibited resistant reactions to 43 isolates (95.5%) and susceptible reactions to 2 isolates. Karki & Sharp (1986), using isolates obtained from Montana (USA) and other countries, reported different reactions on genotypes CI7584 and CI9819. McLean et al (2012) reported Chebec and CI16150 genotypes as moderately resistant. McLean et al (2014a) reported different reactions of the genotype CI5286 to the isolates.

Table 1- Response of barley differential genotypes to 45 *Pyrenophora teres* f. *maculata* isolates. For evaluation, a 1-9 scale developed by Tekauz (1985) was used. Numbers are mean of 3 replications. R, resistant; S, susceptible

	Isolate numbers and the provinces where the isolates obtained																	
Barley genotypes	*PTM 1*	*Konya*	*PTM 2*	*Şanlıurfa*	*PTM 3 Ankara*		*PTM 4 Kahramanmaraş*		*PTM 5 Aksaray*		*PTM 6*	*Mardin*	*PTM 7*	*Diyarbakır*	*PTM 8*	*Şanlıurfa*	*PTM 9*	*Eskişehir*
1 Arimont	5	R	3	R	3	R	5	R	5	R	5	R	1	R	2	R	1	R
2 Cape	3	R	3	R	3	R	5	R	3	R	5	R	3	R	1	R	3	R
3 Chebec	1	R	2	R	1	R	3	R	2	R	3	R	1	R	2	R	2	R
4 CI3546	3	R	3	R	5	R	5	R	5	R	5	R	2	R	2	R	2	R
5 CI11458	2	R	3	R	3	R	7	S	3	R	7	S	2	R	2	R	1	R
6 CI5286	3	R	2	R	1	R	5	R	2	R	3	R	2	R	1	R	1	R
7 CI5791	1	R	1	R	5	R	5	R	2	R	5	R	1	R	1	R	1	R
8 CI7584	2	R	3	R	1	R	5	R	2	R	5	R	1	R	2	R	2	R
9 CI9214	2	R	1	R	5	R	5	R	3	R	3	R	2	R	2	R	2	R
10 CI9776	1	R	1	R	2	R	5	R	3	R	5	R	1	R	1	R	2	R
11 CI9819	2	R	3	R	3	R	5	R	2	R	5	R	2	R	2	R	1	R
12 CI16150	2	R	1	R	3	R	5	R	5	R	3	R	1	R	2	R	2	R
13 Galleon	3	R	1	R	2	R	5	R	3	R	2	R	1	R	2	R	2	R
14 Haruna Nijo	3	R	2	R	3	R	5	R	5	R	7	S	3	R	2	R	2	R
15 Keel	3	R	1	R	3	R	3	R	5	R	3	R	2	R	1	R	1	R
16 Kombar	5	R	1	R	2	R	5	R	3	R	5	R	1	R	2	R	2	R
17 Skiff	3	R	2	R	2	R	3	R	3	R	3	R	1	R	2	R	1	R
18 Steptoe	5	R	3	R	3	R	5	R	5	R	7	S	2	R	3	R	1	R
19 Stirling	5	R	2	R	5	R	5	R	3	R	7	S	1	R	2	R	1	R
20 Summitt	3	R	2	R	3	R	7	S	2	R	2	R	2	R	1	R	1	R
21 Torrens	3	R	1	R	5	R	5	R	2	R	5	R	2	R	1	R	1	R
22 TR250	3	R	1	R	3	R	5	R	2	R	3	R	3	R	1	R	1	R
23 Bülbül 89	7	S	3	R	7	S	7	S	7	S	5	R	3	R	3	R	3	R

Table 1 (Continued)- Response of barley differential genotypes to 45 *Pyrenophora teres* f. *maculata* isolates. For evaluation, a 1-9 scale developed by Tekauz (1985) was used. Numbers are mean of 3 replications. R, resistant; S, susceptible

	Isolate numbers and the provinces where the isolates obtained																	
Barley genotypes	*PTM 10 Kayseri*		*PTM 11 Mardin*		*PTM 12 Hordeum spontaneum Kilis*		*PTM 13 Gaziantep*		*PTM 14 Konya*		*PTM 15 Ankara*		*PTM 16 Sivas*		*PTM 17 Hordeum spontaneum Şanlıurfa*		*PTM 18 Ankara*	
1 Arimont	3	R	5	R	5	R	7	S	1	R	3	R	5	R	8	S	5	R
2 Cape	1	R	7	S	5	R	5	R	1	R	2	R	3	R	8	S	3	R
3 Chebec	2	R	1	R	1	R	7	S	1	R	3	R	5	R	7	S	3	R
4 CI3546	2	R	7	S	5	R	5	R	3	R	3	R	3	R	7	S	3	R
5 CI11458	2	R	7	S	7	S	7	S	2	R	2	R	5	R	8	S	3	R
6 CI5286	1	R	5	R	5	R	5	R	1	R	2	R	3	R	7	S	3	R
7 CI5791	1	R	5	R	5	R	7	S	3	R	3	R	3	R	8	S	3	R
8 CI7584	2	R	3	R	5	R	5	R	1	R	3	R	2	R	7	S	2	R
9 CI9214	1	R	5	R	3	R	5	R	1	R	2	R	2	R	7	S	5	R
10 CI9776	1	R	5	R	5	R	7	S	1	R	3	R	3	R	7	S	7	S
11 CI9819	2	R	5	R	5	R	7	S	2	R	2	R	3	R	7	S	3	R
12 CI16150	2	R	5	R	5	R	8	S	2	R	2	R	2	R	7	S	5	R
13 Galleon	1	R	3	R	5	R	8	S	2	R	3	R	5	R	7	S	7	S
14 Haruna Nijo	1	R	5	R	7	S	7	S	1	R	1	R	3	R	8	S	5	R
15 Keel	2	R	3	R	5	R	8	S	1	R	3	R	5	R	7	S	5	R
16 Kombar	1	R	8	S	7	S	8	S	3	R	3	R	3	R	8	S	3	R
17 Skiff	2	R	3	R	3	R	7	S	2	R	5	R	5	R	7	S	5	R
18 Steptoe	1	R	5	R	5	R	8	S	2	R	7	S	7	S	8	S	7	S
19 Stirling	2	R	7	S	7	S	7	S	2	R	3	R	5	R	8	S	5	R
20 Summitt	2	R	7	S	7	S	7	S	2	R	3	R	3	R	8	S	5	R
21 Torrens	1	R	5	R	5	R	5	R	2	R	5	R	5	R	8	S	5	R
22 TR250	2	R	5	R	5	R	5	R	1	R	2	R	2	R	7	S	3	R
23 Bülbül 89	7	S	7	S	5	R	8	S	2	R	7	S	8	S	8	S	8	S

Table 1 (Continued)- Response of barley differential genotypes to 45 *Pyrenophora teres* f. *maculata* isolates. For evaluation, a 1-9 scale developed by Tekauz (1985) was used. Numbers are mean of 3 replications. R, resistant; S, susceptible

	Isolate numbers and the provinces where the isolates obtained																	
Barley genotypes	*PTM19*	*Eskişehir*	*PTM 20*	*Diyarbakır*	*PTM 21*	*Kahramanmaraş*	*PTM 22*	*Kayseri*	*PTM 23*	*Çankırı*	*PTM 24*	*Kırşehir*	*PTM 25*	*Afyonkarahisar*	*PTM 26*	*Edirne*	*PTM 27*	*Çankırı*
1 Arimont	2	R	3	R	5	R	1	R	5	R	2	R	2	R	5	R	5	R
2 Cape	2	R	3	R	5	R	1	R	3	R	1	R	2	R	7	S	3	R
3 Chebec	2	R	2	R	3	R	1	R	3	R	2	R	2	R	5	R	3	R
4 CI3546	2	R	5	R	5	R	2	R	5	R	2	R	2	R	7	S	3	R
5 CI11458	2	R	5	R	5	R	3	R	3	R	2	R	2	R	8	S	5	R
6 CI5286	2	R	3	R	3	R	1	R	3	R	1	R	2	R	5	R	3	R
7 CI5791	2	R	5	R	5	R	3	R	3	R	1	R	2	R	5	R	3	R
8 CI7584	1	R	5	R	3	R	2	R	3	R	1	R	2	R	5	R	3	R
9 CI9214	2	R	3	R	2	R	2	R	2	R	2	R	2	R	5	R	5	R
10 CI9776	1	R	5	R	2	R	1	R	3	R	1	R	1	R	7	S	3	R
11 CI9819	2	R	5	R	5	R	2	R	3	R	2	R	1	R	5	R	3	R
12 CI16150	2	R	5	R	2	R	1	R	3	R	1	R	2	R	5	R	3	R
13 Galleon	1	R	5	R	2	R	2	R	5	R	2	R	1	R	7	S	5	R
14 Haruna Nijo	1	R	3	R	5	R	2	R	3	R	1	R	2	R	7	S	3	R
15 Keel	3	R	2	R	1	R	1	R	3	R	2	R	1	R	5	R	7	S
16 Kombar	3	R	5	R	5	R	2	R	3	R	3	R	2	R	7	S	7	S
17 Skiff	3	R	3	R	3	R	3	R	5	R	3	R	1	R	5	R	5	R
18 Steptoe	5	R	5	R	3	R	2	R	7	S	3	R	3	R	7	S	8	S
19 Stirling	3	R	5	R	5	R	1	R	5	R	2	R	2	R	7	S	5	R
20 Summitt	2	R	3	R	3	R	2	R	3	R	3	R	1	R	7	S	3	R
21 Torrens	1	R	3	R	5	R	2	R	5	R	3	R	2	R	7	S	5	R
22 TR250	1	R	5	R	3	R	2	R	2	R	2	R	1	R	5	R	3	R
23 Bülbül 89	5	R	5	R	3	R	2	R	7	S	7	S	5	R	5	R	8	S

Table 1 (Continued)- Response of barley differential genotypes to 45 *Pyrenophora teres* f. *maculata* isolates. For evaluation, a 1-9 scale developed by Tekauz (1985) was used. Numbers are mean of 3 replications. R, resistant; S, susceptible

	Isolate numbers and the provinces where the isolates obtained																	
Barley genotypes	*PTM 28*	*Şanlıurfa*	*PTM 29*	*Şanlıurfa*	*PTM 30*	*Niğde*	*PTM 31*	*Ankara*	*PTM 32*	*Diyarbakır*	*PTM 33*	*Kırıkkale*	*PTM 34*	*Diyarbakır*	*PTM 35*	*Adıyaman*	*PTM 36*	*Ankara*
1 Arimont	5	R	3	R	1	R	1	R	5	R	1	R	1	R	1	R	2	R
2 Cape	7	S	5	R	1	R	1	R	5	R	1	R	1	R	2	R	1	R
3 Chebec	5	R	3	R	1	R	1	R	5	R	1	R	1	R	1	R	2	R
4 CI3546	7	S	3	R	1	R	2	R	3	R	1	R	2	R	3	R	1	R
5 CI11458	7	S	7	S	2	R	1	R	5	R	2	R	1	R	1	R	2	R
6 CI5286	5	R	3	R	2	R	1	R	3	R	1	R	1	R	2	R	1	R
7 CI5791	7	S	5	R	1	R	2	R	5	R	2	R	1	R	1	R	1	R
8 CI7584	7	S	5	R	1	R	1	R	5	R	2	R	2	R	2	R	1	R
9 CI9214	7	S	5	R	1	R	1	R	7	S	2	R	1	R	1	R	1	R
10 CI9776	5	R	5	R	1	R	1	R	5	R	2	R	1	R	1	R	2	R
11 CI9819	5	R	5	R	1	R	2	R	5	R	2	R	1	R	2	R	1	R
12 CI16150	5	R	5	R	1	R	1	R	3	R	1	R	1	R	2	R	2	R
13 Galleon	8	S	5	R	2	R	1	R	5	R	2	R	2	R	2	R	2	R
14 Haruna Nijo	7	S	5	R	1	R	1	R	5	R	2	R	2	R	2	R	2	R
15 Keel	7	S	5	R	2	R	1	R	5	R	2	R	2	R	2	R	2	R
16 Kombar	7	S	5	R	1	R	1	R	5	R	2	R	1	R	1	R	2	R
17 Skiff	7	S	3	R	1	R	1	R	5	R	2	R	2	R	2	R	2	R
18 Steptoe	7	S	5	R	2	R	5	R	5	R	7	S	5	R	2	R	3	R
19 Stirling	7	S	5	R	1	R	2	R	5	R	2	R	2	R	2	R	1	R
20 Summitt	7	S	5	R	1	R	1	R	5	R	2	R	1	R	2	R	1	R
21 Torrens	7	S	5	R	1	R	1	R	5	R	2	R	1	R	2	R	1	R
22 TR250	7	S	3	R	1	R	1	R	5	R	1	R	1	R	1	R	1	R
23 Bülbül 89	5	R	3	R	1	R	5	R	5	R	2	R	3	R	1	R	3	R

Table 1 (Continued)- Response of barley differential genotypes to 45 *Pyrenophora teres* f. *maculata* isolates. For evaluation, a 1-9 scale developed by Tekauz (1985) was used. Numbers are mean of 3 replications. R, resistant; S, susceptible

	Isolate numbers and the provinces where the isolates obtained																	
Barley genotypes	*PTM 37*	*Konya*	*PTM 38*	*Konya*	*PTM 39*	*Konya*	*PTM 40*	*Kırşehir*	*PTM 41*	*Diyarbakır*	*PTM 42*	*Yozgat*	*PTM 43*	*Diyarbakır*	*PTM 44*	*Konya*	*PTM 45*	*Eskişehir*
1 Arimont	3	R	1	R	1	R	1	R	7	S	5	R	2	R	2	R	5	R
2 Cape	3	R	2	R	2	R	1	R	7	S	5	R	1	R	3	R	3	R
3 Chebec	2	R	1	R	2	R	1	R	5	R	3	R	1	R	5	R	3	R
4 CI3546	2	R	2	R	3	R	1	R	5	R	2	R	5	R	3	R	3	R
5 CI11458	2	R	2	R	2	R	5	R	5	R	3	R	2	R	3	R	3	R
6 CI5286	2	R	1	R	1	R	1	R	7	S	3	R	5	R	3	R	3	R
7 CI5791	2	R	1	R	1	R	1	R	5	R	5	R	2	R	3	R	3	R
8 CI7584	2	R	1	R	1	R	1	R	5	R	5	R	2	R	3	R	3	R
9 CI9214	1	R	1	R	2	R	1	R	7	S	2	R	2	R	3	R	5	R
10 CI9776	2	R	2	R	2	R	1	R	5	R	3	R	2	R	3	R	3	R
11 CI9819	2	R	2	R	2	R	1	R	3	R	5	R	2	R	3	R	3	R
12 CI16150	5	R	2	R	1	R	1	R	5	R	3	R	2	R	3	R	2	R
13 Galleon	2	R	2	R	2	R	1	R	7	S	7	S	2	R	5	R	2	R
14 Haruna Nijo	2	R	1	R	2	R	2	R	7	S	3	R	2	R	3	R	5	R
15 Keel	3	R	3	R	3	R	2	R	7	S	5	R	3	R	7	S	5	R
16 Kombar	2	R	1	R	3	R	1	R	7	S	5	R	1	R	5	R	3	R
17 Skiff	2	R	2	R	3	R	3	R	5	R	5	R	2	R	3	R	5	R
18 Steptoe	7	S	7	S	7	S	5	R	7	S	7	S	2	R	7	S	7	S
19 Stirling	2	R	2	R	1	R	1	R	7	S	7	S	3	R	5	R	3	R
20 Summitt	2	R	2	R	2	R	1	R	7	S	3	R	3	R	5	R	3	R
21 Torrens	1	R	2	R	1	R	1	R	8	S	5	R	2	R	3	R	3	R
22 TR250	2	R	1	R	1	R	1	R	7	S	3	R	1	R	3	R	3	R
23 Bülbül 89	7	S	7	S	7	S	5	R	8	S	5	R	3	R	5	R	7	S

Table 2- Nineteen pathotypes of *Pyrenophora teres* f. *maculata* using a differential set containing 23 barley genotypes and isolate locations

Isolates	*Locations*	*Genotype numbers showing susceptible reactions/ Pathotype numbers*	*Numbers of susceptible genotypes*	*Virulence value*
PTM 30	Niğde	Pathotype 0	0	1.17
PTM 34	Diyarbakır			1.47
PTM 31	Ankara			1.52
PTM 9	Eskişehir			1.56
PTM 36	Ankara			1.60
PTM 35	Adıyaman			1.65
PTM 14	Konya			1.69
PTM 40	Kırşehir			1.69
PTM 7	Diyarbakır			1.73
PTM 8	Şanlıurfa			1.73
PTM 22	Kayseri			1.78
PTM 25	Afyonkarahisar			1.86
PTM 2	Şanlıurfa			1.95
PTM 19	Eskişehir			2.17
PTM 43	Diyarbakır			2.26
PTM 21	Kahramanmaraş			3.60
TM 20	Diyarbakır			4.04
PTM 33	Kırıkkale	Pathotype 18	1	1.91
PTM 10	Kayseri	Pathotype 23	1	1.82
PTM 24	Kırşehir			2.13
PTM 1	Konya			3.04
PTM 3	Ankara	Pathotype 23	1	3.17
PTM 5	Aksaray			3.34
PTM 29	Şanlıurfa	Pathotype 5	1	4.47
PTM 32	Diyarbakır	Pathotype 9	1	4.82
PTM 38	Konya	Pathotype 18-23	2	2.08
PTM 39	Konya			2.26
PTM 37	Konya			2.60
PTM 15	Ankara			3.17
PTM 45	Eskişehir			3.69
PTM 23	Çankırı			3.78
PTM 16	Sivas			3.91
PTM 44	Konya	Pathotype 15-18	2	3.82
PTM 42	Yozgat	Pathotype 13-18-19	3	4.30
PTM 4	Kahramanmaraş	Pathotype 5-20-23	3	5
PTM 27	Çankırı	Pathotype 15-16-18-23	4	4.39
PTM 6	Mardin	Pathotype 5-14-18-19	4	4.47
PTM 18	Ankara	Pathotype 10-13-18-23	4	4.47
PTM 12 *Hordeum spontaneum*	Kilis	Pathotype 5-14-16-19-20	5	5.08
PTM 11	Mardin	Pathotype 2-4-5-16-19-20-23	7	5.13
PTM 26	Edirne	Pathotype 2-4-5-10-13-14-16-18-19-20-21	11	6
PTM 41	Diyarbakır	Pathotype 1-2-6-9-13-14-15-16-18-19-20-21-22-23	14	6.21
PTM 28	Şanlıurfa	Pathotype 2-4-5-7-8-9-13-14-15-16-17-18-19-20-21-22	16	6.43
PTM 13	Gaziantep	Pathotype 1-3-5-7-10-11-12-13-14-15-16-17-18-19-20-23	16	6.65
PTM 17 *Hordeum spontaneum*	Şanlıurfa	Pathotype 1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-20-21-22-23	23	7.47

Genotypes CI3576, CI9214, CI9776 and Torrens exhibited resistant reactions to 91% of the isolates in our current study. These genotypes showed susceptible reactions to 4 isolates. Akhavan et al (2016) reported that CI9214 genotype was resistant to all *Ptm* isolates except two. In another study, genotypes CI9214 and CI9776 showed a resistant reaction to all isolates used (Karki & Sharp 1986). Differential cultivar Torrens exhibited moderately resistant-moderately susceptible reactions (McLean et al 2012) and different infection responses among the isolates were observed (McLean et al 2014a).

In our current study, Arimont, CI5791, Skiff and TR250 genotypes exhibited resistant reactions to 42 isolates (93%). These genotypes showed susceptible reactions to 3 isolates. Akhavan et al (2016) reported the virulence of 19 (70.4%) *Ptm* isolates on genotype CI5791. Cultivar Arimont was reported as susceptible in a previous study (Karki & Sharp 1986). Cultivar Skiff was reported as generally moderately resistant and genotype TR250 was reported as moderately susceptible (McLean et al 2012).

Cape, Keel, Galleon, Haruna Nijo, Kombar, Summitt, CI11458 and Stirling genotypes exhibited low infection responses to 88%, 86%, 84%, 84%, 82%, 82%, 80% and 80% of the isolates, respectively, in our current study. In other studies, cultivar Keel was found resistant to all isolates, however, genotypes Cape, CI11458 and Summitt were moderately susceptible and cultivar Galleon was moderately resistant. Cultivar Kombar exhibited a susceptible reaction to more than half of the isolates (McLean et al 2012, 2014a). Cultivar Stirling showed different reactions to different isolates (Gupta et al 2012).

In our current study, cultivar Steptoe was susceptible to 40% of the isolates. In Akhavan et al (2016) study, this cultivar was susceptible to 81.5% of the isolates.

Barley cultivars and genotypes Cape, CI11458, CI5791, CI7584, CI9819, Kombar and Bülbül 89 were also used in Çelik Oğuz & Karakaya (2017) study. In their study, genotypes Cape, CI11458, CI5791, CI7584, CI9819, Kombar and Bülbül 89 showed susceptible reactions to 10, 16, 9, 13, 10, 20 and 44 out of 50 isolates, respectively. In our current study, genotypes Cape, CI11458, CI5791, CI7584, CI9819, Kombar and Bülbül 89 exhibited susceptible reactions to 5, 9, 3, 2, 2, 8 and 19 out of 45 isolates, respectively.

In the current study, cultivars Steptoe and Bülbül 89 exhibited susceptible reactions to 18 and 19 isolates, respectively. These cultivars were the most susceptible cultivars. Cultivar Bülbül 89 could be used as universal susceptible genotype in an international *Ptm* differential set. The genotypes used in this study were useful in differentiating *Ptm* pathotypes.

4. Conclusions

For determination of *Pyrenophora teres* f. *maculata* pathotypes, a differential set consisted of 22 international cultivars and genotypes and a susceptible Turkish barley variety Bülbül 89 were tested using 45 isolates obtained from different regions of Turkey. Nineteen pathotypes were determined out of 45 isolates used. Cultivar Bülbül 89 could be used as universal susceptible genotype in an international *Ptm* differential set. The genotypes used in this study were useful in differentiating *Ptm* pathotypes.

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Effect of Fertilization on Weed Infestation, Morphological and Productive Traits of Different Alternative Small Grains

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ABSTRACT
This examination aims to determinate influence of fertilizers toward weed infestation, morphological and productive traits of different alternative small grains, as well as to examine the correlation of the studied traits of alternative small grains with weed infestation in organic production. The field experiment was conducted on luvic chernozem in completely randomized blocks, repeatad four times, in the three year period (2013/2014-2015/2016). Examination was carried out on one winter cultivars of naked barley, spelt, durum, compactum wheat and triticale, and the experiment included fertilization with microbiological fertilizer (5.0 L ha^{-1}) as well as the combined application of microbiological (5.0 L ha^{-1}) and organic fertilizer (3.0 t ha^{-1}). Results obtained pointed out that examined factors have important influence toward number and the dry weed weight, but the influence toward diversity weeds is negligible. The smallest the total number of weeds (12.7 plants m^{-2}), as well as the dry weight of weeds (18.5 g m^{-2}) was noted in the spelt ($P<0.01$). The considerably significant smaller the total number of weeds on the variant F_2 (14.7 plants m^{-2}) compared to F_1 (15.4 plants m^{-2}) and F_0 (15.5 plants m^{-2}), while dry weed weight on the variants F_1 (23.7 g m^{-2}) and F_2 (23.8 g m^{-2}) significant higher comparing to control F_0 (20.7 g m^{-2}). The use of fertilizers have significatly influenced the increase of the stem height (5.5-10.0%), spike length (6.4-9.9%), weight of the plant (9.5-20.8%) and the weight of grains in the spike (7.8-16.9%). The negative dependence of the weeds number ($r= -0.69$) and its dry weight ($r= -0.39$) related to the height of stem, and, also, negative dependence between weight of grains in the spike with the weed number ($r= -0.32$) has been perceived. These results of examination showed that proper selection of the genotypes with the application of fertilisers could have a significant effect on the weeds in the organic production alternative small grains.

Keywords: Alternative small grains; Organic farming; Fertilizers; Weed infestation

1. Introduction

The selection of species and cultivars of cereals suitable to specific conditions of organic agriculture demands different approach comparing to that used in conventional and intensive system of production (Przystalski et al 2008; Roljevic Nikolic et al 2018). This is because there are fewer opportunities in organic production to compensate for yield decrease caused by diseases, low nutrient levels, and weeds (Wolfe et al 2008). An important part of the weed control strategy in the organic production system is the selection of cultivars that compete with weeds for limited resources, produce exudates that inhibit growth of weeds and reduce economic losses by maintaining a high level of grain yield (Andrew et al 2015). During the last three decades the significant development was achieved in understanding the morphological and physiological traits of cereals which provide foundation for their competitive ability against weeds. Examinations conducted globally are pointing toward those cereal`s cultivars with fast initial growth, height of stem, large leaf surface and intensive tillering, to be the most competitive. It is determined that such a varietes are reducing the photosyntetic active radiation through their above-ground parts, creating shade for weeds and thus reducing their biomass and number

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(Korres & Froud-Williams 2002; Vandeleur & Gill 2004; Mason et al 2007; Zerner et al 2016). Therefore, the imperatives in organic production is knowledge of crop characteristics in order to establish stable production.

Apart from genotype, soil fertility and availability of nutrients can also affect the diversity and dynamics of the weed community (Jornsgard et al 1996; Blackshaw et al 2005). Garcia-Martin et al (2007) have noted that by reducing the availability of nutrients in the initial stages of vegetation, weeds are placed in an extremely unfavorable position in relation to crops, and in this respect organic sources of nutrients can have an impact on the weed community.

On the other hand, some studies conducted with mineral fertilizers have shown that higher levels of their application positively affect the seed germination (Jornsgard et al 1996) and the weed number (Ross & Van Acker 2005), and that the mineral fertilization have a stimulating effect on weed infestation. When it comes to weed biomass, Jornsgard et al (1996) state that weed biomass in winter wheat could be increased, unchanged, or reduced with increased soil N depending on weed and crop. The aim of this research is to determine if in the terms of organic production, the use of fertilization might influence weed infestation, morphological and productive traits of alternative small grains, simulatenously, investigate connection between traits of different alternative small grains and the weed infestation.

2. Material and Methods

This research was carried out on „Radmilovac“ (44º45’N, 20º35’E Serbia) experimental field of the Faculty of Agriculture in Belgrade, during the period 2013/2014-2015/2016. The average annual air temperature in this period was 12.7 °C, while the average rainfall was 670.6 mm.

A two-factor field trial was arranged using the completely randomized blocks was employed in four replications, with the area of the elementary plot amounting to 6 m^2. The soil type was luvic chernozem (WRB 2014), with the following characteristics: pH-(H_2O) 8.04, total content of N 13%, available forms of phosphorus 22.18 mg P_2O_5 100 g^{-1} dry weight and potassium 19.10 mg K_2O 100 g^{-1} dw, content of humus in the topsoil layer 2.45%. Winter cultivars of different alternative small grains are grown in four crop rotation: maize → winter wheat → spring barley+red clover → red clover. Previous crop was maize (*Zea mays* L.) in all three seasons.

Tillage method with the plough was carried out at a depth of 25 cm, from 15-20 October during all three years. Pre-sowing preparation was carried out by disc harrow and harrow. The sowing was done manually, from 20-25 October.

The examined factors were different species of alternative small grains and fertilization. Five domestic cultivars of different alternative small grains are included in this research: *Hordeum vulgare* var. *nudum* (cv. Golijat, 450 seeds m^{-2}); *Triticum durum* (cv. Dolap, 600 seeds m^{-2}); *Triticum aestivum* ssp. *compactum* (cv. Bambi, 600 seeds m^{-2}); *Triticum aestivum* ssp. *spelta* (cv. Nirvana, 550 seeds m^{-2}); *Triticale* sp. (cv. Odisej, 550 seeds m^{-2}).

Two varinats of fertilization were applied: F_1 - application of foliar microbiological fertilizer in the spring, in phenofase BBCH 31-33 (5.0 L ha^{-1}), F_2 - ploughing under biohumus in autumn (3.0 t ha^{-1}) and application of foliar microbiological fertilizer in the spring in phenofase BBCH 31-33 (5.0 L ha^{-1}). Control (F_0) was without of fertilizer. Properties of organic fertilizer (commercial name „Biohumus Royal offert“, producer „Altamed“ Serbia) was: pH 8.63, minimum content of: N 2.2%. P_2O_5 4.8% and K_2O 2.8%. Features of microbiological fertilizer (commercial name „Slavol“, producer „Agrounik“ Serbia) was: *Bacillus megaterium* 10^{-6} cm^3, *Bacillus licheniformis* 10^{-6} cm^3, *Bacillus suptilis* 10^{-6} cm^3, *Azotobacter chtoococcum* 10^{-6} cm^3, *Azotobacter vinelandi* 10^{-6} cm^3, *Derxia* sp. 10^{6} cm^3.

Weed infestation is followed by: floristic composition of weed community, number of perennial species and number of annual species (species m^{-2}), number od weeds (plants m^{-2}) and dry weed weight (g m^{-2}) noticed during the whole examined period. The floristic composition of weed community is determined by method of squared before small grains heading.

Weeds were cut and roots discarded. The weight was measured (g m^{-2}) in fresh, and after drying in natural conditions, along with the weight in air dry condition. Measures for weed suppression were not carried out.

During all three years of examination, seven to ten days before harvest, 10 plants were randomly sampled in order to determine the morphological and productive traits of all alternative small grains in each variant of fertilization. The

following parameters were measured: (1) the stem height to the spike (cm), (2) the length of the spike (cm), (3) the weight of stem and the spike–weight of plants (g), (4) the weight of grains per spike (g).

Data processing was performed in Statistica statistical package. A method for analyzing variance (*F test*) for dual factor examination was used, and the significance of differences between treatments was tested with the LSD test at significance level $P<0.01$ and $P<0.05$. The method of simple linear correlation and regression analysis was used to examine the dependence between the parameters tested.

3. Results and Discussion

3.1. Floristic composition of weed community of diferent alternative small grains in organic production

After the observation during this three-year period, it can be concluded that a weed community consists of relatively large number of species (26) in alternative small grains, representing one of organic field production characteristics. Annual weeds make 61.5%, and perennial 38.5% of the total number of recorded species. According to Koocheki et al (2009), in organic and rational crop cultivation systems, perennial weeds account for 66% and 56% of the total weed population, while in intensive crop cultivation systems they are significantly lower. During all three years of the examined period, preceding crop was maize (*Zea mays* L), row crop, thus explaining the higher representation of annual weeds. The most prevalent annual weed are *Stellaria media* (L.) Vill., *Veronica persica* Poir., *Capsella bursa pastoris* L., and perennial *Agropyrum repens* L., *Lepidim draba* L. and *Convolvulus arvensis* L. (Table 1). These weed species infestate a lot of various crops, particularly cereals and row crops.

Table 1- Floristic composition of weed community in cereals

Weed species	*Fertilization*		
	F_0	*F_1*	*F_2*
Annual weeds (m^{-2})			
Apium graveolens L.	2.0		2.0
Ambrosia artemisiifolia L.	3.3		1.5
Avena fatua L.	2.8	3.0	
Bilderdykia convolvulus (L.) Dum.	2.0	2.0	1.9
Capsella bursa pastoris (L.) Medic.	2.5	2.3	2.3
Chamomila recutita L.	1.5	1.5	1.0
Chenopodium album L.		2.0	1.8
Consolida regalis S.F. Gray	2.5	2.0	1.0
Erigeron canadensis L.	1.5	1.8	1.5
Galium aparine L.			2.0
Papaver rhoeas L.		1.0	2.0
Polygonum aviculare L.	1.8	2.5	2.0
Sinapis arvensis L.	2.1	2.7	1.6
Sonchus oleraceus L.	1.5	2.0	1.9
Stellaria media (L.) Vill.	3.2	4.3	3.9
Veronica persica Poir.	4.6	2.5	3.4
Perennial weeds (m^{-2})			
Agropyrum repens (L.) Beauv.	5.3	2.3	6.0
Cirsium arvense (L.) Scop.	2.0	2.9	3.3
Convolvulus arvensis L.	4.3	2.5	3.2
Euphorbia cyparissias L.	1.0		
Lepidium draba L.	5.0	2.8	3.5
Rubus caesius L.			3.0
Sonchus arvensis L.	1.8	2.0	
Sorghum halepense (L.) Pers.	1.5	1.0	2.3
Taraxacum officinale Weber.	3.0		1.0
Trifolium pratense L.	3.0	3.1	3.0
Total number of weed species	22	20	23
Total number of weeds (no. m^{-2})	58.1	46.1	55.1
Number of annual weeds (no. m^{-2})	31.3	29.6	29.8
Number of perennial weeds (no. m^{-2})	26.8	16.5	25.3
Dry weed weight (g m^{-2})	20.7	23.7	23.8

The proper selection of species and cultivars of crops is an important part of the strategy in the action against weeds, especially in production systems characterized by reduced protection (Andruszczak et al 2012; Andrew et al 2015). The results of the study have shown that the genotype has a slight influence on the diversity of the weed community in conditions of organic production. Only statistically significant differences in the number of perennial weeds were established, whereby it is with the spelt (0.9 species m^{-2}) significantly lower compared to triticale (2.0 species m^{-2}) and compactum wheat (1.8 species m^{-2}) (Table 2). Examining the impact of different tillage methods on the weed community in organic production Sans et al (2011) have established a smaller share of perennial weeds in the crop of spelt compared to common wheat. Research conducted by other authors is also indicative of the lesser weed infestation of spelt compared to other cereals (Szewczyk 2013).

Table 2- Weed infestation of winter cultivars various cereals

Fertilizer	*F_O*	*F_1*	*F_2*		*F_O*	*F_1*	*F_2*	
Genotype	*The number of perennial weeds (species m^{-2})*			*Average*	*The number of annual weeds (species m^{-2})*			*Average*
Golijat	1.5	0.7	1.8	1.3	4.3	4.8	4.3	4.4
Dolap	1.2	2.3	1.5	1.6	4.5	3.0	4.0	3.8
Bambi	1.8	2.0	1.5	1.8	3.8	3.8	4.3	3.9
Nirvana	0.6	1.8	0.5	0.9	3.5	4.0	4.3	3.9
Odisej	1.4	2.1	2.5	2.0	3.8	3.5	3.8	3.7
Average	1.3	1.8	1.6		3.9	3.8	4.1	
	The total number of weeds (plants m^{-2})			*Average*	*Dry weed weight (g m^{-2})*			*Average*
Golijat	15.5	18.8	15.5	16.6	24.9	26.2	25.0	25.4
Dolap	16.6	18.5	15.5	16.7	30.8	21.9	26.1	26.2
Bambi	14.3	13.5	14.8	14.2	13.6	21.9	25.2	20.2
Nirvana	13.3	12.8	12.0	12.7	12.1	18.0	25.4	18.5
Odisej	18.3	13.5	15.5	15.8	22.2	30.2	17.3	23.3
Average	15.5	15.4	14.7		20.7	23.7	23.8	

On the other hand, the number of weeds and their dry weight was significantly influenced by investigated factors as their interaction is producing significant effect (Table 3). The results indicate significant differences between the alternative small grains in the number of weeds, with the largest number being recorded in durum wheat (16.7 plants m^{-2}) and the lowest in spelt (12.7 plants m^{-2}) (Table 2). A significant influence of fertilization on the total number of weeds was determined, whereby it smaller on the variant F_2 (14.7 plants m^{-2}) compared to F_1 (15.4 plants m^{-2}) and F_0 (15.5 plants m^{-2}) ($P<0.05$). In these production conditions, the GxF interaction showed a very significant impact on the number of weeds, which was most pronounced in triticale, as the number of weeds on varieties F_1 and F_2 was 26.2% and 15.3% lower compared to control. On the other hand, in naked barley and durum wheat number of weeds at F_1 variant was significantly higher in comparison with the F_0 (21.3% and 11.4%), while in spelt and compactum wheat the obtained differences between the variants of fertilization were not significant.

During the examination of weed infestation among crops of winter spelt wheat cultivars grown under different conditions of mineral fertilization and chemical plant protection, Andruszczak et al (2012) have underlined that application of higher rates of mineral fertilizers have slightly increased the number of weeds. Similar results are noticed by other authors (Jornsgard et al 1996).

The analysis of the variance of dry weeds weight indicates that there are very significant differences between the investigated genotypes (Table 3). The highest dry weight of the weed was recorded in durum wheat (26.2 g m^{-2}), and the lowest in the spelt (18.5 g m^{-2}) (Table 2). A large dry weight of the weed recorded in the cv. Dolap was expected, given the origin of durum wheat and its adaptation to a warmer climate with less precipitation (Kaya & Turkoz 2016). On the other hand, examinations of other authors also indicate the high competitiveness of spelt against weeds in conventional (Andruszczak et al 2012; Szewczyk 2013) and organic production (Zuk-Golaszewska et al 2015).

Fertilization is a factor that significantly affects the weeds weight (Table 3). Very significant differences were found between control (20.7 g m^{-2}) and fertilization variants (23.7 g m^{-2} and 23.8 g m^{-2}) (Table 2). Also, the obtained

differences between fertilization variants in the examined alternative small grains were significant (GxF) (Table 2 and Table 3). The largest differences were found in the compactum wheat and spelt, wherein the dry weight of the weed on the F_1 variant was higher by 61.0% and 48.8%, while in the variant F_2 to 85.3% and 109.9% compared to control. Significantly lower dry weight of the weed on varieties with applied fertilizers compared to control was recorded only in hard wheat (28.9% and 15.3%). On the other hand, some authors have not noticed significant differences in the biomass of weeds between different levels of mineral fertilizers, because in such circumstances it was dependent on both crops and the types of weeds (Jornsgard et al 1996; Andruszczak et al 2012).

Table 3- The statistical significance of differences of the tested parameters (*F* test and LSD test)

Parameters	*Factor/Interaction*		*G*	*F*	*GxF*
The number of perennial weeds (species m^{-2})	*F* test		*	ns	ns
	LSD	0.05	0.725	-	-
		0.01	0.994	-	-
The number of annual weeds (species m^{-2})	*F* test		ns	ns	ns
	LSD	0.05	-	-	-
		0.01	-	-	-
The total number of weeds (plants m^{-2})	*F* test		**	*	**
	LSD	0.05	0.828	0.642	1.435
		0.01	1.136	0.880	1.967
Dry weed weight (g m^{-2})	*F* test		**	**	**
	LSD	0.05	0.433	0.612	0.866
		0.01	0.594	0.840	1.187
Stem height (cm)	*F* test		**	**	ns
	LSD	0.05	2.178	3.080	-
		0.01	2.986	4.223	-
Spike length (cm)	*F* test		**	**	ns
	LSD	0.05	0.280	0.396	-
		0.01	0.384	0.543	-
Weight stem and length (g)	*F* test		**	**	ns
	LSD	0.05	0.198	0.280	-
		0.01	0.271	0.384	-
Weight of grains per spike (g)	*F* test		**	**	ns
		0.05	0.07	0.09	-
		0.01	0.09	0.13	-

G-genotypes (Golijat, Dolap, Bambi, Nirvana, Odisej); F-fertilizers (F_0 control, F_1 microbiological fertilizer, F_2 organic + microbiological fertilizer); ns, P>0.05, *, P<0.05, **, P<0.01

3.2. Morphological and productive traits of different alternative small grains in organic production

The height of the stem, as well as the overall height of the plant, represents an important traits for cereals, significantly influencing the formation of total yield (Zerner et al 2016). In the framework of the investigated alternative small grains, the spelt is distinguished by the highest stem (94.2 cm) and the longest spike (10.3 cm). The largest plant weight and grain weight in the spike were recorded in triticale, cv. Odisej (4.1 g and 1.6 g). On the other hand, the weight of the plant (2.0 g) and the grain weight in the spike (0.9 g) of naked barley (cv. Golijat), is significantly lower compared to other genotypes (Table 4).

Significant differences in the investigated traits were also established between fertilization variants. The use of fertilizers significantly influenced the increase in the height of the stem (5.5-10.0%), the length of the spike (6.4-9.9%), the plant weight (9.5-20.8%) and the grain weight in the spike (7.8-16.9%), in particular the variant F_2. Jablonskytė-Raščė et al (2013) have found that the combined application of organic fertilizer and bio-activators significantly affects

the length and weight of spike of common and compactum wheat. Significantly higher weight of grains per spike wheat on intensively fertilized treatments was obtained by Jaćimović et al (2008).

Table 4- Morphological and productive traits of winter cultivars tested cereals

Fertilizer	*F0*	*F1*	*F2*	*Average*	*F0*	*F1*	*F2*	*Average*
Genotype	*Stem height (cm)*				*Spike length (cm)*			
Golijat	55.6	59.3	62.1	59.0	8.5	9.5	9.6	9.2
Dolap	63.7	64.9	67.2	65.3	6.7	6.8	6.9	6.8
Bambi	74.0	81.8	84.8	80.2	4.0	4.1	4.1	4.1
Nirvana	90.8	93.1	98.8	94.2	9.7	10.2	10.8	10.3
Odisej	73.7	78.3	80.8	77.6	9.4	10.1	10.6	10.0
Average	71.6	75.5	78.7		7.7	8.1	8.4	
	Weight stem and length (g)			*Average*	*Weight of grains per spike (g)*			*Average*
Golijat	1.9	2.0	2.1	2.0	0.9	0.9	0.9	0.9
Dolap	2.9	3.1	3.4	3.1	1.3	1.4	1.6	1.4
Bambi	3.3	3.7	4.0	3.7	1.4	1.5	1.6	1.5
Nirvana	2.8	2.9	3.3	3.0	1.3	1.4	1.6	1.4
Odisej	3.6	4.1	4.7	4.1	1.5	1.6	1.8	1.6
Average	2.9	3.2	3.5		1.3	1.4	1.5	

Although the GxF interaction did not have a significant impact on the examined morphological and productive traits, the application of fertilizers showed a positive effect on all types of alternative small grains. The best effect of fertilization on the stem height, the weight stem and length, as well as the weight of grains per spike was found in the compactum wheat, while the biggest difference between fertilization variants in the spike length was recorded in the naked barley.

3.3 Correlation and regression analysis

The results of the correlation analysis indicate a positive correlation between the morphological and productive properties of alternative small grains (Table 5). In addition, the negative correlation between the height stem and the number of weeds m^{-2} (- 0.69), as well as the dry weeds weigh (- 0.39) is noticed. Regression analysis is often used biostatistik technique in plant breeding (Pakize et al 2015). Based on the regression equation, it was found that the increase in the height stem by 1 cm leads to a decrease in the number of weeds for 0.11 plants m^{-2} ($\hat{y}i= 23.77–0.11* xi$) and the dry weed weight for 0.16 g m^{-2} ($\hat{y}i= 34.86–0.16* xi$). The dependencies between the parameters examined were very significant, which was determined by testing the obtained coefficients of simple linear correlation: the dependence of the number of weeds on the height stem (r= - 0.69) and the dependence of dry weight of weeds on the height stem (r= - 0.39). In the tests of some authors (Korres & Froud-Williams 2002; Konvalina et al 2007; Hoad & Davies 2008) it is affiliated that cultivars with high stem reduce the penetration of photosynthetic active radiation to lower levels, whereby the weeds become shadowed, and consequently their number and biomass decrease in relation to shorter cultivars of cereals. Thus, the shorter cultivars are less suitable for organic production (Mason et al 2007). Also, a negative correlation relationship between the number of weeds and the grain weight in the spike (- 0.32) was established, where the regression equation showed that by increasing the number of weeds per 1 plant m^{-2} the grain weight in the spike decreases by 0.04 g ($\hat{y}i= 1.999-1.041 * xi$). By testing the linear correlation coefficient, a statistically significant dependence was found between these two parameters (r= - 0.32). In organic wheat production

negative correlation of weed biomass with productive cereal properties has been determined (Mason et al 2007; Roljević Nikolić et al 2017).

Table 5- Correlation matrix of tested parameters

Weed Parameters	*No. of perennial weeds*	*No. of annual weeds*	*No. of weeds*	*Dry weed weight*	*Stem height*	*Spike length*	*Weight stem and length*	*Weight of grains per spike*
No. of perennial weeds	1	0.37**	0.27^{ns}	-0.04^{ns}	-0.11^{ns}	-0.15^{ns}	0.29^{ns}	0.22^{ns}
No. of annual weeds	0.37**	1	0.30*	0.26^{ns}	-0.14^{ns}	0.03^{ns}	-0.25^{ns}	-0.27^{ns}
No. of weeds	0.27^{ns}	0.30*	1	0.21^{ns}	-0.69**	-0.05^{ns}	-0.23^{ns}	-0.32*
Dry weed weight	-0.04^{ns}	0.26^{ns}	0.21^{ns}	1	-0.39**	0.04^{ns}	-0.11	-0.13
Stem height	-0.11^{ns}	-0.14^{ns}	-0.69**	-0.39**	1	0.17^{ns}	0.52**	0.61**
Spike length	-0.15^{ns}	0.03^{ns}	-0.05^{ns}	0.04^{ns}	0.17^{ns}	1	-0.12^{ns}	-0.11^{ns}
Weight stem and length	0.29^{ns}	-0.25^{ns}	-0.23^{ns}	-0.11^{ns}	0.52**	-0.12^{ns}	1	0.93**
Weight of grains per spike	0.22^{ns}	-0.27^{ns}	-0.32*	-0.13^{ns}	0.61**	-0.11^{ns}	0.93**	1

LSD α 0,05/2 =1,96; α 0,01/2 =2,58

4. Conclusions

The results of this study showed that in the organic alternative small grains production the use of proper fertilization and selection of the cultivars with adequate morphological and productive traits, might create significant influence on the number and dry weight of weeds, while the impact on the diversity of the weed community is negligible. In addition, the application of the examined fertilizer variants has a very positive effect on the studied morphological and productive properties of grain. A negative correlation between the stem height and the number (- 0.69) and dry weight of weeds (- 0.39) was established. Consequently, the smallest number (13.7 plants m^{-2}) and dry weight of weeds (18.5 g) were recorded in the spelt, which has the highest stem (94.2 cm) within the investigated cultivars. The obtained results indicate that the height stems has a significant contribution to the suppression of weeds. On the other hand, the weight of grains per spike depends on the number of weeds (- 0.32), which indicates that the mechanisms that contribute to control and suppression of weeds is of key importance for the establishment of stable production in organic agriculture.

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Combining Abilities and Heterotic Groups for Seed Yield and Yield Components in Pea (P*isum sativum* L.)

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ABSTRACT
The aim of this study was to investigate the combining ability and hybrid performance for seed yield and yield components in pea (*Pisum sativum* L.) genotypes. Field experiments were carried out in 2016-2017 during the winter growth period at Bursa Uludag University,Faculty of Agriculture,Agricultural Research and Application Center in Bursa, Turkey. In the study, four female (Debrecen3, Sel 3-25, USA5, and Vesela) and five male (Ardahan, Gap Pembesi, Kirazli, Milwa, and USA1) pea genotypes were used as parents. The all plants were planted on November 8, 2016. The experiment was carried out in the randomized complete block design with three replications. Plant height (cm), pods per plant (number), seeds per pod (number), seeds per plant (number), seed yield (g plant^{-1}), and 1000-seed weight (g) of the plants were determined. In conclusion, the Sel 3-25, Ardahan, Gap pembesi, and Kirazli genotypes were decided to be suitable for use in future hybridization studies for seed yield due to high general combining ability. Also, Debrecen3xUSA1, Sel 3-25xGap pembesi, Sel 3-25xKirazli, USA5xArdahan, USA5xMilwa, and VeselaxKirazli hybrids were determined for seed yield as the best hybrid combinations due to high specific combining ability.

Keywords: Pea; Seed yield; Yield components; Combining ability; Heterotic groups

1. Introduction

Pea (*Pisum sativum* L.) is an important plant. Because it has high adaptability and seed yield, seeds and hay are used as animal fodder, seeds have excellent potential as a cheap source of high-quality protein (Kahraman et al 2015; Shaalan et al 2018). It is a good crop rotation plant and it can be used as green fertilizer plant (Uzun & Acikgoz 1998). Number of the studies related to peas has increased because of pea's superior qualities. However, in order to improve the yield potential in forage pea, it is crucial to develop new genotypes. High genetic diversity in pea is important for success in the development of a new cultivar. The genetic variation among pea cultivars is low due to the limited number of parent plants used in breeding programs and the narrow gene pool (Espósito et al 2007; Şimşek & Ceyhan 2017). In self-pollinated plants like pea, hybridization is one of the applications used to increase genetic variation (Askandar et al 2018). It is very important to selection suitable female and male parents to development of high yielding genotypes. Combining ability analysis provides is a substantial way of selecting superior parents in plant breeding (Bisht & Singh 2011; Kosev 2013; Kumar et al 2017; Kafadar et al 2019). In general, high-quality cultivars are obtained from parents with high combining ability. Studies of combining ability, such as general combining ability and specific combining ability, are used to determine the hybrid performance of parent lines (Kosev 2013; Ceyhan et al 2014). The general combining ability is related to the additive effects of genes, while the specific combining ability is related to the dominance effects of genes

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(Sarode et al 2009; Ceyhan & Kahraman 2013). The general combining ability of the parent plants helps developing genotypes with superior characteristics, while the specific combining ability gives information about the performance of the hybrid combinations (Cruz & Regazzi 1994; Maarouf 2009; Sarode et al 2009). Heterosis, is the basis of hybrid breeding. In hybrid breeding, superior hybrids are selected by investigating their hybrid vigour. Heterosis and heterobeltiosis are of great importance in establishing the hybrid progenies (Yadav et al 2015). Heterosis is the superiority of the hybrid (F1) over its parents in terms of a certain trait. Heterobeltiosis is the superiority of the hybrid progeny over the superior parent. In self-pollinating plants like pea, hybridization is difficult and the use of heterosis in this plants is quite limited. Nevertheless, heterosis is used to determine the superiority of the hybrid progenies in a new variety (Rebika 2017). The Line x Tester analysis is one of the methods used in the determination of the general and specific combining abilities and hybrid vigor (Ceyhan & Avci 2005; Rashid et al 2007). In this method, the parents used as female are called lines and the parents–used as male are called testers. This method gives information about the effects of general and specific combining abilities and the genetic mechanism controlling the seed yield and some other traits (Espósito et al 2013; Mishra et al 2014). In the method, line and tester are crossed in all combinations. The combining ability with heterosis and heterobeltiosis values for seed yield and yield characteristics in pea has been reported by many previous research (Zaman & Hazarika 2005; Kosev et al 2012; Kosev 2014; Mishra et al 2014; Kosev & Naydenova 2015; Joshi et al 2015; Askandar et al 2018). In their studies on pea, Bisht & Singh (2011), Kosev (2013), and Rebica et al (2013) stated that general combining ability was important in seed yield and yield characteristics. Borah (2009) and Kumar et al (2017) found that some pea hybrids had significant specific combining ability effects for yield and yield characteristics. Sarawat et al (1994), Ceyhan & Avci (2005), and Rebika (2017) also determined significant values, which is positive or negative, for heterosis and heterobeltiosis in pea.

The aim of this study was to determine the amount of heterosis in twenty hybrids obtained from four line and five tester genotypes and to select parents and crosses having good combining abilities.

2. Material and Methods

The study was carried out in the experimental fields of the Bursa Uludag University, Faculty of Agriculture, Department of Field Crops in Bursa, Turkey (40°11′ North, 29°04′ East) during the 2016-2017 growing season. During the growing period (November-July) total precipitation was 461.00 mm; average temperature was 12.23 °C; relative humidity was 71.78%. The average long-term total precipitation during the plant growth period was 581.7 mm; the mean temperature was 13.0 °C, and the relative humidity was 69.6%. The study soil was clay-loam, non-saline, lime-poor, and had neutral reactive, very low in organic matter content, and sufficient amounts of available potassium and phosphorus.

In the experiment, four female (Debrecen3, Sel 3-25, USA5, and Vesela) and five male (Ardahan, Gap Pembesi, Kirazli, Milwa, and USA1) field pea genotypes were used. All female genotypes were leafed and white flowers. Origins of Debrecen3, Sel 3-25, USA5 and Vesela were Hungary, Turkey, USA and Bulgarian, respectively. Among Turkey origin male genotypes, Ardahan and Gap pembesi were leafed and purple flowers, while Kirazli was semi-leafless and had purple flower. The flower color of the Milwa, Poland origin, and USA1, USA origin, genotypes were pink. The Milwa was semi-leafless and USA1 was leafed. The hybridizations were carried out in 2013-2015 according to the Line X Tester method in the greenhouse and field conditions. The parents and the F1 hybrids were sown by hand on 8 November 2016. The experimental design was a randomized complete block with three replications. There were four-rows in a plot. The row length was 2 m while row to row was 70 cm and plant to plant 50 cm. Before seeding, 30 kg N ha^{-1} fertilizer was applied as fertilizer. Weeds were controlled by hand four times. No irrigation was applied during the growing season. A herbicide (cypermethrin) was applied twice against Bruchus at the time of flowering. The plant height (cm), pods per plant, seeds per pod, seeds per plant, seed yield per plant (g) and 1000 seed weight (g) were determined for all plants at harvest time. For the data obtained with the experiment, a variance analysis was performed according to the randomized block design and the JUMP package program was used for the analysis. The 1% and 5% probability levels were used in the significance tests. The least significant difference (LSD) was calculated at the 5% probability level. The statistically-different groups were determined using the LSD test. The combining ability analysis was carried out as suggested by Singh & Chaudhary (1977). For this purpose, the data was analyzed using the TARPOPGEN package program. The significance of heterosis and heterobeltiosis values of hybrids were determined at 0.05 and 0.01 levels using t test.

3. Results and Discussion

In the study, the plant height (cm), pods per plant, seeds per pod, seeds per plant, seed yield per plant (g) and 1000 seed weight (g) of the parents and hybrids were determined. In addition, combining ability and heterosis values of the genotypes were established. The variance analysis results (mean square) of plant height, pods per plant, seeds per pod, seeds per plant, seed yield per plant and 1000 seed weight are given in Table 1. As seen in the Table 1, the plant height, pods per plant, seeds per pod, seeds per plant, seed yield per plant and 1000 seed weight values of the genotypes were significant at the 1% level. All traits of the parent genotypes were significant at the 1% probability level. The 1000 seed weight of the parents versus crosses were significant at the 5% level, while the plant height, pods per plant, seeds per pod, seeds per plant and seed yield per plant values were significant at the 1% level. The crosses were significant at the 1% probability level for all characteristics. The difference between the lines was significant at the 5% probability level only for 1000 seed weight values. The plant height, pods per plant, seeds per plant and 1000 seed weight of the testers were significant at the 1% probability level. The line x testers were statistically significant 1% probability level for all characteristics. The mean values of the plant height, pods per plant, seeds per pod, seeds per plant, seed yield per plant and 1000 seed weight for the crosses and parent plants are shown in Table 2. The mean values of the crosses were higher than the mean values of the parent genotypes for all characteristics. Similar results were also obtained in previous studies (Ceyhan & Avci 2005; Ceyhan et al 2008; Ceyhan & Kahraman 2013; Mishra et al 2014; Kumar et al 2017; Şimşek & Ceyhan 2017; Shaalan et al 2018). The VeselaxUSA1 and Sel 3-25xArdahan crosses had the highest plant height. The highest number of pods per plant was obtained from the Sel 3-25xUSA1 and Debrecen3xArdahan crosses. The maximum number of seeds per pod was determined from the Debrecen3 line. The Sel 3-25xUSA1 cross had the highest seeds per plant value. The highest seeds per plant was obtained from the Vesela line and Sel 3-25xGap pembesi and Sel 3-25xKirazli crosses. The 1000 seed weight of USA5xUSA1 cross was the highest. The general combining and specific combining ability related to seed yield and seed yield components in pea are given Table 3. The Debrecen3 line had positive significant general combining ability effect for the number of seeds per pod. Female parent the Sel 3-25 line gave significant positive general combining ability effect for pods per plant, seeds per plant, seed yield per plant and 1000 seed weight. The USA5 genotype had a significantly positive general combining ability effect for 1000 seed weight. Significant general combining effect for plant height and pods per plant were obtained from the Vesela line. The Ardahan tester showed positive significant general combining ability effect for plant height, pods per plant, seeds per plant and seed yield per plant characteristics. Male parent the Gap pembesi had positive significant general combining ability effect for plant height, seed yield per plant and 1000 seed weight. The Kirazli tester showed positive significant general combining ability effect for plant height and seed yield per plant. Positive significant general combining effect for 1000 seed weight were determined from Milwa genotype. Male parent the USA1 tester gave significant positive general combining ability effect for pods per plant, seeds per plant and 1000 seed weight (Table 3). In this study, at least one parent genotype had a significantly positive general combining ability effect for all characteristics. The parent genotypes with a significantly positive general combining ability effect contribute to the increase in the investigated characteristics in hybridization studies. A significant general combining ability indicate the importance of additive gene effects. Ceyhan & Avci (2005), Zaman & Hazarika (2005), Ceyhan et al (2008), Bisht & Singh (2011), Kosev (2013), and Rebica et al (2013) reported that the parent genotypes had significant general combining ability for above traits. Positive and significant specific combining effects were Debrecen3xArdahan, Sel 3-25xArdahan, USA5xGap pembesi, USA5xKirazli, VeselaxMliwa and VeselaxUSA1 hybrids for plant height. The Debrecen3xArdahan, Sel 3-25xUSA1, USA5xGap pembesi, USA5xKirazli and USA5xMilwa hybrids showed positive significant specific combining ability effect for pods per plant. The VeselaxArdahan hybrid for seeds per pod; Debrecen3xArdahan, Sel 3-25xUSA1, USA5xArdahan, USA5xGap pembesi, USA5xMilwa, VeselaxKirazli and VeselaxUSA1 hybrids for seeds per plant; Debrecen3xUSA1, Sel 3-25xGap Pembesi, Sel 3-25xKirazli, USA5xArdahan, USA5xMilwa and VeselaxKirazli hybrids for seed yield per plant; Sel 3-25xGap Pembesi, Sel 3-25xKirazli, Sel 3-25xMilwa, USA5xUSA1, VeselaxArdahan and VeselaxKirazli hybrids for 1000 seed weight had positive and significant specific combining effect (Table 3). Significant special combining ability explain that non-additive effects (dominant or epistatic) are important in the inheritance of investigated characters. Significant specific combining ability effects of hybrids is very important in selecting the cross. In previous studies with pea, significant specific combining ability effects have determined (Borah 2009; Kosev 2013; Mishra et al 2014; Kumar et al 2017). The heterosis and heterobeltiosis values with respect to the plant length, pods per plant, seeds per pod, seeds per plant, grain yield, and 1000-grain weight values of the hybrids are shown in Table 4. The heterosis values varied between -9.9 and 135.4% and the heterobeltiosis values varied between -28.1 and 82.7% for plant height. Both the heterosis and heterobeltiosis values of the plant height trait of the Debrecen3xArdahan, Sel 3-25xArdahan and USA5xArdahan hybrids were positive and statistically significant. In the study, heterosis values ranged from -41.8 to 331.0% and heterobeltiosis values ranged from -58.0 to 173.8% for pods per plant. The Debrecen3xArdahan, Sel 3-25xArdahan, Sel 3-25xUSA1,

USA5xArdahan, VeselaxArdahan, and VeselaxUSA1 hybrids had positive significant heterosis and heterobeltiosis values for pods per plant. The heterosis and heterobeltiosis values of all hybrids were not statistically significant for seeds per pod. Positive and significant heterosis and heterobeltiosis values were observed for Debrecen3x Ardahan, Sel 3-25xArdahan, Sel 3-25xGap pembesi, Sel 3-25xUSA1, USA5xArdahan, VeselaxArdahan, and VeselaxUSA1 hybrids in seeds per plant. For this trait, the heterosis values varied between -33.5 and 664.7% and the heterobeltiosis values varied between -45.7 and 251.7%. The heterosis and heterobeltiosis values ranged between -70.3 and 194.3% and -79.9 and 178.3% for seed yield per plant, respectively. The heterosis (194.3%) and heterobeltiosis (178.3%) values of Sel 3-25 x Kirazli hybrid and only heterosis (135.8%) value of the Sel 3-25xGap pembesi hybrid were positive and statistically significant for seed yield per plant. Generally, hybrids with positive specific combining ability have given positive heterosis and heterobeltiosis values in this study. Sel 3-25xArdahan hybrid had negative and significant (-36.5%) and USA5xUSA1 hybrid had positive and significant (32.9%) heterosis values for 1000 seed weight. Negative and significant heterobeltiosis values were observed for USA5xKirazli (-22.8%) and VeselaxArdahan (-35.4%) hybrids in 1000 seed weight (Table 4). Hybrids that have significant and positive or negative specific combining ability and heterosis and heterobeltiosis values are expected to show a high variability in the investigated traits in the following generations. In future selection studies, the use of hybrid populations with a wide genetic variability will lead to faster genetic improvement and successful results. In their studies on peas, Sarawat et al (1994), Ceyhan & Avci (2005), Ceyhan et al (2008), Joshi et al (2015), and Rebika (2017) determined positively and negatively significant heterosis and heterobeltiosis values for seed yield and yield components.

Table 1- Analysis of variance for Line x tester in pea (mean squares)

Source of variation	*Degre of freedom*	*Plant height (cm)*	*Pods per plant (no.)*	*Seeds per pod (no.)*	*Seeds per plant (no.)*	*Seed yield per plant (g)*	*1000 seed weight (g)*
Replications	2	148.2767**	25.3134^{ns}	0.4445^{ns}	1401.1351^{ns}	16.9657^{ns}	23.0445^{ns}
Genotypes	28	4875.9222**	7336.9817**	1.7530**	99742.3378**	1394.4133**	10289.0805**
Parents	8	4164.1053**	2039.0312**	2.7224**	16546.3490**	1624.0960**	11184.4031**
Parents vs crosses	1	22951.2618**	21273.1139**	8.1791**	473890.3562**	1117.1174**	295.1376*
Crosses	19	4224.3010**	8834.2171**	1.0066**	115080.2269**	1312.2994**	10438.0995**
Lines	3	3045.2586^{ns}	5365.2798^{ns}	1.4961^{ns}	61172.6218^{ns}	2220.5307^{ns}	9543.7372*
Testers	4	12133.1972**	30027.9021**	0.7317^{ns}	344914.7057**	952.5595^{ns}	34696.5091**
Line x Testers	12	1882.7628**	2636.8898**	0.9758**	51945.6532**	1205.1548**	2575.5535**
Error	56	28.4234	35.1313	0.3021	533.4480	54.1769	57.5032

ns, non significant; *, significant at P= 0.05 probability level; **, significant at P= 0.01 probability level

Table 2- Means seed yield and seed yield components in pea

Parents	*Plant height (cm)*	*Pods per plant (no.)*	*Seeds per pod (no.)*	*Seeds per plant (no.)*	*Seed yield per plant (g)*	*1000 seed weight (g)*
Lines						
Debrecen3	41.83 r	13.50 q	7.88 a	75.25 o	18.82 n	138.87 m
Sel 3-25	50.32 qr	17.32 pq	5.49 i-k	141.50 mn	30.51 k-n	231.25 e
USA5	46.13 r	15.88 q	6.75 b-h	80.23 o	43.87 f-j	255.03 cd
Vesela	139.00 ef	62.11 gh	5.00 k	242.09 h-j	99.34 a	155.47 l
Tester						
Ardahan	91.25 l	87.50 d	6.00 h-j	306.50 g	59.45 c-e	68.15 p
Gap pembesi	117.79 ij	61.67 gh	6.27 f-i	192.63 kl	49.27 e-i	221.27 ef
Kirazli	129.00 gh	49.17 i-k	6.50 c-h	158.33 l-n	34.24 jk	180.03 k
Milwa	62.33 op	37.50 lm	4.72 k	153.33 mn	37.89 h-k	252.10 d
USA1	77.35 m	64.00 fg	6.00 h-j	210.25 jk	35.53 jk	207.50 gh
Crosses						
Debrecen3xArdahan	149.50 cd	173.83 a	6.33 e-i	694.92 b	55.92 c-e	88.15 o
Debrecen3xGap pembesi	123.22 hi	35.17 l-n	7.55 ab	237.11 ij	31.86 j-m	204.17 hi
Debrecen3xKirazli	108.15 k	21.70 o-q	7.20 a-e	123.35 n	32.38 j-l	176.87 k
Debrecen3xMilwa	61.15 op	29.31 m-o	7.18 a-e	151.00 mn	31.94 j-m	218.80 fg
Debrecen3xUSA1	75.00 mn	72.25 ef	7.33 a-c	245.00 h-j	62.88 cd	205.00 hi
Sel 3-25xArdahan	166.67 a	158.67 b	6.83 b-h	560.50 cd	52.00 d-g	95.05 o
Sel 3-25xGap pembesi	140.88 d-f	81.75 de	6.48 c-h	362.63 f	94.06 a	243.98 d
Sel 3-25xKirazli	122.17 hi	56.73 g-j	6.38 d-i	269.66 g-i	95.29 a	228.75 ef
Sel 3-25xMilwa	67.38 no	40.85 kl	7.34 a-c	172.38 lm	62.26 cd	266.70 bc
Sel 3-25xUSA1	88.83 l	175.25 a	7.00 a-g	739.50 a	49.88 e-h	205.35 hi
USA5xArdahan	154.61 bc	134.17 c	6.75 b-h	576.25 c	75.29 b	123.03 n
USA5xGap pembesi	161.50 ab	48.50 jk	6.83 b-h	274.25 g-i	55.57 c-f	206.30 h
USA5xKirazli	146.46 c-e	40.09 kl	6.02 h-j	139.50 mn	37.46 i-k	196.90 h-j
USA5xMilwa	68.92 m-o	58.22 g-i	7.13 a-f	275.00 gh	66.56 bc	275.05 b
USA5xUSA1	55.63 pq	26.88 n-p	6.42 d-h	142.88 mn	21.75 l-n	307.57 a
VeselaxArdahan	149.67 c	164.05 b	7.25 a-d	524.91 de	52.42 d-g	100.47 o
VeselaxGap pembesi	135.00 fg	53.60 h-j	7.00 a-g	289.83 g	58.02 c-e	193.15 ij
VeselaxKirazli	126.33 g-i	58.00 g-j	6.25 f-i	243.33 h-l	66.21 bc	188.15 jk
VeselaxMilwa	110.88 jk	29.00 m-o	6.13 g-i	131.50 n	41.84 g-k	198.78 h-j
VeselaxUSA1	168.00 a	126.25 c	5.17 jk	504.00 e	20.00 mn	156.70 l

Table 3- General combining ability (GCA) and specific combining ability (SCA) related to seed yield and seed yield components in pea

Parents	*Plant height (cm)*	*Pods per plant(no.)*	*Seeds perpod (no.)*	*Seeds per plant (no.)*	*Seed yield per plant(g)*	*1000 seed weight(g)*
Lines						GCA
Debrecen3	-15.593**	-12.781**	0.389**	-42.599**	-10.185**	-15.348**
Sel 3-25	-1.811	23.417**	0.077	88.058**	17.519**	14.023**
USA5	-1.574	-17.663**	-0.102	-51.299**	-1.854	27.826**
Vesela	18.978**	7.027**	-0.364*	5.84	-5.481**	-26.521**
Tester						
Ardahan	36.113**	78.447**	0.062	256.271**	5.727**	-92.269**
Gap pembesi	21.154**	-24.379**	0.237	-41.92**	6.699**	17.956**
Kirazli	6.781**	-35.104**	-0.269	-138.915**	4.655*	3.723
Milwa	-41.916**	-39.888**	0.221	-150.405**	-2.529	45.881**
USA1	-22.132**	20.924**	-0.251	74.97**	-14.553**	24.71**
Crosses						SCA
Debrecen3xArdahan	9.98**	28.932**	-0.85**	148.371**	7.195	1.823
Debrecen3xGap pembesi	-1.338	-6.905*	0.197	- 11.245	-17.836**	7.614
Debrecen3xKirazli	-2.035	-9.648**	0.35	-28.01*	-15.272**	-5.452
Debrecen3xMilwa	-0.335	2.746	-0.163	11.13	-8.522*	-5.677
Debrecen3xUSA1	-6.272*	-15.125**	0.466	-120.245**	34.435**	1.693
Sel 3-25xArdahan	13.368**	-22.429**	-0.035	-116.703**	-24.425**	-20.648**
Sel 3-25xGap pembesi	2.543	3.48	-0.561	-16.385	16.664**	18.061**
Sel 3-25xKirazli	-1.797	-10.816**	-0.158	-12.36	19.938**	17.061**
Sel 3-25xMilwa	-7.893*	-21.912**	0.309	-98.15**	-5.909	12.853**
Sel 3-25xUSA1	-6.221*	51.677**	0.444	243.598**	-6.268	-27.327**
USA5xArdahan	1.074	-5.85	0.06	38.404**	18.238**	-6.467
USA5xGap pembesi	22.923**	11.309**	-0.033	34.595*	-2.457	-33.426**
USA5xKirazli	22.257**	13.62**	-0.344	-3.16	-18.523**	-28.592**
USA5xMilwa	-6.59*	36.538**	0.277	143.83**	17.764**	7.399
USA5xUSA1	-39.664**	-55.617**	0.039	-213.669**	-15.022**	61.087**
VeselaxArdahan	-24.421**	-0.653	0.825*	-70.072**	-1.009	25.293**
VeselaxGap pembesi	-24.129**	-7.884*	0.396	-6.964	3.63	7.751
VeselaxKirazli	-18.425**	6.844	0.152	43.531**	13.857**	16.984**
VeselaxMilwa	14.818**	-17.372**	-0.424	-56.81**	-3.333	-14.574**
VeselaxUSA1	52.157**	19.066	-0.949**	90.316**	-13.145**	-35.453**

*, significant at p=0.05 probability level; **, significant at p=0.01 probability level

Table 4- Heterosis (H_s) (%) and heterobeltiosis (H_b) (%) values of hybrids for seed yield and seed yield componenets in pea

Crosses	*Plant height (cm)*		*Pods per plant (no.)*		*Seeds per pod (no.)*		*Seeds per plant (no.)*		*Seed yield per plant (g)*		*1000 seed weight (g)*	
	H_s	H_b	H_s	H_b	H_s	H_b	H_s	H_b	H_s	H_b	H_s	H_b
DxA***	124.7**	63.8**	244.2**	98.7**	-8.8	-19.7	664.7**	126.7**	42.9	-5.9	-14.8	-36.5
DxGP	54.4*	4.6	-6.4	-42.9	6.6	-4.2	77.0	23.1	-6.4	-35.3	13.4	-7.7
DxK	26.6	-16.2	-30.8	-55.9	0.1	-8.6	5.6	-22.1	22.1	-5.4	10.9	-1.8
DxM	17.4	-1.9	14.9	-21.8	13.9	-8.9	32.1	-1.5	12.6	-15.7	11.9	-13.2
DxU1	25.9	-3.1	86.5	12.9	5.6	-6.9	71.6	16.5	131.4	76.9	18.4	-1.2
SxA	135.4**	82.7**	202.8**	81.3**	10.9	13.8	352.0**	82.9**	15.6	-12.5	-36.5*	-58.9
SxGP	67.5**	19.6	106.9*	32.6	10.2	3.4	117.0*	88.3*	135.8*	90.9	7.8	5.5
SxK	36.3	-5.3	70.6	15.4	6.3	-1.9	79.9	70.3	194.3*	178.3*	11.2	-1.1
SxM	19.6	8.1	49.0	8.9	43.6	33.7	16.9	12.4	82.1	64.3	10.4	5.8
SxU1	39.1	14.8	331.0**	173.8**	21.7	16.7	320.5**	251.7**	51.2	40.4	-6.4	-11.2
U5xA	125.1**	69.4**	159.6**	53.3*	5.8	0	198.0**	88.0**	45.7	26.6	-23.9	-51.8
U5xGP	97.1**	37.1*	25.1	-21.4	4.9	1.2	101.0	42.4	19.3	12.8	-13.4	-19.1
U5xK	67.3**	13.5	23.2	-18.5	-9.2	-10.8	16.9	-11.9	-4.1	-14.6	-9.5	-22.8*
U5xM	27.1	10.6	118.1	55.3	24.2	5.6	135.5	79.4	62.8	51.7	8.5	7.9
U5xU1	-9.9	-28.1	-32.7	-58.0	0.6	-4.9	-1.6	-32.0	-45.2	-50.4	32.9**	20.6
VxA	30.0	7.7	119.3**	87.5**	31.8	20.8	91.4**	71.3**	-33.9	-47.2	-10.1	-35.4*
VxGP	5.1	-2.9	-13.4	-13.7	24.1	11.6	33.3	0.2	-21.9	-41.6	2.5	-12.7
VxK	-5.7	-9.1	4.2	-6.6	8.7	-3.9	21.5	0.5	-0.9	-33.4	12.2	4.5
VxM	10.1	-20.2	-41.8	-53.3	26.1	22.6	-33.5	-45.7	-39.0	-57.9*	-2.5	-21.2
VxU1	55.3**	20.9	100.2**	97.3**	-6.0	-13.8	120.8**	108.2**	-70.3	-79.9**	-13.7	-24.5

***, DxA= Debrecen3xArdahan; DxGP= Debrecen3xGap pembesi; DxK= Debrecen3xKirazli; DxM= Debrecen3xMilwa; DxU1=Debrecen3xUSA1; SxA=Sel 3-25xArdahan; SxGP=Sel 3-25xGap pembesi; SxK=Sel 3-25xKirazli; SxM=Sel 3-25xMilwa; SxU1=Sel 3-25xUSA1; U5xA= USA5xArdahan; U5xGP= USA5xGap pembesi; U5xK=USA5xKirazli; U5xM=USA5xMilwa; U5xU1=USA5xUSA1; VxA=VeselaxArdahan; VxGP=VeselaxGap pembesi; VxK=VeselaxKirazli; VxM=VeselaxMilwa; VxU1=VeselaxUSA1; *, significant at p=0.05 probability level; **, significant at p=0.01 probability level

4. Conclusions

As a result, Sel 3-25, Ardahan, Gap pembesi, and Kirazli parents had significant general combining ability and these parents should be used in future hybridization studies. In addition, Debrecen3xUSA1, Sel 3-25xGap pembesi, Sel 3-25xKirazli, USA5xArdahan, USA5xMilwa, and VeselaxKirazli hybrids which have significant general combining ability and heterosis values might be considered promising for future breeding studies.

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Effects of Different Germinated seeds Flour on Mineral, Phytic Acid and Total Phenolic Content of Cookies

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ABSTRACT
The effects of germination at different germination period (1, 3 and 5 days) on physical, chemical and nutritional properties of three different seeds (wheat, rye and lentil) were investigated. Germination caused significant increase in ash, protein, total phenolic content, minerals (Ca, Mg, Fe, Zn, K, P) and phytic acid loss. As the germination period increased, L* values decreased, a*, b* and SI values increased. Germinated seed flours (GSF) were substituted for wheat flour at different ratios (0, 5, 10 and 15%) in cookie formulation to improve nutritional properties. The addition of GSF in cookie formulation gave lower phytic acid content than the control cookie sample. The highest calcium and magnesium content of the cookies were determined with germinated rye flour (GRF); the highest iron, potassium and zinc value were obtained with germinated green lentil flour (GGLF). The use of 5 % level had caused similar taste values to the control cookie samples.

Keywords: Germinating; Mineral; Phytic acid; Total phenolic content

1. Introduction

Cereals that are used in products such as bread, pasta and cookies and had a very important place in human nutrition due to the energy are the edible constituent of the grain. Legumes can be used effectively to meet the protein deficiency required for healthy and balanced nutrition in the world and in our country they are our important plant sources. Various processes (fermentation, germination, autoclaving, milling, heat application) are applied in order to ensure more efficient use of this important plant sources thus nutritional content is enriched and antimicrobial factors are reduced. Nowadays, the production and consumption of the germinated products in the world are increasing in both variety and quantity, as a result of the tendencies towards functional foods. One of the main reasons for this is that the germination process is not expensive and does not require complicated equipment (Lorenz 1980). In literature, some of the nutritionally important components such as vitamins, minerals, dietary fiber, flavonoids, phenolic acids and many antioxidant components, omega 3 type of fatty acids of plant seeds and cereals sprouts increases during germination, and also increase the functional properties of the products are stated (Siro et al 2008; Öztürk 2008). Wheat sprouts compared to wheat; in addition to higher vitamin content, it is stated that it has higher phenolic substance, higher quality protein, more aromatic amino acids and more polyunsaturated fatty acids (Öztürk 2008; Yang 2000). The phytase activity increases (Reddy et al 1982) and the phytic acid content decreases and the digestibility of the fibers and proteins changes during germination. Furthermore, the increase in amino acids and ascorbic acid contributes to the bioavailability of trace minerals (Lintschinger et al 1997). During germination, beta carotene, vitamin C and E content is increasing (Yang 2000).

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In studies, rye (Katina et al 2007), lentils (Urbano et al 1995), beans, peas (López-Amorós & Estrella 2006), cowpea, chickpeas and mung beans (Ghavidel & Prakash 2007), oats (Wilhelmson et al 2001), paddy (Kim et al 2012), lupine (Cunha-Queda & Beirao da Costa 1994), barley (Sung et al 2005), brown rice (Moongngarm & Saetung 2010) germinated.

Nutritional properties increased with germination process and germinated seeds are stated to be valuable resources of natural bioactive compounds and antioxidants. The amount of anti-nutrients decreased during the germination and after the germination, compounds with beneficial and phytochemical properties were formed. These substances are particularly important in many diseases prevention such as cancer. Thus, functional foods that protection of health and positively affected on human health can be developed by germination.

In this study, cereals and legumes (wheat, rye and green lentils) were germinated at different germination period (1, 3 and 5 days), dried and milled. Flours of cereal and legumes seeds were replaced by wheat flour at different ratios (0, 5, 10 and 15%) and cookie production was carried out to improve nutritional and functional properties.

2. Material and Methods

2.1. Material

To germinate the seeds; wheat, rye and green lentil were purchased from local market in Konya, Turkey. Wheat flour, shortening, powdered sugar, salt vanilla and sodium bicarbonate were purchased from local markets in Konya for cookie production.

2.2. Germination of seeds

Wheat, rye and lentil seeds were cleaned from all foreign material, broken and diseased grains. Seeds were germinated according to the jar method. Briefly, 100 g of cleaned seeds were placed in 500 mL of 0.07% sodium hypochlorite (NaClO) for 30 min to sanitize and then washed ten times in tap water to neutralize pH and filtered with excess water. Then, the seeds were soaked in 1000 mL distilled water for 5 hours and shaken for 30 min so as to draw water. The jars were covered with a thin cheese cloth so that the seeds were contacted with air. Then water (kernels: water ratio of 1:2) was added. After rinsing several times, the water was removed from the jar. The seeds were sprouted in the dark at room temperature with watering every 12 h for 1, 3, 5 days. At the end of 1, 3, 5 days germinated seeds were dried in 50 ºC (Nüve FN-400) and grounded in a hammer mill. Ungerminated seeds were used as a control (0 day). The germinated seed flours (GSF) and wheat flour were placed in a large polyethylene bag and mixed thoroughly by hand.

2.3. Production of cookies

To prepare cookie samples; AACCI (Approved Method No: 10-54.01) method were used with some modifications. The dough was prepared in a laboratory mixer following a standard formulation by addition level of 0, 5, 10, and 15% GSF (5 day germinated wheat, rye and green lentil flour). As a result of the preliminary experiments, it was decided that the 5-day germination period was the most suitable GSF for the use of cookie production. 100 g wheat flour (according to 14% moisture content), 40 g shortening, 40 g powdered sugar, 2 g sodium metabisulphite, 1.25 g salt, 1 g milk powder 0.5 g vanillin and variable water were mixed for 10 min at 125 rpm. Cookie dough were sheeted to 5 mm thickness with a rolling pin and shaped with a dough cutter (50-mm diameter) and baked on aluminum trays in an oven (Profilo, HG1503T) at 150 °C for 16 min. The cookies were cooled for 30 min at room temperature and immediately used for further analyses.

Cookies were evaluated for physical characteristics, including diameter (mm) and thickness (mm) measured with a digital micrometer (0.001 mm, Mitutoyo, Minoto-Ku, Tokyo, Japan). Spread ratio was calculated as diameter divided by thickness of the cookies.

The cookie hardness and fracturability were investigated using the standard method AACC 74-09.01 by texture analyzer (TA-XT plus, Stable Microsystems, England). Hardness (as fracture force) of cookies with 3-point bending test using 3-point bending rig, trigger force of 50 g, and load cell of 5 kg. (Pretest speed: 1.0 mm s^{-1}, test speed: 3.0 mm s^{-1}, posttest speed: 10.0 mm s^{-1}, distance: 5 mm).

2.4. Color measurements

Konica Minolta Chroma Meter (Model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) was used for color measurement of cookies. The surface color of cookie was determined as average L* (lightness), a* (redness), b* (yellowness) values. a* and b* values were used to calculate the saturation index (SI) ($SI = \sqrt{a*^2 + b*^2}$) and hue angle ($H = \tan^{-1}(b*/a*)$) values.

2.5. Proximal composition

Chemical composition of ungerminated seeds, GSF and cookie samples were determined following AACCI method (AACC 2000), for moisture (44-12), ash (08-01.01) and protein (46-12.01).

2.6. Nutritional analysis

Mineral content (potassium, magnesium, calcium, phosphorus, iron, and zinc) was determined in cookies using a wet digestion with closed system according to Skujins (1998) and results were expressed in mg 100 g^{-1} sample. Phytic acid content of cookie samples was analyzed according to Haugh & Lantzsch (1983) using colorimetric method. Total phenolic content of the cookies were determined based on Folin-Ciocalteu colorimetric method as described by Gao et al (2002) and Beta et al (2005).

2.7. Sensorial evaluation

Cookies were submitted to sensory analysis by twenty (20) semi-trained panelists (50% male, 50% female) that familiar with the quality aspects of baked products. Panelists evaluated typical attributes for cookie such as color, appearance, friability, taste, odor and general appreciation using a 5-point hedonic scale.

2.8. Statistical analysis

The significance of the variations observed among cookie samples was tested according to three way analysis and one way analysis of variance (ANOVA) using software program (JUMP, version 5.0).

3. Results and Discussion

The characteristics of GSF at 3 different germination periods were shown in Table 1. A significant ($P<0.01$) difference in L*, a*, b* and SI values were obtained according to germinated seeds and germination period as a variance factors. Lightness values ranged from 79.31 for GRF to 82.73 for germinated wheat flour (GWF). According to germinated seeds variance, while GWF showed the highest lightness value, germinated rye flour (GRF) and germinated green lentil flour (GGLF) had the highest redness and yellowness, respectively. GGLF also showed the highest saturation index and hue angle vales among the GSF. After 5 day germination period, lightness values decreased, while the redness, yellowness and saturation index values increased. As expected, decrease in lightness, probably due to the Maillard reaction (between free amino acids and sugar content) during drying. The increase of the saturation index value could be due to the increase of reducing sugar during germination. Similarly Öztürk (2008) reported a decrease in L* values and increase in a* and b* values after germination. Likewise, Ertaş (2015) and Shin et al (2013) observed similar color changes for lupine and soy bean, respectively.

According to ANOVA results, germinated seeds and germination period factors affected the ash, protein, phytic acid and total phenolic content of GSF ($P<0.01$) significantly. Ash content of GSF varied between 1.26 and 2.80%. GGLF possessed higher (2.80%) ash content than the other GSF while the lowest ash values determined with GWF. Increasing germination period increased the ash content of GSF and the biggest change in ash content was observed after 5 day of germination. GGLF showed the highest protein content among the samples. This is due to its high protein content of green lentil compared to the other grains. During germination, increasing in protein content was observed. This result is in accordance with the study of Rosa-Millán et al (2019) who reported the significant increase in protein content of germinated bean compared to ungerminated bean. Total phenolic contents of GSF changed between 2056.38 mg GAE kg^{-1} (for GWF) and 3377.00 mg GAE kg^{-1} (for GGLF).

After 5 day germination period significantly higher total phenolic content of GSF (57.17%) was observed in comparison to ungerminated seed. Tarzi et al (2012) described that the phenolic compounds of acetone, hexane and methanolic extracts of germinated chickpeas were increased as 53.7, 60.1 and 92.8% during the germination, respectively. During germination marginal increase (104.95%) was observed with GGLF (data not shown). In a study of Zilic et al (2014) total phenolic content of 5 day germinated wheat sample was increased from 1431 mg GAE kg^{-1} to 1627 mg GAE kg^{-1}. Also an increase in total phenolic content in germinated rye sample compared to raw rye sample reported before by Katina et al (2007). Yeo & Shadidi (2017) found that the total phenolic content of lentil seed as 6.75 GAE mg g^{-1}.

Table 1-Effects of germinated seeds and germination period on the color and nutritional properties of germinated seeds[1]

Properties	*Germinated seeds flour*			*Ungerminated*	*Germination period (day)*		
	GWF[2]	*GRF*	*GGLF*	*seed (mean)*	*1*	*3*	*5*
L*	82.73^{a}	79.31^{b}	79.50^{b}	83.40^{a}	83.12^{a}	80.61^{b}	74.92^{c}
a*	1.33^{b}	2.11^{a}	-0.75^{c}	0.14^{d}	0.85^{b}	0.56^{c}	1.78^{a}
b*	12.33^{c}	13.65^{b}	21.39^{a}	14.60^{c}	15.61^{b}	15.66^{b}	17.29^{a}
SI[3]	12.41^{c}	13.81^{b}	21.43^{a}	14.66^{c}	15.73^{b}	15.75^{b}	17.41^{a}
Hue angle	83.68^{b}	81.09^{c}	92.05^{a}	85.99^{ab}	85.78^{b}	86.85^{a}	85.83^{a}
Ash (%)	1.26^{c}	1.83^{b}	2.80^{a}	1.75^{c}	1.99^{b}	2.05^{b}	2.07^{a}
Protein[4] (%)	13.46^{b}	13.00^{c}	22.67^{a}	15.31^{c}	17.16^{a}	15.98^{b}	17.05^{a}
TPC5 (mg GAE kg-1)	2056.4^{c}	2665.0^{b}	3377.0^{a}	2185.2^{d}	2347.2^{c}	2817.8^{b}	3434.3^{a}
Phytic acid (mg 100 g^{-1})	865.8^{b}	654.2^{c}	963.2^{a}	1250.9^{a}	1136.1^{b}	593.4^{c}	330.5^{d}
Ca (mg 100 g^{-1})	71.92^{c}	84.89^{b}	93.65^{a}	74.39^{d}	81.62^{c}	84.24^{b}	93.68^{a}
Mg (mg 100 g^{-1})	132.57^{c}	160.39^{a}	145.07^{b}	110.23^{d}	151.06^{c}	156.79^{b}	165.95^{a}
K (mg 100 g^{-1})	319.49^{c}	521.17^{b}	1016.26^{a}	562.98^{d}	611.97^{c}	656.09^{a}	644.85^{b}
P (mg 100 g^{-1})	264.53^{c}	358.01^{b}	432.38^{a}	316.62^{d}	350.58^{c}	373.24^{a}	366.13^{b}
Zn (mg 100 g^{-1})	1.92^{c}	3.05^{b}	3.38^{a}	2.17^{d}	3.05^{b}	3.20^{a}	2.72^{c}
Fe (mg 100 g^{-1})	2.23^{c}	3.17^{b}	6.58^{a}	3.54^{d}	3.94^{c}	4.20^{b}	4.30^{a}

[1], Means with same letter within row sharing a common letter are not significantly different (P<0.05). GWF, Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour; SI[3], Saturation index 4 N×5.70 for cereal flours; N×6.25 for non-cereal flours; TPC[5], Total Phenolic content.

Phytic acid contents of GSF changed between 654.15 mg 100 g^{-1} (for GRF) and 963.21 mg 100 g^{-1} (for GGLF). During germination phytic acid decreased and the lowest phytic acid value was observed after 5 day germination period. This is due to the increasing activity of phytase enzyme during germination. At the end of the germination period of approximately 7-8 days, the complete phytate content disintegration is reported (Ashton & Williams 1958). In this study, the phytic acid reduction of GRF, GWF and GGLF were 65, 74 and 79% after 5 day germination, respectively (data not shown). The primary task of phytase in germinated grains is to provide inorganic phytate phosphate in the early stages of germination (Frolich et al 1988). The enzymatic hydrolysis of the phytate in seed has great prospects for phosphorus metabolism during the germination of cereals (Yamagata et al 1980). In another study performed by Ghavidel & Prakash (2007), it was shown that the phytic acid content of raw and germinated lentil samples were 0.197 g 100 g^{-1} and 0.157 g 100 g^{-1} respectively, and phytic acid content decreased during germination.

GGLF gave the highest calcium, potassium, phosphorus, zinc and iron content. The highest magnesium amount was observed with GRF during the germination period. Calcium, magnesium and iron content increased by increasing germination period. While potassium, phosphorus and zinc content of the GSF increased until the 3rd day of germination, but after 3 days, a decrease of these parameters was observed. Surface color of cookies played a key-role for baking properties to determine the consumer preferences. L*, a*, b*, SI (saturation index) and hue angle values of cookies made with GSF are given in Table 2. The average lightness value of the cookies made with GSF ranged from 63.81 to 67.48 (Table 2).As shown in Table 2, the use of GWF in cookie formulation resulted an increase in lightness and hue angle values compared to the other GSF. In cookie products, reducing lightness value could be expected due to caramelization and Maillard browning reactions that resulting in the darkening of product, generally (Manzocco et al 2000), but in this study, using GSF in the cookie formulation resulted in increase for lightness. Color variation of the cookies may be explained through surface smoothness. Purlis & Salvadori (2009) reported that smooth surfaces were more effective to enhance the lightness than shrinkage. According to these antecedents using GSF resulted in smoother surface than control cookies and the lightness of cookies was clearer and brighter. While the cookies made with GWF resulted the highest lightness and hue angle values, the cookies made with GGLF gave the highest redness (a*),

yellowness (b*) and saturation index values. The highest redness value of GGLF added cookies might be due to the low moisture content of cookie samples of GGLF. Pérez et al (2013) reported that the high water content in cookie results the cookie take more time to reach the water activity value corresponding to the maximum Maillard reaction rate.

Table 2-Effects of germinated seed flours and addition level on color properties of cookie samples[1]

Germinated seeds flour / Addition level (%)	*L**	*a**	*b**	*SI (Saturation index)*	*Hue angle*
Germinated seeds flour					
GWF[2]	67.48^{a}	4.29^{c}	26.31^{b}	26.66^{b}	80.75^{a}
GRF	63.81^{c}	6.11^{b}	25.64^{c}	26.38^{b}	76.59^{c}
GGLF	66.11^{b}	6.32^{a}	27.30^{a}	28.06^{a}	77.07^{b}
Addition level (%)					
0	62.53^{c}	4.90^{c}	25.59^{c}	26.06^{c}	79.15^{b}
5	69.35^{a}	5.02^{c}	27.35^{a}	27.81^{a}	79.59^{a}
10	65.69^{b}	6.27^{a}	26.35^{b}	27.15^{b}	76.82^{c}
15	65.63^{b}	6.11^{b}	26.37^{b}	27.12^{b}	76.99^{c}

[1], Means with same letter within column are not significantly different (P<0.05); GWF[2], Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour

Significant increases (P<0.01) were observed with GSF addition in lightness, redness, yellowness and saturation index values of cookie samples. The highest lightness, yellowness and saturation index values were obtained with a 5% addition level while the highest redness values were obtained with a 10% addition level.

The results were presented in Table 3 and shown that the diameters, thickness and spread ratio values of the cookie samples supplemented with GSF varied between 47.10 and 47.24 mm, 7.82 and 7.97 mm, 5.93 and 6.02, respectively. It can be seen that there were a significant (P<0.01) increase in diameter of the samples with 10 and 15% addition level of GSF compared to control. The cookies made with GRF gave the highest thickness values while GRF addition resulted the lowest spread ratio values of cookies. The cookies containing GGLF and GWF gave the highest spread ratio values.

Table 3-Effects of germinated seed flours and addition level on textural properties of cookie samples[1]

Germinated seeds flour / Addition level (%)	*Diameter (mm)*	*Thickness (mm)*	*Spread Ratio*	*Hardness (g)*	*Fracturability (g)*
Germinated seeds flour					
GWF[2]	47.24^{a}	7.86^{ab}	6.01^{a}	3428.55^{b}	39.87^{ab}
GRF	47.23^{a}	7.97^{a}	5.93^{b}	3704.88^{a}	40.13^{a}
GGLF	47.10^{a}	7.82^{b}	6.02^{a}	3209.49^{c}	39.43^{b}
Addition level (%)					
0	47.05^{b}	7.89^{a}	5.96^{ab}	3452.81^{b}	39.72^{a}
5	46.63^{b}	7.92^{a}	5.89^{b}	3774.91^{a}	39.84^{a}
10	47.53^{a}	7.80^{b}	6.10^{a}	3428.29^{b}	39.61^{a}
15	47.53^{a}	7.93^{a}	5.99^{ab}	3134.92^{c}	40.09^{a}

[1], Means with same letter within column are not significantly different (P<0.05); GWF[2], Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour

Data on the textural characteristics of cookies enriched with GSF are presented in Table 3. The tested cookies showed significant (P<0.01) differences according to GSF type and addition level of GSF with respect to hardness. The hardness of the cookies was found between 3209.49 and 3704.88 g and the fracturability values were observed between 39.43 and 40.13 g. The lowest hardness and fracturability value of the cookies was observed for cookies containing GGLF, while the GRF addition resulted in the highest hardness and fracturability values. Wani et al (2012) reported that, high hardness values are related to protein content, high protein content resulted in a more hard structure and this result was due to the interaction between protein and starch. Another is the fiber content that affects the texture.

GRF addition decreased the spread ratio and increased the hardness of cookies that may be due to dilution of gluten or less protein content of rye compare to the other seeds and less water availability for hydration. As the addition level of GSF increased in cookies, fracturability values were not affected while hardness of cookies increased at 5% addition level and decreased at higher levels. It is thought that the fluctuations in the hardness values are caused by the differences in the moisture content of the cookie samples.

Baranzelli et al (2018) noted that the increase in the germination time reduced tenacity and increased extensibility of the flours. Also we know that tenacious gluten composites were used to make bread, whereas extensible gluten composites were used for cake and cookie production (Melini et al 2017; Sanchez-Garcia et al 2015). So that addition of GSF is more suitable for cookie making than bread making.

The moisture and ash values of cookies made with GSF were given in Figure 1. The moisture value of the cookie samples were between 2.30% and 5.42%, the average was 3.90±1.23%. The cookies made with GRF gave the highest moisture content. The usage of GGLF gave the lowest moisture content. Cornejo & Rosel (2013) mentioned that germinated rice flour gave lower moisture content than raw rice flour. The ash amounts of the cookie samples varied between 1.55 and 1.73%. The values of ash did not significantly ($P>0.05$) varied among the three GSF. The results showed that an increase was observed in the ash values according to addition level of GSF. This result was associated to the increase in the relative proportion of minerals due to the loss of other seed storage reserves reported by Borek et al (2006).

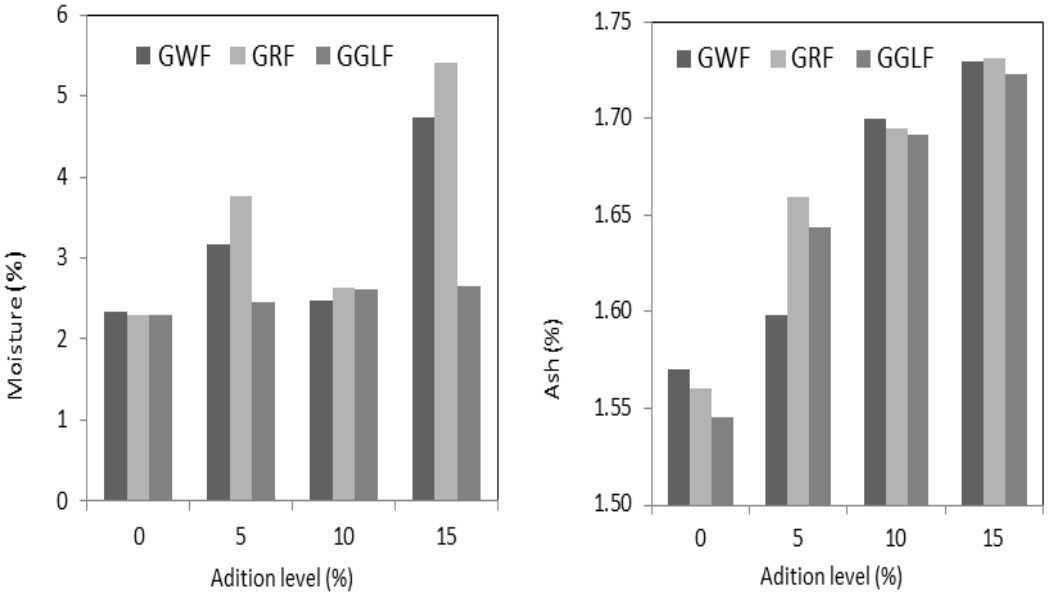

Figure 1- Moisture and ash contents of cookie samples (GWF, Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour)

Nutritional analyzes were performed at the end of the 5th day germination period and with only 15% substitution rate of the GSF added cookie samples. The effects of GSF addition on the phytic acid content of cookies are summarized in Table 4. The amount of phytic acid in the control cookie and GWF, GRF, GGLF added cookie samples were 222.60±5.94, 195.30±2.97, 203.70±2.97, 191.10±2.97 mg 100 g^{-1}, respectively. The lowest amount of phytic acid was determined with GGLF and GWF added cookie samples, while the highest amount of phytic acid was obtained from control cookie sample (made with ungerminated wheat flour). Germination process decreases the phytic acid content due to the increasing phytase enzyme during germination. A comparable observation was described by Azeke et al (2011) who found that during germination, the level of phytase activity increased and reached its maximal value after six (5-fold), five (7-fold), and eight (6-fold) days of germination for maize, millet, and wheat and also on the seven (16- and 3-fold respectively) for rice and sorghum. Usually, legume based food product contain higher phytate contents than cereal-based food products. But in present study, GGLF added cookie samples gave lower phytic acid content than cereal based control cookie sample. Germination process is effective for decreasing the phytic acid content of legume based products.

Table 4-Effects of germinated seed flours on mineral contents of cookie samples[1]

Germinated Seed Flours	*Phytic Acid (mg 100g^{-1})*	*TPC[3] (mg GAE kg[1])*	*Calcium (Ca) (mg 100g^{-1})*	*Iron (Fe) (mg 100g^{-1})*	*Potassium (K) (mg 100g^{-1})*	*Magnesium (Mg) (mg 100g^{-1})*	*Phosphorus (P) (mg 100g^{-1})*	*Zinc (Zn) (mg 100g^{-1})*
Control	222±5.94^{a}	713.5±6.36^{d}	32.04±0.02^{d}	1.54±0.03^{b}	122.23±0.08^{d}	19.66±0.02^{d}	557.16±0.72^{a}	0.84±0.01^{c}
GWF[2]	195±2,97^{c}	838±4.24^{c}	41.42±0.01^{b}	1.47±0.01 ^{c}	146.27±0.17^{c}	31.62±0.03^{b}	507.17±0.63^{c}	1.01±0.01^{a}
GRF	203±2.97^{b}	904.5±4.95^{b}	54.50±0.01^{a}	1.04±0.01^{d}	162.49±0.36^{b}	39.05±0.01^{a}	303.64±0.66^{d}	0.95±0.01^{b}
GGLF	191±2.97^{c}	1095±4.24^{a}	36.71±0.01^{c}	1.83±0.02 ^{a}	195.07±0.28^{a}	26.97±0.01^{c}	559.90±0.90^{b}	1.00±0.02^{a}

[1], means with same letter within column are not significantly different (P<0.05); [2], GWF: Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour; [3], TPC, Total Phenolic content

The total phenolic content (TPC) of cookie samples was presented in Table 4. Important phenolic compounds found in cereals; phenolic acids, flavonoids and tannins, which are concentrated in the bran fraction of cereal grains, are found in free, conjugated, and bound forms. Total phenolic content of control and GWF, GRF, GGLF added cookie samples were 713.50±6.36, 838.00±4.24, 904.50±4.95 and 1095.00±4.24 GAE mg kg^{-1}, respectively. TPC increased by 17.5, 27, 55% with GWF, GRF and GGLF addition in cookie formulation compared to control, respectively. As reported by Gawlik-Dziki et al (2016) after 4 days germination process, total phenolic content of wheat samples increased from 2.25 GAE mg g^{-1}to 4.35 GAE mg g^{-1}. GGLF with rich TPC had a more dominant effect on TPC of cookie samples compared to the other GSF. Lentil has higher total phenolic and tannin content compared to other legumes. The TPC of lentils is thought to contribute significantly to the total antioxidant activity. The cotyledon of the lentil mainly contains non-flavonoid phenolic compounds, while the shell layer contains flavonoids.

Table 4 shows some mineral contents (Ca, Fe, K, Mg, P and Zn) in each of the cookies enriched with 15% level of GSF. Control cookie samples were determined as mg 100g^{-1}; 32.04±0.02 calcium, 19.66±0.02 magnesium, 122.23±0.08 potassium, 557.16±0.72 phosphorus, 0.84±0.01 zinc and 1.54±0.03 irons. The addition of GSF in cookie formulation increased the Ca, K, Mg and Zn content cookies compared to the control cookie sample. According to test results of cookie samples; the highest calcium and magnesium values were observed with cookies made GRF; the highest iron, potassium and zinc values were determined with GGLF cookies, while the lowest calcium, potassium, magnesium and zinc values were obtained from control cookie samples. The average daily mineral quantities are as follows: Calcium 1000 mg, iron 18 mg, potassium 3500 mg, magnesium 400 mg, phosphorus 1000 mg, zinc 15 mg. 100 grams of cookies made with GRF supplied 5.45% of the daily calcium content, 9.76% of the magnesium content, 4.64% of the amount of potassium, 30.36% of the phosphorus content, 6.33% of the zinc content, 5.78% of the amount of iron. 100 grams of cookies made with GGLF supplied 3.67% of the daily calcium content, 6.74% of the magnesium content, 5.57% of the amount of potassium, 55.99% of the amount of phosphorus, 6.67% of the zinc amount, 10.17% of the amount of iron.

Sensory analyzes were performed in cookies enriched with 0, 5, 10 and 15% GSF and shown in Table 5. The average organoleptic values of cookie samples evaluated between 1-5 points; the color values were 4.22-4.62, the appearance values were 4.23-4.45, the friability was 4.40-4.54, the taste was 4.10-4.57, the odor was 4.35-4.78 and the overall appreciation values were found between 4.32 and 4.51. The general appreciation of the cookies produced with the addition of GSF was evaluated as not different from each other. Generally, the highest appreciation value was found in 5% GWF added cookies and control cookies. In particular, the GWF added cookie received the highest (P<0.05) score for friability and taste, and the lowest score for odor. The cookies made with the GGLF gave the highest color and odor values, while the lowest scores were received for appearance with GGLF. The highest friability score was determined in cookie samples with supplemented GWF, while the most appreciated taste scores were determined in these samples. The increasing addition level did not affect the appearance of cookies. Color is one of the most important visual characteristic of cookies that strongly influences consumer's choice. Color scores was found similar scores up to 10% substitution rate, whereas more than 10% addition level decreased color score. Taste values showed similar scores with the control cookie samples up to 5% addition level, adding more than 5% level, resulting in a decrease in taste scores. Odor scores decreased with increasing addition level. In general, the addition of GSF to 5% level resulted in general appreciation scores similar to the control cookie samples, which led to a decrease in the probability of more addition levels.

Table 5-Effects of germinated seed flours and addition level on sensorial cookie properties[1]

Germinated seeds flour / Addition level (%)	*Color*	*Appearance*	*Friability*	*Taste*	*Odor*	*General appreciation*
Germinated seeds flour						
GWF[2]	4.40[b]	4.45[a]	4.54[a]	4.53[a]	4.53[b]	4.49[a]
GRF	4.22[c]	4.41[a]	4.43[b]	4.24[c]	4.45[b]	4.35[a]
GGLF	4.58[a]	4.23[b]	4.45[b]	4.38[b]	4.68[a]	4.45[a]
Addition level (%)						
0	4.62[a]	4.38[a]	4.40[b]	4.50[a]	4.78[a]	4.50[a]
5	4.45[a]	4.40[a]	4.53[a]	4.57[a]	4.62[b]	4.51[a]
10	4.38[a]	4.27[a]	4.50[a]	4.35[b]	4.45[c]	4.39[b]
15	4.28[b]	4.40[a]	4.45[ab]	4.10[c]	4.35[d]	4.32[c]

[1], Means with same letter within column are not significantly different (P<0.05); GWF [2], Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour

4. Conclusions

The GSF is an alternative source in cookie formulation for nutritional enrichment. In this study, chemical, nutritional and sensorial properties of cookies enriched with GSF were investigated. Germination improved the nutritional quality such as ash, protein, total phenolic content, minerals and decreased the anti-nutritional factor as phytic acid. According to sensorial evaluation, the addition of GSF to 5% level resulted in general appreciation scores similar to the control cookie samples. Result of this study revealed that more nutritious cookies can be produced by up to 5% addition level of GSF and this formulation of cookie can be beneficially affects the nutritive composition and also does not impair sensorial attributes of cookies.

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On-Farm Assessment of Soil Quality in Low and High Grazing Under Integrated Crop-Livestock System in South Dakota

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ABSTRACT
Integrated crop-livestock system (ICLS) has the potential to enhance soils quality by improving soil chemical, physical, and biological parameters especially soil organic carbon. objective of this study was to assess the impact of low and high stocking rates (number of animal per hectare) under ICLS on soil quality parameters at the farm scale and the approach of farmers in Getysburg, Roscoe and Selby sites for this system. Study sites located at three different farms that has low stocking rate of cattle grazing. Data from this study showed that low stocking rate under ICLS increased soil organic carbon (SOC) from 20.7 to 28.3 g kg^{-1}, and total nitrogen (TN) from 2.06 to 2.60 g kg^{-1} at the surface 0-5 cm depth. However, high stocking rates under ICLS decreased the SOC. Low stocking rate under ICLS increased the soil N but it did not impact on soil P significantly. High stocking rate decreased the BG and MBC but low stocking rate increased. High stocking rate increased the soil penetration resistance 2.43 to 2.83 MPa. Further, data showed that the low stocking rate under ICLS improved the soil quality index (SQI) while high stocking rate under ICLS decreased it. This study showed that ICLS with low stocking density can be beneficial in enhancing soil quality at the farm scale.

Keywords: Integrated crop-livestock system, soil organic carbon, soil quality

1. Introduction

Integrated crop-livestock system (ICLS) has numerous benefits such as higher crop production, improved soil quality (Russelle et al 2007), enhanced soil structure and fertility, weed control, reduced damage of insects and diseases, and high-quality of feed for the livestock (Bullock 1992; Humphreys 1994; McKenzie et al 1999). Due to these advantages, ICLS has gained increased attention among various research professionals and the producers. However, grazing under ICLS can positively or negatively impact soils depending upon how it is being managed.

The moderate or low stocking rate which means number of animal per hectare of grazing can contribute to the improvement of soil quality and economic benefits (Savadogo et al 2007; Follett & Reed 2010). This type of grazing can enhance the SOC (Russelle et al 2007), and water infiltration (Hiernaux et al 1999). However, heavy grazing (intensive or high stocking rate) can decrease SOC and TN contents (Cui et al 2005; Han et al 2008), soil wet aggregate stability (WAS) and soil moisture content (Johnston et al 1971; Warren et al 1986), increase soil compaction resistance (SPR) due to treading by livestock and hence reduce water infiltration rate (Katsvairo et al 2006). Further, it decreases the root biomass due to high compaction (Han et al 2008), soil phosphorus (P) (Hiernaux et al 1999) and biomass productivity and increases the soil erosion due to high soil compaction (Savadogo et al 2007). Agronomy News defined

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the soil quality as "the capacity of soil to function". Soil quality context is evaluated as soil chemical, pyhscial and biological paramaters. ICLS is one of the important agriculture practice to imrove the soil health.

Thus, specific objective of this study was to evaluate the impacts of different livestock stocking density under the ICLS on soil quality based on the select indicators in South Dakota and calculate the SQI value by using soil management assessment framework (SMAF) tool.

2. Material and Methods

2.1. Study sites

The study sites were chosen from three different farms located at Roscoe, Gettysburg, and Selby in South Dakota, USA. Each site has two different treatments that include ICLS and non-ICLS as the control. At the Roscoe, the mean daily temperature was 6 ^{o}C and mean annual precipitation was 444 mm (NRCS 2009), and soils were classified as fine-loamy, mixed, superactive, frigid Typic Argiustolls with 3-6% slope (USDA 2018). This site has low stocking rate (0.69 AU ha^{-1}). Grazing area in ICLS was the no-till system that was grazed during every winter (cover crops) for 7-10 years. Crops in the ICLS included corn-soybean-wheat-cover crop-alfalfa rotation. Control treatment was also in a no-till system without grazing non-cover crop under the corn-soybean rotation. Soils at the Gettysburg site were classified as fine-silty, mixed, superactive, mesic Typic Argiustolls with 2-6% slope. The mean daily temperature was 7 ^{o}C, and the mean annual precipitation was 507 mm (NRCS 2009). This site has high stocking rate (43 number of animals/hectare). Grazing area in ICLS was in a no-till system that was grazed during every winter for 16 years. Crop system in the ICLS was winter wheat-cover crop-corn-sunflower rotation. Control area was also no-till tillage system that was corn-fallow-winter wheat-cover crop rotation. Soils at the Selby site were classified as fine-silty, mixed, superactive, frigid Typic Argiustolls with 2-6% slope. The mean daily temperature was 7 ^{o}C and the average annual precipitation was 444 mm (NRCS 2009). This site has high stocking rate (42.5 number of animal/hectare). Grazing area (15 years) in ICLS was the no-till system that was corn-cover crop rotation. Control treatment was also no-till system that was grazed one time in last 8-yr. Crop system in the control treatment was corn-winter wheat-sunflower rotation.

2.2. Soil sampling and analysis

Soil samples were collected in June 2017 and 2018 from all the study sites (Roscoe, Gettysburg, and Selby) at the 0-5 and 5-15 cm depths by using a soil auger and grid soil sampling. The auger soil samples were air-dried and ground to pass through a 2-mm sieve. Samples were ground using a roller mill through a 0.5-mm sieve to analyze the total soil C and N (sum of nitrate, nitrite, organic and ammonia) concentrations using dry combustion techniques with a LECO TruSpec C/N analyzer (LECO 2002). The TC was considered as SOC because pH was below 6.5. Undisturbed soil samples were used to determined soil bulk density (BD) by using core method (Grossman & Reinsch 2002). Extractable soils N (mineral nitrogen), P were analyzed using the standard operating methods (Bray and Kurtz 1945; Haby et al 1990; Warncke and Brown 1998). Soil wet aggregate stability (WAS) was analyzed using the method described by (Kemper & Rosenau 1986). Chloroform fumigation direct extraction method (CFDE) was used for analyzing microbial biomass carbon (MBC) (Beck et al 1997). Beta-glucosidase enzyme activity (BG) was analyzed using the method descripted by (Deng & Tabatabai 1994). Soil Penetration Resistance (SPR) was measured by using penetrometer (Bradford 1986). Further, Soil Management Assessment Framework (SMAF) method was used to determine the effect of integrated crop-livestock system on soil quality. SMAF consist of three steps; indicator selection, indicator interpretation, and integration into a soil quality index value (SQI) (Andrews 1998). In the first step, Minumun Data Set (MDS) indicators are selected using the parameters from the database (Bellocchi et al 2002; Schadt et al 2002). In the second step, the selected MDS indicators are created the graph by using nonlinear scoring curves (Karlen & Stott 1994). Last step gives final results to integrate all the indicator scores using former interpretation with index value (Karlen et al 1997). Their values were scored on a 0-1 scale based on the algorithms in the SMAF model. Score 1 is highest soil qaulity index value while score 0 is lowest soil quality index value.

These scores depend on soil texture, temperature, rainfall, slopes, and season (Andrews et al 2004). Soil Quality Index (SQI) value based on the SMAF method was calculated using the formula given by (Karlen et al 2014): SQI = Sum of SMAF scores/Number of indicators.

2.3. Statistical analysis

The effects of grazing under ICLS on soil quality parameters and SQI values for each depth in 2017 and 2018 were evaluated using scores using Analysis of Variance (ANOVA) in the SAS9.4 software (SAS 2013). Mean separation were analyzed using a protected PDIFF option (t-test) within the LSMEANS statement following PROC GLM procedure in SAS9.4. Statistical differences among the treatments were explained significant at α=0.10 significant level. This significant level is very common in studies because variability is high in farmers sites (Halpern et al 2010) . Also, SMAF model was used to calculate the SQI value and after SAS9.4 software (SAS 2013) was used for statistical analysis.

3. Results

Data for SOC and TN under different treatments for all the three sites at the 0-5 and 5-15 cm depths in 2017 and 2018 are presented in Table 1. In 2017, grazing in ICLS significantly impacted SOC at the Gettysburg, Roscoe, and Selby sites at 0-5 and 5-15 cm depth, except for Selby site at 5-15 cm depth. High stocking decreased the SOC for 0-5 cm depth at the Gettysburg and Selby, whereas, low stocking density increased it at the Roscoe site. A similar trend was observed in 2018 where low stocking density increased the SOC but high stocking density decrease it, however, differences were not always significant.

Table 1- Mean soil organic carbon (SOC) and total nitrogen (TN) under grazing and control (non-grazing) treatments at the 0-5 and 5-15 cm depths in 2017 and 2018 at the Gettysburg, Roscoe, and Selby in South Dakota, USA

Locations	*Stocking Rate*	*Treatments*	*SOC (g kg^{-1})*				*TN (g kg^{-1})*			
			2017		*2018*		*2017*		*2018*	
			0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm
Gettysburg	High	Grazing	26.6^{b†}	19.9^{b}	25.6^{a}	18.2^{a}	2.40^{b}	1.89^{b}	2.42^{a}	1.84^{a}
		Control	29.3^{a}	23.3^{a}	22.3^{a}	14.7^{a}	2.63^{a}	2.10^{a}	2.16^{a}	1.60^{a}
Roscoe	Low	Grazing	28.3^{a}	23.5^{a}	27.2^{a}	22.9^{a}	2.60^{a}	1.88^{a}	2.38^{a}	1.84^{a}
		Control	20.7^{b}	16.8^{b}	19.2^{b}	15.2^{b}	2.06^{b}	1.55^{a}	2.10^{b}	1.37^{b}
Selby	High	Grazing	26.7^{b}	16.6^{a}	23.4^{b}	14.6^{b}	2.43^{b}	1.61^{b}	2.33^{b}	1.43^{b}
		Control	31.2^{a}	20.7^{a}	27.3^{a}	21.7^{a}	2.87^{a}	2.01^{a}	2.69^{a}	1.99^{a}

†Means within the same column followed by different small letters for each study sites are significantly different at P<0.10 for the grazing treatment

Data for total nitrogen (TN) under the grazing treatment at the 0-5 and 5-15 cm depths at all three sites in 2017 and 2018 are presented in Table 1. Data showed that low stocking density at the Roscoe site significantly increased the TN at 0-5 and 5-15 cm depths for 2017 (26 and 21%) and 2018 (13 and 34%), except that it was not different in 2017 at 5-15 cm depth. However, high stocking density decreased the TN at 0-5 cm and 5-15 cm depths in 2017 and 2018 for Gettysburg and Selby sites, however, differences were not significant at the Gettysburg site for 2018.

Data for soil N and P under different treatments for all the three sites at the 0-5 and 5-15 cm depths in 2017 and 2018 are presented in Table 2. In 2017, grazing under ICLS significantly impacted soil N at all the sites for 0-5 and 5-15 cm depths, except for Roscoe site at the 5-15 cm depth. High and low stocking increased the soil N at the Roscoe and Selby site, whereas, high stocking density decreased it at the Gettysburg site for two depths. There was a similar trend in 2018 although differences were not always significant.

Low and high stocking density at the Roscoe and Selby sites increased the soil P at both the soils depths for either year except that differences were not significant at the 0-5 and 5-15 cm depths for 2018. However, high stocking density decreased the soil P at 0-5 cm depth for Gettysburg site.

Table 2- Mean extractable soil nitrogen (N) and soil phosphorus (P) under grazing and control (non-grazing) treatments at the 0-5 and 5-15 cm depths in 2017 and 2018 at the Gettysburg, Roscoe, and Selby in South Dakota, USA

Locations	*Stocking Rate*	*Treatments*	*Soil N (mg kg^{-1})*				*P (mg kg^{-1})*			
			2017		*2018*		*2017*		*2018*	
			0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm
Gettysburg	High	Grazing	17.2^{b†}	11.5^{b}	12.4^{b}	11.9^{a}	14.5^{b}	8.13^{a}	14.3^{a}	3.33^{a}
		Control	28.5^{a}	20.5^{a}	28.8^{a}	7.63^{a}	31.7^{a}	13.1^{a}	21.3^{a}	4.23^{a}
Roscoe	Low	Grazing	40.6^{a}	26.5^{a}	26.2^{a}	11.1^{a}	11.9^{a}	8.56^{a}	5.03^{a}	2.35^{a}
		Control	24.8^{b}	13.3^{a}	15.4^{b}	5.33^{b}	11.2^{a}	5.56^{b}	5.33^{a}	1.67^{a}
Selby	High	Grazing	29.3^{a}	11.4^{a}	39.5^{a}	10.7^{a}	34.4^{a}	8.88^{a}	26.7^{a}	2.33^{a}
		Control	17.4^{b}	5.61^{b}	42.4^{a}	8.63^{b}	10.5^{b}	5.44^{b}	7.00^{b}	1.67^{a}

†Means within the same column followed by different small letters for each study sites are significantly different at P<0.10 for the grazing treatment.

Data for BG, MBC, BD, SPR, and WAS under different treatments for all three sites at the 0-5 cm depth in 2017 and 2018 are presented in Table 3. Data showed that high stocking density grazing decreased the BG (2017) or no impact (2018), however, low stocking density has no impact (2017) or increased (2018) the BG. A similar trend was observed for the MBC, however, differences were not always significant. Data showed that grazing did not impact soil BD and SPR, except that low stocking density decreased the soil BD in 2017 and high stocking density at Selby increased the SPR in 2017. Furthermore, grazing increased the WAS in 2017 but unaffected in 2018 as compared to the control treatments for all the sites. Data for SQI under different treatments for all the three sites at the 0-5 and 5-15 cm depths in 2017 and 2018 are presented in Figure 1. In 2017, grazing in ICLS significantly impacted the SQI at Gettysburg, Roscoe, and Selby sites at 0-5 and 5-15 cm depths, except for Selby site at the 5-15 cm depth. High stocking rate decreased the SQI at the both depths for Gettysburg and Selby sites, whereas, low stocking density increased the SQI at the Roscoe site. There was a similar trend in 2018 and high stocking rate decreased the SQI at the 0-5 cm depth, except for Selby site. At the 5-15 cm depth, grazing in ICLS did not significantly impact the SQI.

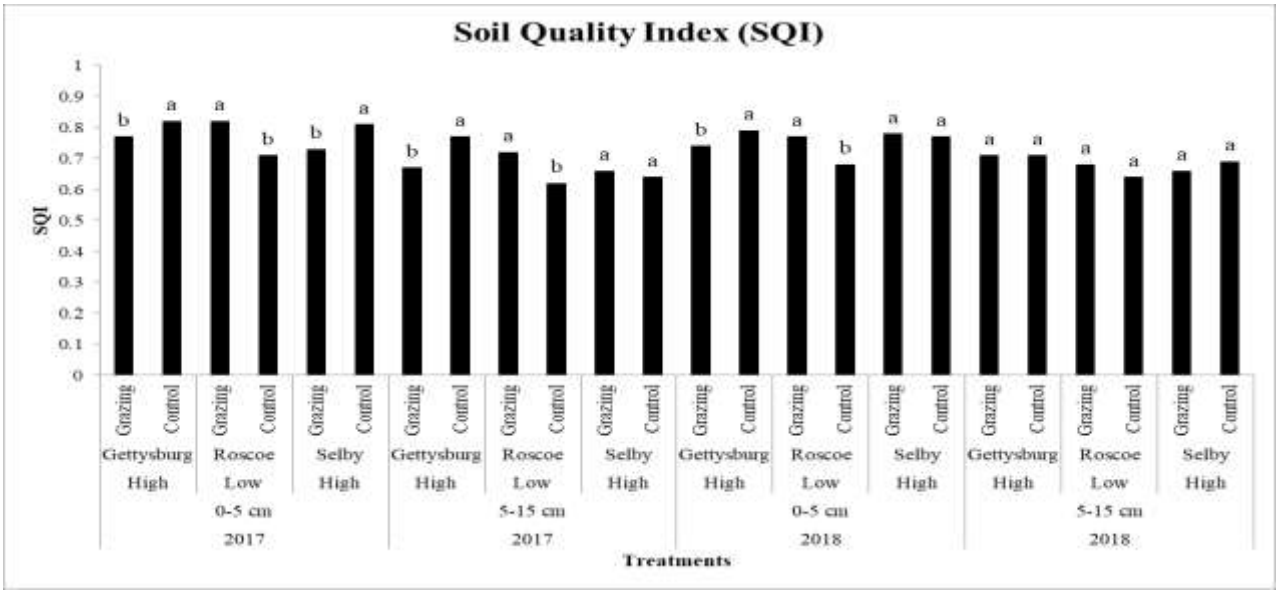

Figure 1- Mean soil quality index (SQI) under grazing and control (non-grazing) treatments at the 0-5 and 5-15 cm depths in 2017 and 2018 at the Gettysburg, Roscoe, and Selby in South Dakota, USA

Table 3- Mean beta-glucosidase (BG), microbial biomass carbon (MBC), bulk density (BD), soil penetration resistance (SPR) and wet aggregate stability (WAS) under grazing and control (non-grazing) treatments at the 0-5 cm depth in 2017 and 2018 at the Gettysburg, Roscoe, and Selby in South Dakota, USA

Locations	*Stocking Rate*	*Treatments*	*BG (μg ml^{-1})*		*MBC (μg g^{-1})*		*BD (g cm^{-3})*		*SPR (MPa)*		*WAS (%)*	
			2017	*2018*	*2017*	*2018*	*2017*	*2018*	*2017*	*2018*	*2017*	*2018*
Gettysburg	High	Grazing	24.6^{b†}	73.0^{a}	73.01^{b}	119.1^{b}	1.34^{a}	1.33^{a}	2.36^{a}	2.37^{a}	81.5^{a}	84.5^{a}
		Control	36.3^{a}	89.7^{a}	118.0^{a}	220.5^{a}	1.38^{a}	1.43^{a}	2.48^{a}	2.47^{a}	78.2^{b}	78.1^{a}
Roscoe	Low	Grazing	20.1^{a}	97.4^{a}	249.8^{a}	227.0^{a}	1.48^{b}	1.37^{a}	1.93^{a}	2.53^{a}	90.3^{a}	91.2^{a}
		Control	22.7^{a}	77.0^{b}	69.72^{b}	150.8^{b}	1.52^{a}	1.37^{a}	2.29^{a}	2.16^{a}	84.9^{b}	82.6^{a}
Selby	High	Grazing	22.6^{b}	35.0^{b}	65.04^{b}	236.1^{a}	1.48^{a}	1.41^{a}	2.83^{a}	2.14^{a}	88.3^{a}	84.2^{a}
		Control	40.2^{a}	99.6^{a}	168.7^{a}	234.2^{a}	1.34^{a}	1.40^{a}	2.43^{b}	2.17^{a}	81.8^{b}	78.9^{a}

†Means within the same column followed by different small letters for each study sites are significantly different at $P < 0.10$ for the grazing treatment

4. Discussion

For SOC, heavy grazing can reduce plant residue and increase soil compaction and reduce SOC (Hiernaux et al 1999). Increased compaction can reduce the SOC content by decreasing resistance to deformation and rebound effects (Soane 1990).Our findings are in accord with some previous results that showed that SOC content was decreased with the increase in grazing intensity (Cui et al 2005; Han et al 2008). Stark et al 2002 reported that SOC did not improve by reindeer grazing due to overgrazing or unsuitable timing. Similar to our finding, a previous study demonstrated that moderate grazing improved the SOC in Tibetan Plateau (Hafner et al 2012).

For TN, similar findings were reported by Hoffmann et al 2008 from a study that showed the TN reduced by 0.4 kg ha^{-1} because grazing reduced the crop residue left on the soil. Grazing, when used appropriately, can enhance TN in mixed-grass rangeland (Schuman et al 1999). Further, it depends on type of the plant, legumes or cover crop used for grazing because these crops can enhance the nitrogen in the soil.

For soil N, a study showed that grazing positively affected soil N availability compared to the non-grazing treatment in Yellowstone National Park (Hamilton III & Frank 2001). Seagle et al 1992 showed that the soil N content under high and low stocking rate was higher than the non-grazing treatment under grasslands. For soil P, similar findings were reported by Marrs et al 1989; Neff et al 2005.

For soil BG and MBC, studies showed that increasing low grazing intensity increased the BG in subarctic tundra in Fennoscandia (Stark et al 2015). Further, grazing enhanced the MBC due to increased available nitrogen (Lopez et al 1977). However, intensive grazing reduced the MBC (Holt 1997). Similarly, Bardgett and Leemans 1995 also reported that intensive grazing reduced the MBC by 44% due to the fact that heavy grazing can reduce plant residue and increase soil compaction and decrease MBC (Hiernaux et al 1999).

For soil SPR and BD, grazing treatments increased the SPR due to compaction from animals on the soil (Mulholland & Fullen 1991; Chanasyk & Naeth 1995; Broersma et al 2000). Grazing treatments with high stocking rate increased the soil BD (Willatt & Pullar 1984; Chanasyk & Naeth 1995; Zhou et al 2010; Pulido et al 2018). Another study conducted in Inner Mongolia showed that intensive grazing increased the BD (Steffens et al 2008).

For SQI, ıt has been reported that heavy grazing (high stocking rate) can decrease the soil quality (Han et al 2008) because high stocking rate can decrease the soil physical quality (da Silva et al 2003). Similar to our findings, a study showed that the low or moderate grazing had the highest SQI values as compared to the non-grazing treatment; and non-grazing treatment had the higher SQI value compared to heavy grazing in the USA (Wienhold et al 2004). This was partially due to the differences in stocking rate for grazing.

5. Conclusions

This study was conducted to explore the responses of high and low stocking rates under ICLS on soil quality parameters at three different locations (Gettysburg, Roscoe, and Selby) in South Dakota. Data showed that low stocking rate increased the SOC and TN while high stocking rate decreased the SOC and TN compared to the non-grazing treatments. High stocking rate decreased the BG and MBC content than the non-grazing treatment. In addition, high stocking density grazing increased and low stocking density decreased the BD and SPR, however, differences were not always significant. Grazing treatments significantly influenced soil N and P, and low and high stocking rate increased the soil N and P content compared to non-grazing treatment, except Gettysburg site. Results showed that grazing treatments significantly influenced the soil quality. High stocking rate decreased the SQI value while low stocking rate increased it compared to the non-grazing treatment at the 0-5 and 5-15 cm depths. Data from this study conclude that low stocking grazing is beneficial in enhancing the soil quality. Our study and previous study showed that low stocking rate under ICLS can be helful for improving soil quality.

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Abbreviation and Symbol	
ICLS	Integrated crop-livestock system
SOC	Soil organic carbon
SMAF	Soil Management Assessment Framework
SQI	Soil Quality Index
TN	Total Nitrogen

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Physiological Responses of Gamma-Irradiated Onion Bulbs during Storage

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ABSTRACT
The present study aimed to evaluate the effects of different doses of gamma radiation on some physiological characters of onion genotypes and the expression of *PAL* gene in the best interaction of gamma irradiation dose and onion genotype treatment. To this aim, four onion genotypes (White-Qom, White-Neyshabour, Red-Ridge-Lump, and Red-Ray-Corrugated) irradiated at 0, 30, 60, 90, 120, and 150 Gy. After four months of storage (at 10-15 °C and 70% relative humidity), the effects of gamma rays on the dry matter (DM), protein content, phenylalanine ammonia lyase (PAL) and peroxidase (POD) were investigated. In addition, the expression of *PAL* gene in the best interaction of gamma irradiation dose and onion genotype treatment was assessed. The result indicated that POD activity was increased by most of the gamma irradiation levels; however, the protein content and PAL activity were decreased. Moreover, dry matter content was found to be highly genotype-dependent. A linear regression (R^2= 0.82) between PAL activity and gamma irradiation levels, was observed. PAL activity decreased with increasing in gamma irradiation level, while the expression rate of *PAL* gene was not significantly changed between irradiated and non-irradiated control, indicating that the radiation might not have direct effects on the gene regulatory elements. These results suggest that gamma irradiation could reduce the PAL activity possibly by controlling abiotic stress sources such as fungal and bacterial stresses.

Keywords: Gamma radiation; *Allium cepa*; PAL activity; Gene expression

1. Introduction

Onion (*Allium cepa* L.) is one of the most important vegetable crops, a member of the Liliaceae family (Sabiu et al 2019). Onion is commonly used for its flavor, nutritional and medicinal values. Jones & Mann (1964) proposed that Pakistan and Iran are the onion primary center of origin. Since ancient times, it is highly consumed in Iran (Fakhri et al 2018). Iran with 2,379,000 tons annually onion production, was among the top four producer countries (FAO 2017).

Biochemical changes in the onion bulb tissues exposed to different adverse environmental conditions caused a reduction in their quality (Benkeblia et al 2003; Benkeblia et al 2004). Gamma irradiation have been widely used as a safe and effective method to increase storage time and shelf life in some crops such as onion (Nouri & Toofanian 2001), potato (Frazier et al 2006), pistachio (Akbari et al 2018), peach (Khan et al 2018), and pomegranate (Ashtari et al 2019).

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The effect of gamma irradiation on storage life is due to control of fungal and bacterial infections (Pereira et al 2015; Fernandes et al 2016) and delay in ripening and growth inhibition (Kader 1986). Also, studies have shown that the gamma irradiation can affect physiological, chemical, biological changes in the crops (Di Stefano et al 2014; Pereira et al 2016).

Dry matter is one of the most important quality criteria in onion. Studies showed that gamma irradiation had a positive effect on onion dry matter resulted in increasing the storage life (Rutherford & Whittle 1984). Non-structural carbohydrates are the main compound of dry matter (Darbyshire & Henry 1981). Ionized bulbs can increase the storage life for several months, whereas it can affect the carbohydrates content (Benkeblia et al 2004). When the crops tissues damaged by cutting, grinding or pulping, phenolic compounds produce by phenylalanine ammonia lyase (PAL), then they oxidized by polyphenol oxidase (PPO) and peroxidase (POD) which polymerize to brown pigments (Mai & Glomb 2013).

PAL is a key enzyme in the phenyl propanoid biosynthesis pathway, which catalyzes the elimination of ammonia from $_{L}$-phenylalanine to give *trans*-cinnamate, which is the first step in the biosynthesis of plant-specific phenylpropanoid derivatives such as phenolic compounds (Benkeblia 2000; Hamamouch & Adil 2019). PAL activity was found to vary in different plant development stage and various stresses (Li et al 2017). The main role of POD is to oxidize molecules at the expense of H_2O_2. It is possibly involved in the dormancy of onion bulbs and inverse relationships have been reported between the growth rate and POD activity (Benkeblia 2000).

Despite the fact that there are many reports on the application of gamma irradiation on the onion commercial quality traits, however, the effect of gamma irradiation on PAL, POD and protein content has not been investigated. While the germicidal impact of gamma irradiation in more than 70 crops and in many countries has been widely studied for disinfection (Steele 2000; Jeong et al 2016; Jeong et al 2017; Wang et al 2019). The present study aimed to evaluate: 1) the effects of different doses of gamma radiation on POD, protein content, dry matter and phenylalanine ammonia lyase (PAL) during storage life of four onion genotypes, and 2) the expression of *PAL* gene in the best interaction of gamma irradiation dose and onion genotype treatment.

2. Material and Methods

2.1. Experimental conditions

In the current study, four onion genotypes (White-Ghom, White-Neyshabour, Red-Ridge-Lump, and Red-Ray-Corrugated), were subjected to gamma irradiation at Karaj Nuclear Agriculture Research Center in 2016. A factorial experiment was performed based on a completely randomized design with three replications and two factors. The factors included four onion genotypes and six irradiation intensity (0, 30, 60, 90, 120, and 150 Gy), from a Cobalt-60 gamma resource (with a dose rate of 0.3 gray/second and specific activity of 2300 curie). The onion bulbs were packed in mesh bag after irradiation and stored for four months at 10-15 °C and 70% relative humidity. After the onion bulbs storage period, the following characters were recorded POD activity (U g^{-1}), protein content (mg g^{-1}), dry matter (%) and PAL activity (U g^{-1}).

2.2 Peroxidase assay

100 mg of onion tissues were homogenized with 20 mL sodium phosphate buffer (pH 6.8) and grounded by a cold pestle and mortar. The homogenates were transferred into 2 mL Eppendorf tube and then centrifuged at 12000 rpm for 20 min at 4 °C. POD activity measured in a reaction mixture including 100 µL enzyme extract, 10 µL H_2O_2 30% (w v^{-1}), 200 mL Guaiacol and 20 mL phosphate buffer (pH 6) (MacAdam et al 1992). Absorbance was measured at 470 nm for 60 sec (15-sec interval) using a UV-VIS spectrophotometer.

2.3. Protein content

The bulb tissues (600 mg) were homogenized with 4 mL of sodium phosphate buffer (pH 6.8) and centrifuged for 10 min at 15000 rpm in 4 °C. The supernatant was transferred to a new cold 2 mL tube until assessment. Protein content was measured according to the Bradford method using bovine serum albumin (BSA) as a standard (Bradford 1976).

2.4. Phenylalanine ammonia lyase

The PAL was determined according to Cheng & Breen (1991) method. Onion bulbs tissue (10 g) were homogenized in 100 mL acetone and then extracted at 4 ºC with 50 mL of extraction buffer. The extraction buffer was included 38 g L^{-1} sodium borate (pH 8.8), 0.39 g L^{-1} beta-mercaptoethanol, 0.58 g L^{-1} EDTA, and PVPP at g/100 g of fresh weight. After 1 h of extraction, the solution was filtered and centrifuged at 20000×g and kept in 4 ºC for 15 min. After 10 min of pre-incubation, sodium borate buffer (1.5 mL; pH 8.8) and L-phenylalanine (1 mL), were added to 0.5 mL of supernatant. Finally, PAL was measured at 290 nm wavelength by spectrophotometer.

2.5. Dry matter content

The samples were oven-dried at 75 °C for 24 h. The dry matter content was determined using the following formula:

$$\text{Dry matter (\%)} = \frac{\text{Dry weight}}{\text{Initial weight}} \times 100 \quad (1)$$

2.6. RNA extraction and qRT-PCR

Total RNA was extracted from bulb tissues using the RNeasy Plant Mini kit QIAGEN according to the manufacturer's instructions. Genomic DNA removed by treatment with DNase I during the RNA extraction. The quality and quantity of RNA samples were measured by a NanoDrop spectrophotometer and an absorption ratio (A260/A280) of ≥1.9 were used for reverse transcription. cDNA synthesis was performed by 2 µg of total RNA using RevertAid First Strand cDNA Synthesis kit (Thermo Scientific, USA). Real-time PCR was performed on the Corbett Rotor-Gene 6000 using Power SYBR green master mix (Life Technologies). The q-PCR conditions were as follows: 95 °C for 2 min, followed by 42 cycles of 95 °C for 5 sec; 62 °C for 45 sec followed by a melting curve analysis from 72-95 °C with two technical and three biological replicates. B-tubulin was used as the internal reference gene. Relative expression of PAL was calculated by Livak method (Livak & Schmittgen 2001). The used primers are shown in Table 1

Table 1- List of primers used for Real-time PCR analysis

Gene	*Primer*
B-*tubulin*- forward	5'- GGAAGCATGTGCCCGTGCTATATTTG-3'
B-*tubulin*- reverse	5'-ACAATCTGGATCGTGCGCTTCGTCTTT-3'
AcPAL- forward	5'-TACCCAGCAAAAGAGGTAGC-3'
AcPAL- reverse	5'-CTGGAATGTCTGACACCATC-3'

2.7. Statistical analysis

The data were analyzed using SAS (Statistical Analysis System) software (SAS Institute, Cary, NC). The means were compared using the least significant difference (LSD) test (Steel & Torrie 1980). Cluster analysis was performed using the UPGMA method and SPSS 20.0 on Windows (SPSS Inc., Chicago, IL). To find relationships among the measured characters, Pearson correlation coefficient, was used. The correlation analysis was shown as a heat map using MetaboAnalyst (Xia & Wishart 2016).

3. Results and Discussion

3.1. ANOVA and means comparison for the measured characters

The ANOVA demonstrated that there were significant differences ($P<0.01$) among the studied factors including four onion genotypes, six irradiation levels and their interaction for following traits: POD activity, protein content, PAL and dry matter (%) (Table 2). According to Wickens & Keppel (2004), when the interaction of two factors is significant, then less attention is paid to the two main effects and we focused on the interaction effect. Therefore, in the present study, we have focused on the interaction of onion genotypes and irradiation levels. The result of mean comparison indicated that POD activity increased by most of gamma irradiation levels. To find a relationship between POD activity and gamma irradiation, the regression analysis, was applied. The result showed a linear regression ($R^2= 0.73$) between

POD activity and gamma irradiation (Figure 1). The maximum of POD activity in each genotype was related to different irradiation levels, for example, the maximum of the character in white Qom genotype was obtained at 120 Gy irradiation (20.91 U g^{-1}), whereas the maximum of this trait in white Neishabur genotype was founded at 90 Gy irradiation. POD activity was decreased in all genotypes irradiated at 30 Gy than the control treatments. One of the important roles of POD is degrading indole acetic acid (IAA) activity, which is involved in the growth and dormancy of onion bulbs (Nissen 1985; Benkeblia 2000). The result showed that gamma irradiation in different levels increased the POD activity in all the onion genotypes, as the POD activity has inverse relationships with the growth rate (Gardiner & Cleland 1974), therefore the quality and the time of storage of the onion bulbs can be improved by gamma irradiation. POD is an important antioxidant enzyme that plays a role in ROS scavenging. POD activity was increased in *Vicia faba* and *Glycine max* after gamma rays applied (20 Gy) (Moussa 2008; Moussa 2011). There are many reports about the improvement of POD activity using radiation in different plants such as *Raphanus sativus* (Lee et al 2003), *Triticum aestivum* (Hong et al 2018); *Hordeum vulgare* (Wang et al 2018) and *Phoenix dactylifera* (Zarbakhsh & Rastegar 2019).

Table 1- ANOVA of studied traits in four onion genotypes under gamma irradiation

S.O.V	*D.F*	*Mean square*			
		POD	*Protein*	*Dry matter*	*PAL*
Genotype (A)	3	338.25**	22.99**	1.39**	0.67**
Gamma irradiation (B)	5	414.55**	75.79**	1.92**	1.04**
A × B	15	130.78**	15.69**	7.19**	0.98**
Error	48	0.157	0.305	0.318	0.103
C.V (%)	-	5.15	5.64	5.66	17.50

**, significant at P≤0.01 probability level

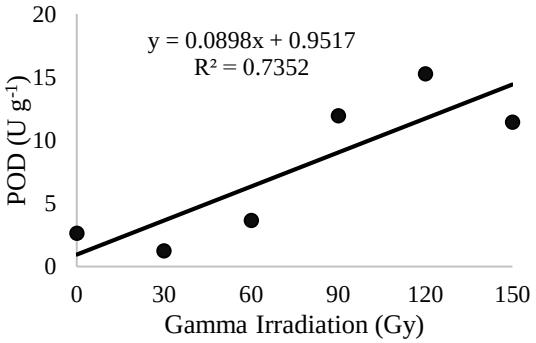

Figure 1- Plot depicting the relation between POD content and gamma irradiation levels in onion

The highest PAL activity in the white-Qom and red-Ray-Corrugated genotypes (3.367 and 2.27 U g^{-1}, respectively) were observed in their control treatments, whereas in white-Neishabur and Red-Ridge-Lump genotypes were observed at 60 and 120 Gy, respectively (Table 3). However, the lowest PAL activity in three genotypes (White-Qom, Red-Ray-Corrugated, and White-Neishabur) was obtained at 150 Gy, whereas in Red-Ridge-Lump genotype was revealed at 90 Gy. Therefore it can be concluded that PAL activity was depended on onion genotype, under normal and irradiated conditions. PAL is known as a key enzyme in the phenylpropanoid pathway related to phenolic compounds synthesis, which accumulates during different plant stress such as tissue damage, nutrient deficiencies, fungal, bacterial, and insect attack (He & Luo 2007; Daayf et al 2012). Tripathi & Variyar (2016) reported that PAL activity remained stable during storage of *Benincasa hispida* under the application of gamma irradiation. The authors revealed that during the plant storage, as the irradiated samples were protected from spoilage, the PAL activity remained unchanged. PAL and POD induction is a defense mechanism of plant tissues from external stress. The results of this study revealed that gamma irradiation improved the PAL and POD activities in the onion storage, which proposes that these enzymes may be involved in the onion defense mechanism. Figure 2 showed the relation between PAL activity and gamma irradiation in White-Qom genotype. A linear regression (R^2= 0.82) between PAL activity and gamma irradiation levels, was observed.

Table 2- Mean comparisons of studied traits in onion genotypes under different irradiation levels

Genotype	*GI (Gy)*	*POD (U g^{-1})*	*Protein (mg g^{-1})*	*DM (%)*	*PAL (U g^{-1})*
White-Qom	0	3.153	13.93	9.803	3.367
	30	2.53	9.657	9.047	2.133
	60	4.577	10.32	10.31	2.10
	90	6.583	10.13	7.577	2.157
	120	20.91	5.61	12.45	1.38
	150	9.62	6.84	11.97	1.257
White-Neishabur	0	4.477	13.11	10.84	1.023
	30	0.727	10.33	9.657	1.66
	60	1.01	12.62	11.49	2.647
	90	19.03	9.803	9.323	2.013
	120	22.84	11.05	10.57	2.123
	150	31.07	8.007	9.083	1.50
Red-Ridge-Lump	0	1.73	11.54	9.183	1.59
	30	1.383	18.09	9.123	1.633
	60	6.32	12.46	8.407	2.193
	90	19.94	8.13	10.23	1.387
	120	9.43	7.35	10.21	2.213
	150	3.36	5.937	10.45	2.00
Red-Ray-Corrugated	0	1.187	12.12	8.623	2.267
	30	0.313	9.37	12.85	0.99
	60	2.623	10.82	9.857	1.953
	90	2.177	4.00	10.19	2.067
	120	7.817	7.753	7.807	1.347
	150	1.653	6.183	9.877	0.943
LSD (0.01)		0.87	1.21	1.23	0.70

GI, gamma irradiation; DM, dry matter

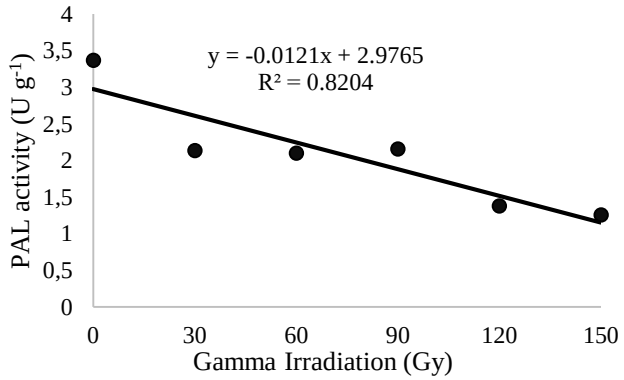

Figure 2- Plot depicting the relation between PAL activity and gamma irradiation levels in white-Qom

The highest protein content in the White-Qom, White-Neishabur and Red-Ray-Corrugated genotypes (13.93, 13.11, and 12.12 mg g^{-1}, respectively) were observed in their control treatments, whereas, the highest value for this character in Red-Ridge- Lump was observed at 30 Gy (18.09 mg g^{-1}). The result showed that with increasing the gamma irradiation levels, the protein content decreased. To find a relationship between protein content and gamma irradiation, a regression analysis, was used (Figure 3). The result of the regression analysis indicated that there was a linear regression between the onion protein content and gamma irradiation. El-Beltagi et al (2011) reported that with increasing the gamma irradiation doses, the protein synthesis and enzyme activity were disrupted. The results of the present study are in contrast with Ling et al (2008), who found that the citrus samples irradiated at high doses showed higher protein content compared to their control samples. However, in Red-Ridge-Lump genotype at the dose of 30 Gy, the protein content was increased as compared to its control. It seems that the onion genotypes had a different response to gamma irradiation.

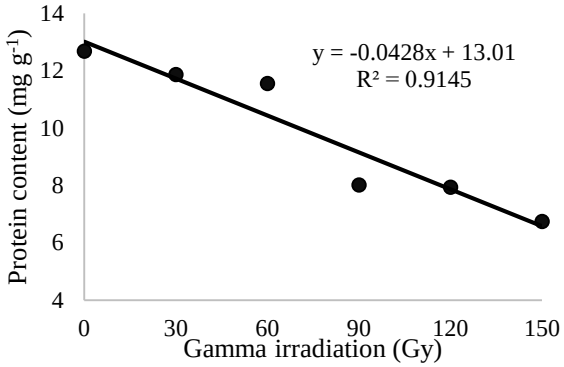

Figure 3- Plot depicting the relation between protein content and gamma irradiation levels in onion

Dry matter is an imperative quality character in onion bulbs, which is supposed to have a relationship with storage time and bulb properties. The results showed that dry matter content was highly genotype-dependent. In the White-Qom genotype, the lowest (7.57%) and the highest (12.45%) dry matter were achieved at doses of 90 and 120 Gy, respectively, which had significant differences with control treatment (9.8%). The interaction of Red-Ray-Corrugated with a dose of 30 Gy had the highest dry matter (12.85%) among the other interaction treatments. Glucose, fructose, and sucrose are the main parts of DM, which are non-structural carbohydrates (Benkeblia et al 2004). These carbohydrates are about 70-80% of the bulb dry matter (Böttcher et al 2018). The compositions of dry matter have changed during the storage period. Also, during frying, it can change to fructose and glucose that are active in non-enzymatic browning (Clark et al 2018). Onions storage for the long term is one of the problems in the frying industry, resulting in the darker and more bitter the fried product (Asefi & Mozaffari 2010).

3.2. Cluster and correlation analyses

The dendrogram was generated using the following measured parameters, PAL, POD, protein content, and dry matter, which grouped the onion genotypes into two clusters (Figure 4). The first group included G1 (White-Ghom) and G2 (White-Neyshabour) genotypes. This group had the highest values of DM, protein content, and POD activity than the second group, which means that the quality of genotypes in this group were better than the genotypes in the second group. Two other genotypes including G3 (Red-Ridge-Lump) and G4 (Red-Ray-Corrugated) were in the second group. Based on the cluster analysis there was a moderate diversity among the four studied onion genotypes. These variation is due to genetic factors among the samples (Ebrahimi et al 2012; Farajpour et al 2017; Boroomand et al 2018; Hassanabadi et al 2019). Among the parameters, DM and POD activity had the highest positive correlation coefficient with each other ($r= 0.76$; $P<0.01$; Figure 5). However, the highest negative correlation was between DM and PAL activity. Protein and POD had a significant correlation with each other ($r= 0.63$; $P<0.05$).

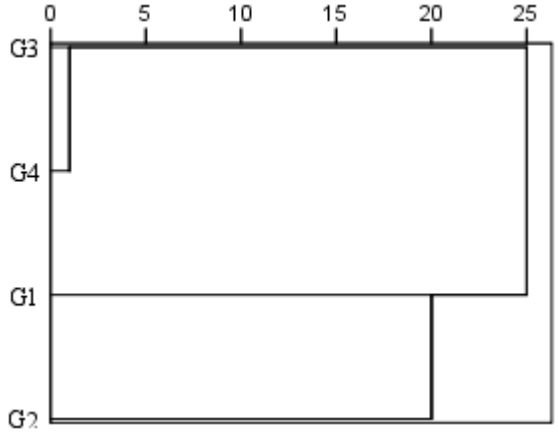

Figure 4- Dendrogram generated based on the four measured characters on the four onion genotypes using UPGMA method

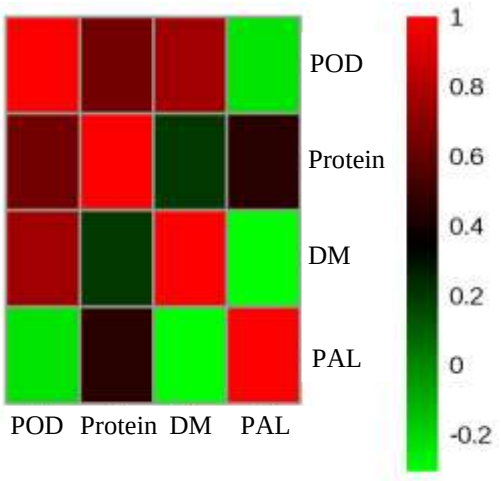

Figure 5- Heat map of the correlations between the four studied characters of the four onion genotypes

3.3. Real-time PCR analysis of PAL gene

In order to evaluate the effects of gamma irradiation on the relative expression of *PAL* gene, quantitative Real-time PCR analysis, was performed. Based on data obtained from PAL activity and sprouting (data not shown), the relative expression of *PAL* gene in White-Qom genotype at 150 Gy, was assessed. Non-irradiated onion bulbs were used as control. Results showed that there was no significant difference between onion bulbs irradiated by 150 Gy and non-irradiated bulbs in terms of *PAL* gene expression (Figure 6). Results revealed that the PAL activity decreased at higher gamma irradiation levels, while the expression level of the *PAL* gene was not significant between irradiated and non-irradiated control bulbs. This may be due to the effects of gamma irradiation in controlling the fungal, bacterial or other sources of stresses in onion bulb, resulting in reduced PAL activity at higher gamma irradiation doses (Calado et al 2014; Jeong et al 2016). Based on the results, the *PAL* gene expression was not affected by gamma irradiation, indicating that the radiation might not have direct effects on the PAL gene regulatory elements. These results suggest that gamma irradiation could reduce the PAL activity possibly by controlling abiotic stress sources such as fungal and bacterial stresses; it can be pointed out as a positive point, which can reduce food safety concerns.

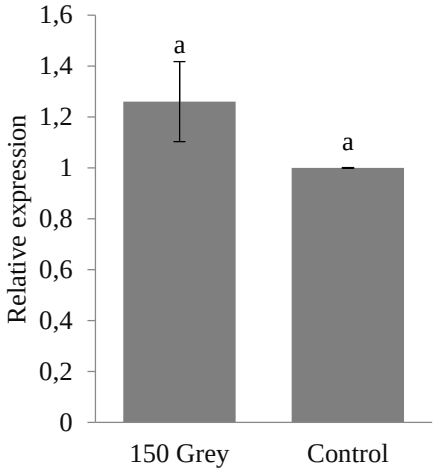

Figure 6- Relative expression of *PAL* gene in White Qom onion genotype treated by 150 Gy gamma irradiation

4. Conclusions

The present study revealed the feasibility of gamma irradiation as an efficient tool for the management of onion storage. The result indicated that POD activity increased by most of the gamma irradiation levels, however, the protein content

and PAL activity decreased. Also, the result showed that onion genotypes had a different response to gamma irradiation. Among the genotypes, White-Ghom and White-Neyshabour had the highest quality. The PAL activity decreased with increasing in gamma irradiation levels, while the expression level of the *PAL* gene was not significant between irradiated and non-irradiated control bulbs. Based on literature review, biotic and abiotic stresses increase the PAL activity. So it can be concluded that gamma irradiation caused to control the source of stresses such as fungal and bacterial. Therefore, with increasing the level of gamma irradiation, the PAL activity was decreased, indicating that the gamma radiation did not make any mutation in the *PAL* gene. So it can be used as a useful method, which can reduce food safety concerns.

Abbreviations and Symbols	
Gy	gray
DM	dry matter
PAL	phenylalanine ammonia lyase
POD	peroxidase
BSA	bovine serum albumin
LSD	Least Significant Difference
ANOVA	analysis of variances

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The Impact of Different Cover Crops, Mechanical Cultivation and Herbicide Treatment on The Soil Quality Variables and Yield in Apple (*Malus domestica* Borkh.) Orchard with a Coarse-Textured Soil

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ABSTRACT
Effects of different cover crops on soil quality parameters and yield of an apple orchard located in Develi town of Kayseri province of Turkey were investigated in this study. *Trifolium repens* L. (TR), *Festuca rubra* subsp. *rubra* (FRR), *Festuca arundinacea* (FA), *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture (TFF), *Vicia villosa* (VV) and *Trifolium meneghinianum* (TM) were used as the cover crops in an apple orchard with sandy loam soil. Experiments also included plots mechanically cultivated (MC), herbicide treatment (HC) and control (C) plot without cover crops. Soil samples were taken from two different depths (0-20 and 20-40 cm) in each plot. Experiments were conducted in randomized complete blocks design with four replications. The cover crop treatments improved soil quality parameters like organic matter (OM), basal soil respiration, bulk density (BD), aggregate stability, saturated hydraulic conductivity (Ks), available water capacity compared to the soil of a nontreated control plot. Mean OM contents at 0-20 cm soil depth was ordered as; HC (0.56%)<C (0.62%)<MC (0.67%)<FRR (1.03%)<TM (1.06%)<TFF (1.09%)<FA (1.10%)<VV (1.34%)<TR (1.36%). The highest cover crop dry biomass mean was found in TFF treatment (45.5 ton ha^{-1}), and the lowest was found in TM treatment (2.6 ton ha^{-1}) in the apple orchard. While the highest mean apple yield was obtained from VV (23.9 ton ha^{-1}) and TR (23.8 ton ha^{-1}) treatments, the lowest mean apple yield (13.7 ton ha^{-1}) was obtained from MC treatment. It was concluded based on the obtained findings that cover crops, especially *Trifolium repens* and *Vicia villosa* could be incorporated into cropping systems to improve soil quality and apple yield.

Keywords: Cover crops; Organic matter; Sandy loam soil; Soil quality; Available water capacity; Yield

1. Introduction

Apple is a product of economic importance in Turkey and the World as whole. Apple production in the world was realized in 4.9 million ha area according to FAO statistics. The apple production in the world was 83.1 million tones in the same year. The most important share in production belongs to China (49.8%), USA (6.2%), Turkey (3.6%), and India (2.7%), respectively. Turkey comes thirth in terms of apple production with 3.6% of the world total production (FAOSTAT 2017). Apple growing areas are generally concentrated in the regions of Niğde-Nevşehir-Kayseri, Amasya-Tokat, Bursa-Yalova-Çanakkale, Antalya and Isparta-Burdur in Turkey (Gül & Erkan 2001). Apples can be grown in almost all parts of the Turkey and apple production constitutes 9% of total fruit areas and 20% of total fruit production. 2.82% of the total apple production of Turkey is supplied from Kayseri province. In the province of Kayseri, approximately 69 938 tons of apple are produced 6088 ha in each year (TURKSTAT 2018). Many soils in Turkish apple orchards are low in organic matter and nutrients, resulting in poor soil structure and nutrient deficiencies in the fruit trees (Zengin et al 2016). This situation

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negatively affects the growth of apple trees, which is detrimental to the yield and the quality of the fruit. The use of cover crops as a source of organic matter and nutrients may be potential solution to these problems. Cover crops have been used to improve soil aquality and reduce nonpoint sources of nutrient pollution, e.g., NO_3 (Daliparthy et al 1994). Therefore, it is significant from both an environmental and economic standpoint to indicate how cover crop systems effect soil organic matter characteristics and the biogeochemical cycling of carbon. Legume and grass species are the most significant annual and perennial forage crops in Turkey and are preferred by farms because of providing sufficient plant cover and improving the percentage of organic matter (Nyakatawa et al 2001). Soil-structure improvements associated with legume based rotations also increase the drought tolerance of soils and moisture-holding capacity (Goldstein 1989).

The ability of soils to provide optimum conditions for plant development and growth is defined as soil fertility or soil quality. Soil quality is expressed in several physicals, chemical and biological attributes such as organic matter content, bulk density, salinity, microbial activity, nutritional composition etc. (Stott et al 2010). Long-term soil fertility in conventional and organic farming can be achieved only with sustainable practices (Price & Norsworthy 2013). Farming systems should generally take effects of agricultural practices on the environment and human health into consideration. Excessive synthetic fertilizers applied in conventional farming may exert serious risks both on soils and environment (Stolze et al 2000). Cover crops can decrease the use of external inputs such as fertilizers and can improve and maintain soil fertility.

Establishing cover crops also has an important effect on improving soil chemical, physical and biological properties and hence on increasing the yields of successive row crops (Fageria et al 2005). Plant residues or cover crop species are commonly used to improve organic carbon contents and soil quality (Demir & Gülser 2015). Crude protein production largely depends on dry matter yield and crude protein concentrations vary with the plant species (Albayrak et al 2008). Leguminous crops are quite rich in nitrogen and can fix atmospheric nitrogen into the soils. In this way, they reduce or replace synthetic fertilizers used for nitrogen supply in conventional farming (Gselman & Kramberger 2008).

Recent environmental and ecological awareness has started a resurgence in cover crop use in Turkey. Although cover crops have been used for centuries, today's modern farmer has grown up in a generation which has replaced the use of cover crops with widespread use of fertilizers and herbicides. Cover crops have an important role in successful sustainable farming systems. Cover crops control soil erosion, improve soil quality and fertility, suppress weeds and provide insect control (Sarrantonio 2007). At the same time cover crops are suitable implements for weed control in orchards (Mennan et al 2009; Işık et al 2009). Using cover crops for weed control in apple orchards is one of the broadly applied alternative methods to the mechanically cultivated and herbicide treatment. Herbicide application and mechanical cultivation treatment are important among the current weed control practices in apple farming. Herbicide and mechanical cultivation treatment are expected to provide weed-free apple fields. However, coverless (bare) apple fields may bring about increased erosion and run-off, reduced organic matter and moisture contents, and damage the soil chemical and physical properties (Keesstra et al 2016). In addition, a few other problems are also associated with the use of herbicides for weed control in apple fields. Evolution of herbicide resistance in weeds and environmental pollution are the most important among these (Annett et al 2014). In addition, the effects of herbicide and mechanical control applications used in weed control on soil quality are not known.

Agricultural lands of Turkey are particularly prone to erosion after the crop harvests in August until the re-establishment of soil cover in November. Severe rainstorms in these months negatively affect physical properties of the bare soil by deteriorating aggregates and clogging macropores leading to an increased erosion risk. Choosing a crop that increases aggregate stability during the growing season is one strategy to reduce the risk for post-harvest erosion (Yakupoglu et al 2011). Thus, crop residue management is a key element of sustainable crop production. Crop residues were incorporated into cropping systems in previous studies to improve and maintain soil structure and fertility. It is important to use cover crops to achieve the objectives of sustainable cropping systems (Ruffo & Bollero 2003). Therefore, new approaches should be evaluated for sustainable soil management, environmental protection, and human health. While there are many studies on cover crops, number of studies about the effects of the *Trifolium repens* L. (TR), *Festuca rubra rubra* L. (FRR), *Festuca arundinacea* (FA), *Trifolium repens* (40%)+*Festuca rubra rubra* (30%)+*Festuca arundinacea* (30%) mixture (TFF), *Vicia villosa* (VV) *and Trifolium meneghinianum* (TM) on soil quality of an apple orchard is quite limited. The objectives of this study were: i) to determine the changes in some soil quality variables of a sandy loam soil of an apple orchard with different cover crop treatments including plots mechanically cultivated (MC), herbicide treatment (HC) and control plot (C), ii) to determine the relationships between soil quality variables iii) to determine the effects of all treatments on apple yield.

2. Material and Methods

2.1. Experimental site

Experiments were conducted in the experimental semi-dwarf apple orchard of Erciyes University Agricultural Faculty between the years of 2012-2014. Experimental site is located between 38.22 N - 35.27 E in Develi town of Kayseri province of Turkey. The annual average temperature was 10.6 °C and the average annual precipitation were 384.3 mm. At the beginning of this study in 2012, the apple trees in the orchard were 7 years old. Each plot was 35 m^2 (5×7 m) and consisted of 5 semi-dwarf apple trees.

2.2. Cover crop treatments

The cover crop treatments consisted of *Trifolium repens* L. (TR), *Festuca rubra rubra* L. (FRR), *Festuca arundinacea* (FA), *Trifolium repens* (40%) + *Festuca rubra rubra* (30%) + *Festuca arundinacea* (30%) mixture (TFF), *Vicia villosa* (VV) and *Trifolium meneghinianum* (TM). *Vicia villosa* and *Trifolium meneghinianum* are annual legume plants and *Trifolium repens* L. are perennial legume plants. *Festuca rubra rubra* L. and *Festuca arundinacea* are perennial grass cover plants. The experiment was established in a randomized complete block design with four replications including plots mechanically cultivated (MC), herbicide treatment (HC) and control plots without cover crops (C). Consecutive plots were separated with a buffer zone with no cover crop. Soil preparation was made according to local practices for Orchards. Cover crops were manually seeded on 10 April 2012, annual species (VV and TM) seeded again 27 October 2012, and 2 November 2013. The cover crop seeds were broadcasted and then incorporated into the soil with a shallow cultivator. Primary tillage and chisel plowing, followed by disking with a harrow. Seeding rate was 50 kg ha^{-1} for *Trifolium repens* L., 80 kg ha^{-1} for *Festuca rubra rubra* L., 70 kg ha^{-1} *Trifolium repens* (40%) + *Festuca rubra rubra* (30%) + *Festuca arundinacea* (30%) mixture, 100 kg ha^{-1} *Vicia villosa* and 40 kg ha^{-1} *Trifolium meneghinianum. Trifolium meneghinianum* seeds were obtained from Black Sea Agricultural Research Institute and the others were purchased from private seed companies. The cover crop treatments were maintained in the same plots throughout the experiments. During the vegetation period, no fertilizer was applied. Cover crops mowed when *Festuca* spp. in the flag leaf periods, others were at the beginning of the flowering periods (Işık et al 2014; Tursun et al 2018). Cover crops were mowed and incorporated into the soil by disking. Apple harvest was performed on 11 October 2012 and 19 October 2013, but not in 2014 due to hail storm decimated almost all of the yield. Prior to treatments (mowing) of the cover crops, above ground biomass samples of the cover crops were collected from randomly selected 50 x 50 cm quadrat in each plot. Cover crops were dried at 70 °C for 48 h to obtain dry biomass (Mennan et al 2009; Işık et al 2009). Glyphosate isopropylamine salt (360 g a.i L^{-1}) was applied at 2880 mL ha^{-1} (1.39 kg a.i ha^{-1}) in the herbicide control plots. Glyphosate was applied at 3 atm pressure (303.97 kPa) with 250 L ha^{-1} spraying volume with a portable hand sprayer (Honda WJR 2225 model).

2.3. Soil sampling

Soil samples were taken from 0-20 and 20-40 cm depths in each plot 90 d after plant harvest using a corkscrew-shaped soil drill and soil quality parameters were determined. Samples were sieved through 2 mm sieve and made ready for physicochemical analyses. Some physical and chemical properties of the soil at the start of the experiment were given in Table 1. Soil analyses revealed that soils (at 0-20 cm) in the experimental field site have a coarse-textured soil (74.40% Sand, 15.23% Silt, 10.37% Clay), were slightly alkaline (7.57), and low organic matter (0.61) contents (Soil Survey Staff 2014).

Table 1- Some physical and chemical properties of the soil at the start of the experiment

Soil properties	*Depth, cm*		*Soil properties*	*Depth, cm*	
	0-20	*20-40*		*0-20*	*20-40*
Sand, %	74.40	73.51	OM, %	0.61	0.53
Silt, %	15.23	15.41	Ca, me 100 g^{-1}	6.24	6.36
Clay, %	10.37	11.08	Mg, me 100 g^{-1}	2.40	1.96
Soil texture	SL	SL	Na me 100 g^{-1}	0.32	0.31
BD, gr cm^{-3}	1.52	1.50	K, me 100 g^{-1}	0.25	0.20
pH (1:1)	7.57	7.55	$CaCO_3$, %	0.71	0.67
$EC_{25°C}$, ds m^{-1}	0.45	0.44	P, mg kg^{-1}	7.33	6.91

BD, Bulk density; SL: Sandy loam; pH, Soil reaction; EC, Electrical conductivity; OM, Organic matter; Ca, Exchangeable calcium; Mg, Exchangeable magnesium; Na, Exchangeable sodium; K, Exchangeable potassium; P, Available phosphorus

2.4. Soil chemical analysis

Soil reaction (pH) was measured by using a pH meter with glass electrode in a 1:1 (w:v) ratio soil-water suspension (Jackson 1958). Electrical conductivity ($EC_{25°C}$) was measured with an EC meter in a 1:1 (w:v) ratio soil-water suspension (Richards 1954).

Exchangeable cations (Ca, Mg, K, Na) were determined with the 1N ammonia acetate (NH_4OAc) extraction (Rowell 1996). Available P contents were determined through extraction with 0.5 M $NaHCO_3$ at pH 8.5 by Olsen's method (Olsen et al 1954). Organic matter was determined by the modified Walkley-Black method (Black 1965). Total N was determined by the LECO model Tru-Spec CHN elemental analyzer (Dumas 1831). Lime content was determined by Scheibler Calcimeter (Nelson 1982).

2.5. Soil biochemical analysis

Basal soil respiration (BSR) was measured in accordance with Isermayer (1952) through measuring CO_2 productions at 22 ºC. The CO_2 production at the end of the 24 h incubation period was expressed in mg CO_2 100 g^{-1}.

2.6. Soil physical analysis

Soil particle size distribution was determined by using Bouyoucos hydrometer method (Bouyoucos 1953). Bulk density (BD) was carried out with the cylinder method (Black 1965). Soil field capacity (FC) and the permanent wilting point (PWP) were determined according to Hillel (1982). After saturating soil samples with tap water for 24 hours, soil water content at the field capacity was determined through equilibrating soil moisture for 24 hours at 33 kPa on a ceramic plate, and the permanent wilting point was measured through equilibrating soil moisture for 96 hours at 1500 kPa on a pressure plate apparatus (Hillel 1982). Available water content (AWC) was then calculated as the difference between FC and PWP (Hillel 1982). Volumetric water content (θ) was estimated from the following equation (Hillel 1982);

θ = gravimetric water content (W) (g H_2O g^{-1} soil at the sampling time) x soil bulk density (g cm^{-3}) (1)

Then, Equation. 2 was used to calculate the total porosity (F) (Hillel 1982):

$$F = 1 - (BD/2.65) \quad (2)$$

Hydrometer method and the following equation were used to determine soil structural stability index (SSI):

$$SSI = \sum b - \sum a \quad (3)$$

Where "b" is percent clay and "a" is silt + clay (Leo 1963). A wet sieving device was used to assess the aggregate stability (AS) (Yoder type) (Kemper & Rosenau 1986). Constant head permeameter was used to measure saturated hydraulic conductivity (Ks) of the soil samples (US Salinity Lab. Staff 1954). Following Darcy equation was sued to calculate saturated hydraulic conductivity (Ks, cm h^{-1});

$$Ks = \frac{Q}{A\,t}\left(\frac{S}{S+H}\right) \quad (4)$$

Where, Q: volume of outflow (cm^3), A: cross sectional area of soil column (cm^2), t: time (hour), S: length of soil column (cm), H: water head over the soil column (cm).

Dry sieving method was used to calculate mean weight diameter (MWD) (Hillel 1982):

$$MWD = \sum_{i=1}^{k} W(i)\,\bar{x}i \quad (5)$$

2.7. Statistical analysis

Experimental results were subjected to statistical analyses with SPSS. Data were subjected to ANOVA and treatment means were compared with Duncan's multiple range test at the 0.01 and 0.05 probability levels. Correlation analyses were performed to express the relationships between experimental parameters (Yurtsever 2011).

3. Results and Discussion

3.1. Soil chemical quality variables

3.1.1. Soil organic matter (SOM)

Cover crop treatments increased soil organic matter contents at 0-20 cm soil depth as compared to the soil of an untreated control plot (Table 2). Cover crop treatments increased SOM content from 0.59% in the control treatment to 1.29% in *Trifolium repens* L. (TR) treatment in the first year of the experiments (2013). Soil organic matter contents at 0-20 cm soil depth in the second year of the experiment (2014) were ordered as; HC (0.59%) < C (0.64%) < MC (0.67%) < FRR (1.06%) < TM (1.09%) < TFF (1.13%) < FA (1.14%) < VV (1.41%) < TR (1.42%) (Table 2). SOM contents varied significantly between experimental years. The mean OM values of the control plots without cover crops were 0.62% for 0-20 cm and 0.53% for 20-40 cm. Percent changes in chemical soil quality parameters as compared to control are provided in Table 3. The soil quality is assessed through physicochemical and biological soil quality parameters. The soil characteristics, environmental factors, and land management practices significantly influence all these quality parameters (Stott et al 2010). Organic carbon is one of the most significant quality indicators for soils because it has a variety of significant impacts on other soil quality attributes. Cropping treatments are also used to improve soil organic carbon contents and thus soil quality and fertility (Demir & Gülser 2015). Organic matter contents significantly increased from 0.64% in the control to 1.42% in *Trifolium repens* (TR) treatment at 0-20 cm soil depth. Gülser (2004) reported that cropping treatments increased the organic matter content from 2.28% for bare soil to 3.18% for bromegrass treatment. In addition, Gülser (2004) determined that such increases in SOM content due to different cropping regimes were obtained in the following order: control < crownvetch < subterranean clover < alfalfa < ryegrass < small burnet < bromegrass. Ramos et al (2010) was reported in a previous study that two cover crop (oat-*Avena sativa* L. and oat-vetch-*Vicia sativa* L.) treatments increased soil organic carbon content (55.6% and 66.7%, respectively) and aggregate stability. Obi (1999) found that cover crops (*Axonopus compressus*, *Cynodon plectostachyum*, *Panicum maximum*, *Pennisetum polystachion*, *Stylosanthes gracilis and Pueraria phaseoloides*) on degraded sandy clay loam soil improved mean organic carbon level (by 28.1%) over value for the control soils. In this study, the greatest increases in soil organic matter contents at 0-20 cm soil depth of apple orchard as compared to the control in both years of the experiment were observed in *Trifolium repens* (TR) treatment respectively with 117.8 and 120.1%. A closely related species, *Trifolium repens* has a robust root system that could provide drought resistance as compared to grass cover crops (Roumet et al 2006). Bertin et al (2003) reported that soil organic matter content was increased in the cover crops through root activity, i.e. exudation of low-molecular weight organic compounds. In addition, the root systems of perennials are more randomly branched and have larger diameter roots than annual (Roumet et al 2006). This may explain the nutrient conservative strategy of perennials and the high nutrient uptake capacities of annuals (Roumet et al 2006). On the other hand, salt tolerance in *Trifolium repens* appears to be correlated with (i) a capacity to restrict and regulate the transport of these ions from the roots to the shoots, leading to lower concentrations of Na^+ and Cl^- in the shoot, and (ii) lower uptake rates of Na^+ and Cl^- per unit of root tissue (Rogers et al 1997).

The differences in SOM contents were not found to be significant for the 20-40 cm soil depth in both years of experiments (Table 4). SOM contents ranged from 0.51% in herbicide treatment to 0.64% in mixture (*T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%)) treatment for the 20-40 cm soil depth in 2013. SOM contents ranged from 0.52% in herbicide treatment to 0.73% in *Festuca arundinacea* (FA) treatment for the 20-40 cm soil depth in 2014 (Table 4).

Table 2- Effects of treatments on chemical soil quality variables at 0-20 cm soil depth

2013										
	*pH, (1:1)***	*EC, dS m^{-1}***	*OM, %***	*N, %***	*NH_4OAc extractable, me 100 g^{-1}*				*P, mg kg^{-1} ***	*BSR, mg CO_2 100 g^{-1}***
					Ca	*Mg*	*K*	*Na*		
TR	7.22e	0.91a	1.29a	0.086a	6.2	2.7	0.32	0.25c	12.4a	5.8a
FRR	7.46bc	0.75b	1.00c	0.065abc	6.4	2.4	0.34	0.26c	11.0a	5.4ab
FA	7.36d	0.87a	1.06bc	0.072abc	6.3	2.2	0.35	0.30abc	11.6a	5.3ab
TFF	7.42cd	0.84b	1.05bc	0.075ab	6.4	2.6	0.34	0.29abc	11.8a	5.6a
VV	7.33d	0.89b	1.26ab	0.085a	6.8	2.5	0.35	0.25c	13.0a	6.0a
TM	7.49bc	0.65b	1.02c	0.075ab	6.7	2.5	0.34	0.27bc	12.1a	5.7a
HC	7.53b	0.46c	0.53d	0.045c	6.8	2.2	0.27	0.35a	7.3b	2.4b
MC	7.54b	0.49c	0.66d	0.049bc	6.3	2.2	0.29	0.29abc	7.3b	2.5b
C	7.65a	0.45c	0.59d	0.047bc	6.7	2.4	0.26	0.34ab	7.2b	2.3b
2014										
	*pH, (1:1)***	*EC, dS m^{-1}***	*OM, %***	*N, %***	*NH_4OAc extractable,me 100 g^{-1}*				*P, mg kg^{-1} ***	*BSR,mg CO_2 100 g^{-1}***
					Ca	*Mg*	*K**	*Na***		
TR	7.17d	0.92a	1.42a	0.100a	6.1	3.0	0.36ab	0.14b	13.3a	9.7a
FRR	7.48bc	0.75ab	1.06b	0.071bc	6.2	3.0	0.36ab	0.24ab	12.2a	7.3bc
FA	7.28cd	0.98a	1.14b	0.074bc	6.7	2.7	0.37a	0.23ab	12.0a	6.2c
TFF	7.39bc	0.92a	1.13b	0.082ab	6.4	2.8	0.36ab	0.19b	12.2a	8.4ab
VV	7.25d	0.99a	1.41a	0.102a	6.1	2.9	0.38a	0.21ab	14.0a	9.8a
TM	7.40bc	0.75ab	1.09b	0.085ab	6.2	2.5	0.36ab	0.15b	12.6a	8.6ab
HC	7.51b	0.45c	0.59c	0.042d	6.6	2.3	0.25b	0.33a	7.0b	2.9d
MC	7.50ab	0.55bc	0.67c	0.056cd	6.3	2.4	0.26b	0.32a	7.6b	4.2d
C	7.62a	0.47c	0.64c	0.053cd	6.1	2.7	0.25b	0.31a	7.4b	3.4d

**, P <0.01; *, P <0.05; TR, *Trifolium repens* L.; FRR, *Festuca rubra* subsp. *Rubra;* FA, *Festuca arundinacea;* TFF, *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture; VV, *Vicia villosa;* TM, *Trifolium meneghinianum;* MC, Mechanically cultivated; HC, Herbicide treatment; C, Control; pH, Soil reaction; EC, Electrical conductivity; OM, Organic matter; N, Total Nitrogen; Ca, Exchangeable calcium; Mg, Exchangeable magnesium; K, Exchangeable potassium; Na, Exchangeable sodium; P, Available phosphorus; BSR, Basal soil respiration; CO_2, Karbondioksit

Table 3- Percent changes in chemical soil quality variables as compared to control at the 0-20 cm soil dept (%)

2013										
Treatments					*NH_4OAc extractable*					
	pH	*EC*	*OM*	*N*	*Ca*	*Mg*	*K*	*Na*	*P*	*BSR*
TR	-5.6	99.2	117.8	83.7	-7.4	14.3	22.8	-25.6	72.0	151.1
FRR	-2.5	65.3	68.4	40.1	-4.4	0.7	28.4	-22.8	52.8	133.2
FA	-3.7	91.5	79.4	54.9	-6.1	-6.4	31.3	-13.3	60.5	129.9
TFF	-3.0	85.9	77.9	61.0	-5.8	9.6	28.4	-14.7	64.1	144.6
VV	-4.1	95.7	113.2	82.4	0.6	4.1	31.3	-26.3	80.6	162.5
TM	-2.1	43.4	72.7	60.5	-0.9	7.7	28.4	-21.3	67.2	147.8
HC	-1.5	1.2	-10.6	-3.5	0.9	-5.6	0.8	2.1	1.3	5.4
MC	-1.4	7.2	11.8	4.6	-6.4	-6.0	8.6	-13.9	1.6	6.5
2014										
TR	-5.9	95.1	120.1	91.3	-0.4	13.6	46.2	-56.0	78.9	181.2
FRR	-1.8	57.5	65.3	35.7	2.2	11.2	47.0	-24.8	64.2	113.4
FA	-4.5	107.2	77.0	41.4	9.7	2.0	48.8	-26.4	61.6	81.4
TFF	-3.1	93.9	75.8	56.2	5.8	6.4	44.2	-40.0	64.5	143.3
VV	-4.9	108.9	119.7	94.8	0.9	9.5	50.9	-31.9	88.1	186.3
TM	-2.9	59.2	70.0	61.9	2.3	-4.3	47.2	-53.4	69.9	151.3
HC	-1.4	-4.1	-8.2	-21.0	8.7	-12.5	0.5	4.0	-5.4	-15.5
MC	-1.5	15.4	3.8	5.7	3.1	-8.9	4.6	0.8	1.6	22.4

TR, *Trifolium repens* L.; FRR, *Festuca rubra* subsp. *rubra*, FA, *Festuca arundinacea;* TFF, *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture; VV, *Vicia villosa*; TM, *Trifolium meneghinianum;* MC, Mechanically cultivated; HC, Herbicide treatment; C, Control; pH, soil reaction; EC, Electrical conductivity; OM, Organic matter; N, Total nitrogen; Ca, Exchangeable calcium; Mg, Exchangeable magnesium; K, Exchangeable potassium; Na, Exchangeable sodium; P, Available phosphorus; BSR, Basal soil respiration

Table 4- Effects of treatments on chemical soil quality variables at 20-40 cm soil depth

2013										
	pH	*$EC_{25°}$,*	*OM,*	*N,*	*NH_4OAc extractable, me 100 g^{-1}*				*P,*	*BSR,*
Treatments	*(1:1)*	*dS m^{-1}*	*%*	*%*	*Ca*	*Mg*	*K*	*Na*	*mg kg^{-1}*	*mg CO_2 100 g^{-1} soil*
TR	7.49	0.429	0.56	0.031	6.21	1.98	0.22	0.30	7.14	0.013
FRR	7.59	0.412	0.60	0.030	6.15	2.12	0.19	0.31	7.31	0.012
FA	7.59	0.473	0.62	0.030	6.70	2.17	0.20	0.35	7.68	0.012
TFF	7.58	0.409	0.64	0.029	6.73	2.07	0.21	0.34	7.13	0.012
VV	7.52	0.443	0.56	0.028	6.09	2.04	0.21	0.30	7.26	0.013
TM	7.64	0.414	0.54	0.030	6.03	2.00	0.22	0.32	7.12	0.013
HC	7.70	0.412	0.51	0.027	6.70	1.91	0.22	0.30	6.94	0.011
MC	7.64	0.400	0.54	0.027	6.63	1.83	0.21	0.32	6.90	0.011
C	7.70	0.406	0.52	0.028	6.95	1.93	0.21	0.32	6.98	0.012
2014										
TR	7.40	0.534	0.60	0.032	6.17	2.20	0.21	0.28	7.55	0.015
FRR	7.39	0.428	0.65	0.032	6.16	2.54	0.21	0.30	7.01	0.015
FA	7.42	0.501	0.73	0.032	6.72	2.42	0.22	0.27	8.06	0.014
TFF	7.45	0.512	0.69	0.032	6.16	2.56	0.20	0.30	7.73	0.015
VV	7.48	0.457	0.68	0.031	6.20	2.51	0.23	0.31	7.25	0.014
TM	7.43	0.509	0.62	0.032	5.99	2.28	0.23	0.27	7.22	0.015
HC	7.45	0.433	0.52	0.029	6.49	2.04	0.21	0.30	6.72	0.012
MC	7.51	0.488	0.59	0.029	6.82	2.10	0.23	0.28	7.05	0.014
C	7.50	0.451	0.56	0.030	6.22	2.26	0.21	0.30	7.14	0.012

TR, *Trifolium repens* L.; FRR, *Festuca rubra* subsp. *rubra*, FA, *Festuca arundinacea*, TFF, *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture; VV, *Vicia villosa;* TM, *Trifolium meneghinianum*; MC, Mechanically cultivated; HC, Herbicide treatment; C, Control; pH, soil reaction; EC, Electrical conductivity; OM, Organic matter; N, Total nitrogen; Ca, Exchangeable calcium; Mg, Exchangeable magnesium; K, Exchangeable potassium; Na, Exchangeable sodium; P, Available phosphorus; BSR, Basal soil respiration; CO_2, Karbondioksit

3.1.2. Total N

Cover crop treatments increased total N at 0-20 cm soil depth as compared to the soil of an untreated control plot in both years of the experiments (Table 2). The highest total N values (0.086%) were seen at 0-20 cm in 2013 in the TR treatment while the lowest total N value (0.045%) was seen in HC treatment (Table 2). Total N significantly increased from 0.053% in the control to 0.102% in VV treatment at 0-20 cm soil depth in 2014. Gülser (2004) found that present increases in the total N over the control were between 8.85% for the crownvetch and 36.46% for the alfalfa treatment. Ramos et al (2010) reported in a previous study that two cover crop (oat- vetch-*Vicia sativa* L. and oat-*Avena sativa* L.) treatments increased total N (32.5%) according to control. In this study, percent increases in the total N over the control soil varied between 40.1-83.7% in 2013 and 35.7-94.8% in 2014 (Table 3). The leguminous family is the most commonly used cover crop because it ensures self sufficiency in N, recycles macro and micronutrients, has usually a deep and extensive root system, is able to extract nutrients from deeper soil layers, and provides large amounts of organic matter in soil (Alagöz & Yılmaz 2009). Soil organic carbon and nitrogen are the main nutritiens used for vegetation growth. These parameters are also used in soil quality assessment and sustainable land use management practices (Liu et al 2011). The soil organic carbon and N not only reflect the soil fertility level but also explain the evolution of the regional ecological system. The relationship between them can be represented as the soil C/N ratio, a sensitive indicator of soil quality and for assessing carbon and nitrogen cycling of soils (Zhang et al 2011). The high soil C/N ratios decelerate decomposition rate of organic matter and nitrogen through limiting soil microbial activity, whereas the low soil C/N ratios accelerate the process of microbial decomposition of organic matter and nitrogen, which is not conducive for carbon sequestration (Wu et al 2001). In this study, the highest soil C/N ratio was observed in FA treatment (8.94) and the lowest was found in HC treatment (6.83) at 0-20 cm soil depth of the apple orchard. For 20-40 cm the soil depth, the soil C/N ratio ranged from 13.08 for the FA to 10.32 for the HC treatment. Mean C/N values of the control plot were found be 7.55 for 0-20 cm and 11.01 for 20-40 cm soil depth. Nitrogen plays a catalyzer role in organic matter formation. In this study, cover crops increased N supply through improving the availability of residual N and through N_2 fixation with legume crops. Cover crops, especially the legumes, fixate atmospheric nitrogen into the soils and thus increase total N contents. The N release from cover crops varies based on lignin, carbohydrate and cellulose content of the residues. Hoagland et al (2008) reported that a cover of mixed legumes established in an apple orchard raised the organic carbon and total N, soil biological activity and potentially available N for trees over a two-year period.

The differences in total N values were not found to be significant for the 20-40 cm soil depth in both years of experiments (Table 4). Total N values at 20-40 cm soil depth varied between 0.027-0.031% with a mean value of 0.029% in 2013 and between 0.029-0.032% with a mean value of 0.031% in 2014 (Table 4).

3.1.3. Soil reaction (pH)

Cover crop treatments significantly reduced pH from 7.65 in control to 7.22 for the *Trifolium repens* (TR) treatment at the 0-20 cm soil depth in 2013 (Table 2). As compared to control, percent decreases in pH values at the 0-20 cm soil depth in 2014 varied between 1.4% in herbicide treated (HC) and 5.9% in *Trifolium repens* (TR) treatments (Table 3). Such decreases were mainly because of CO_2 release into the soil ambient, decomposition of organic wastes and conversion of these organic wastes into carbonic acid (H_2CO_3) through reactions with water. Besides, when the organic matter is mineralized there is a production of organic acids that could raise the soil acidity (Garcia & Rosolem 2010). Gülser (2004) found that values of soil pH importantly reduced with the cropping treatment and percent decreases in pH compared the control soil were between -5.96% for crownvetch and -0.31% for bromegrass treatment. In present study, as compared to control, percent decreases in pH values the aired between -2.1% in TM and -5.6% in TR treatments in 2013 and between 1.8% in FRR and 5.9% in TR treatments in 2014 (Table 3). Cover cropping significantly reduced pH (Demir & Işık 2019a; Demir & Işık 2019b; Demir et al 2019b), due to the acidic root exudates, and this may alter nutrient availability at the root surface (Chapin 1980).

The differences in soil pH values were not found to be significant for the 20-40 cm soil depth in both years of experiments (Table 4). The mean pH values for 20-40 cm soil depth were 7.61 in 2013 and 7.45 in 2014 (Table 4).

3.1.4. Electrical conductivity (EC)

Cover crop treatments increased soil electrical conductivity values at 0-20 cm soil depth as compared to the soil of an untreated control plot (Table 2). The highest EC values was obtained from *Trifolium repens* L. (0.91 dS m^{-1}) in 2013 and *Vicia villosa* (0.99 dS m^{-1}) in 2014. EC significantly increased from 0.47 dS m^{-1} in the control to 0.99 dS m^{-1} in VV treatment at 0-20 cm soil depth in 2014. The greatest increase in the soil EC values was observed in VV (0.99 dS m^{-1}) and the least increase was observed in MC (0.55 dS m^{-1}). The soil electrical conductivity is a significant indicator of dissolved nutrients and can be used to monitor organic matter mineralization (Candemir & Gülser 2010). Previous researchers also reported increasing the EC values with organic matter and compost treatments. Gülser (2004) reported that highest percent change in EC values over the control plots was obtained as 124.60% for alfalfa treatment while lowest percent increase in EC values was 15.97% for bromegrass treatment. In this study, EC values significantly increased with the cropping treatment and percent increases in EC over the control soil were between 43.4% in TM and 99.2% in TR treatments in 2013 and between 57.5% in FRR and 108.9% in VV treatments in 2014 (Table 3).

The differences in EC values were not found to be significant for the 20-40 cm soil depth in both years of experiments (Table 4). EC values ranged from 0.400 dS m^{-1} in mechanically cultivated (MC) treatment to 0.473 dS m^{-1} in *Festuca arundinacea* (FA) treatment for the 20-40 cm soil depth in 2013. EC values ranged from 0.433 dS m^{-1} in herbicide treatment to 0.534 dS m^{-1} in *Trifolium repens* L. (TR) treatment for the 20-40 cm soil depth in 2014 (Table 4).

3.1.5. Exchangeable cations (Ca, Mg, K, Na)

Cover crop treatments increased extractable K at 0-20 cm soil depth as compared to the soil of an untreated control plot (Table 2). In addition, significantly higher exchangeable K was obtained in 2014 than in 2013. While the exchangeable K contents varied between 0.25 me 100 g^{-1} in C and HC and 0.38 me 100 g^{-1} in VV treatments; exchangeable Na contents ranged from 0.33 me 100 g^{-1} in HC to 0.14 me 100 g^{-1} in *Trifolium repens* (TR) treatment in 2014. Cover crop treatments significantly reduced exchangeable Na from 0.31 me 100 g^{-1} in control to 0.14 me 100 g^{-1} in *Trifolium repens* (TR) treatment in 2014 (Table 2). As compared to control, percent decreases in exchangeable Na value at the 0-20 cm soil depth in 2014 varied between 24.8% in FRR and 56.0% in *Trifolium repens* (TR) treatments (Table 3). Demir et al (2019a) cover crop treatments in an apricot orchard with clay soil significantly reduced exchangeable Na and pH from 0.35 me 100 g^{-1} and 7.47 for the bare control treatment to 0.20 me 100 g^{-1} for the *Vicia pannonica* Crantz treatment and to 7.02 for the *Vicia villosa* Roth and *Vicia pannonica* Crantz treatments, respectively. A number of chemical properties

(nutrient availability and cycling, pH, buffering capacity, and cation exchange capacity) are affected by organic matter content in the soil (Tisdall et al 1986).

The differences in the exchangeable cations (Ca, Mg, K, Na) were not found to be significant for the 20-40 cm soil depth in both years of experiments (Table 4). The exchangeable Ca values varied between 6.1-6.8 me 100 g^{-1}, the exchangeable Mg values between 2.2-3.0 me 100 g^{-1}, the exchangeable K values between 0.26-0.38 me 100 g^{-1}, the exchangeable Na values between 0.14-0.35 me 100 g^{-1} (Table 4).

3.1.6. Available P

Cover crop treatments significantly increased available P at 0-20 cm soil depth as compared to the soil of an untreated control plot in both years of experiments (Table 2).

In 2013, available P value was the lowest in the control followed by HC = MC <FRR < FA < TFF < TM < TR < VV treatments (Table 2). Available P significantly increased from 7.4 mg kg^{-1} in the control to 14.0 mg kg^{-1} in VV treatment at 0-20 cm soil depth in 2014 (Table 2). P uptake of succeeding crops was improved by the cover crops (Cavigelli & Thien 2003). Vavoulidu et al (2004) indicated increased available P contents with organic matter treatments. Obi (1999) found that legume and grass cover crops (Axonopus compressus, Cynodon plectostachyum, Panicum maximum, Pennisetum polystachion, Stylosanthes gracilis and Pueraria phaseoloides) on degraded sandy clay loam soil increased mean phosphorus levels (by 112%) compared with the initial conditions. In the present study, cover crop treatments significantly increased P contents and such increases varied between 61.6% in FA and 88.1% in VV treatment (Table 3). Gates & Wilson (1974) reported that available P values was higher for legume cover crops than for grass cover crops, probably due to higher P requirements for legumes due to the mechanisms involved in nitrogen fixation.

The differences in available P were not found to be significant for the 20-40 cm soil depth in both years of experiments (Table 4). Available P values at 20-40 cm soil depth varied between 6.90-7.68 mg kg^{-1} with a mean value of 7.16 mg kg^{-1} in 2013 and between 6.72-8.06 mg kg^{-1} with a mean value of 7.30 mg kg^{-1} in 2014 (Table 4).

3.2. Soil biochemical quality variable

In both years of the experiment, the basal soil respiration (BSR) significantly increased with cover crop treatments. In 2013, BSR value was the lowest (2.3 mg CO_2 100 g^{-1}) in the control followed by C < HC < MC < FA < FRR < TFF < TM < TR < VV treatments. The highest BSR values (9.83 mg CO_2 100 g^{-1}) were seen at 0-20 cm in 2014 in the VV while the lowest BSR value (2.90 mg CO_2 100 g^{-1}) was seen in HC treatment (Table 2). Demir et al (2019a) found that highest basal soil respiration values (41.5 mg CO_2 100 g^{-1}) was obtained in the *Vicia villosa* Roth treatment while the lowest BSR values (12.5 mg CO_2 100 g^{-1}) was in the control in the apricot orchard. In this study, the greatest increase in BSR was observed in VV treatment (186.29%) and the least increase was observed in MC treatment (22.38%) (Table 3). Basal soil respiration is used as an indicator of CO_2 released through the decomposition of organic matter and respiration of the roots. The soil organic matter is largely decomposed through the microbial activity, thus the basal soil respiration is also used as a well-indicator of soil health, organic matter content and organic matter decomposition. The basal soil respiration rates also improved through crop residues and organic matter supplementing agricultural practices (Kladivko 2001).

The differences in the BSR were not found to be significant for the 20-40 cm soil depth in both years of experiments (Table 4). The mean BSR values for 20-40 cm soil depth were 0.012 mg CO_2 100 g^{-1} in 2013 and 0.014 mg CO_2 100 g^{-1} in 2014 (Table 4).

3.3. Soil physical quality variables

3.3.1. Bulk density (BD) and total porosity (F)

There were significant decreases in bulk density (BD) values ($P<0.05$) and significant increases in total

porosity (F) (P<0.01) with cover crop treatments at 0-20 cm soil depth when compared to values of the control (Table 5). As compared to control, the percent changes in bulk density and total porosity are provided in Table 6. BD values at 0-20 cm soil depth in 2013 were ordered as; MC > C=HC > TM > FRR > FA > TFF =TR > VV treatments. The greatest increase in total porosity (16.5%) and the greatest decrease in bulk density (13.0%) were observed in *Trifolium repens* (TR) treatment in 2014. Total porosity values increased with decreasing bulk densities. While the highest BD was found in HC (1.53 g cm^{-3}), the lowest bulk density was obtained in *Trifolium repens* (TR) treatment (1.30 g cm^{-3}) at 0-20 cm soil depth in 2014. Cover crop treatments may significantly improve moisture retention capacity, soil structure, density and consistency. Such treatments also modify soil porosity, conductivity, aeration, infiltration rates and hydraulics. The decay of roots or plant residues increases the size and quantity of macropores (Sultani et al 2007). Gülser (2004) reported that cropping treatments significantly decreased bulk density from 1.45 g cm^{-3} for control plot to 1.27 g cm^{-3} for bromegrass treatment. In addition, due to cropping effects, total porosity significantly increased from 45.28% for control plot to 52% for bromegrass treatment. The increases in total porosity were determined in the following order; control < ryegrass < alfalfa < crownvetch < small burnet < subterranean clover < bromegrass treatments.

Table 5- Effects of treatments on physical soil quality variables at 0-20 cm soil depth

2013										
	*BD, gr cm^{-3}***	*FC, %***	*PWP, %*	*AWC, %**	*F, %***	*Θ, %***	*Ks, cm h^{-1}***	*AS, %**	*MWD, mm*	*SSI, %*
TR	1.33b	19.2a	11.7	7.5a	49.8b	18.3b	10.5b	20.7a	0.45	13.3
FRR	1.37ab	19.1a	11.6	7.5a	48.3c	13.7e	9.8b	19.4ab	0.44	13.1
FA	1.35b	18.9a	11.3	7.6a	49.1c	14.6d	9.5b	19.3ab	0.44	13.0
TFF	1.33b	19.1a	11.4	7.7a	49.8b	16.3c	10.3b	19.5ab	0.44	13.3
VV	1.30b	19.4a	11.9	7.5a	50.9a	18.7a	12.7a	20.2a	0.46	13.4
TM	1.41ab	18.7a	11.3	7.4a	46.8d	14.7d	10.4b	19.7ab	0.45	13.2
HC	1.52a	16.4b	10.4	6.0b	42.6e	10.1g	6.1c	17.1b	0.42	12.1
MC	1.53a	16.5b	10.5	6.0b	42.3e	10.5f	6.8c	17.2b	0.41	12.0
C	1.52a	16.4b	10.4	6.1b	42.6e	10.4f	6.4c	17.2b	0.40	12.1
2014										
	*BD, gr cm^{-3}***	*FC, %***	*PWP, %**	*AWC, %**	*F, %***	*Θ, %***	*Ks, cm h^{-1}***	*AS, %***	*MWD, mm**	*SSI, %***
TR	1.30b	19.7a	12.0a	7.8ab	50.6a	19.0a	12.1ab	21.4a	0.49a	13.4a
FRR	1.39ab	19.3a	11.7ab	7.6ab	47.5c	15.8c	10.8b	19.7b	0.45ab	12.9ab
FA	1.35b	19.5a	11.7ab	7.8a	49.1b	15.4d	10.2b	19.8b	0.45ab	13.1a
TFF	1.31b	19.1a	11.8ab	7.3ab	50.6a	17.4b	12.1ab	20.1ab	0.46ab	13.2a
VV	1.33b	20.0a	12.0a	8.0a	49.8ab	19.1a	13.4a	21.5a	0.50a	13.5a
TM	1.39ab	19.1a	11.8ab	7.3ab	47.6c	15.4d	9.8b	20.6ab	0.46ab	13.3a
HC	1.53a	16.3b	10.3b	6.0b	42.6d	10.1e	6.1c	17.1c	0.41b	12.1b
MC	1.52a	16.5b	10.4b	6.1b	42.6d	10.4e	7.0c	17.4c	0.42b	12.2b
C	1.50a	16.4b	10.3b	6.1b	43.4d	10.1e	6.4c	17.2c	0.41b	12.2b

**, P <0.01; *, P<0.05; TR, *Trifolium repens* L.; FRR, *Festuca rubra* subsp. *Rubra;* FA, *Festuca arundinacea;* TFF, *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture; VV, *Vicia villosa*, TM, *Trifolium meneghinianum;* MC, Mechanically cultivated; HC, Herbicide treatment; C, Control, BD, Bulk density; FC, Field capacity; PWP, Fermanent wilting point; AWC, Available water content; F, Total porosity; θ, Volumetric water content; Ks, Saturated hydraulic conductivity; AS, Aggregate stability; MWD, Mean weight diameter; SSI, Structural stability index

Table 6- Percent changes in physical soil quality variables as compared to control at the 0-20 cm soil dept (%)

2013										
Treatments	*BD*	*FC*	*PWP*	*AWC*	*F*	*Θ*	*Ks*	*AS*	*MWD*	*SSI*
TR	-12.2	16.7	12.9	23.4	16.8	75.7	65.1	20.4	12.7	9.7
FRR	-9.6	16.3	11.9	23.9	13.3	31.4	54.0	12.9	10.0	8.6
FA	-11.2	15.3	9.2	25.7	15.0	39.9	49.0	12.5	9.5	7.6
TFF	-12.2	16.1	9.9	26.7	16.8	56.3	62.8	13.5	9.7	10.3
VV	-14.7	18.5	14.8	24.7	19.5	79.6	99.1	17.8	14.7	10.6
TM	-7.4	13.9	8.8	22.6	9.7	41.4	63.4	14.8	12.3	9.4
HC	0.3	-0.2	0.2	-0.9	0.0	-2.7	-4.4	-0.3	3.6	0.3
MC	1.0	0.8	1.7	-0.7	-0.9	0.7	7.4	0.4	3.0	-1.0
2014										
TR	-13.0	20.7	16.4	27.9	16.5	88.3	89.4	24.3	20.0	10.2
FRR	-7.3	18.0	13.3	25.9	9.6	56.7	69.2	14.6	8.7	5.6
FA	-10.4	19.3	13.5	29.1	13.0	52.8	60.4	15.4	9.5	7.5
TFF	-13.0	16.8	14.9	20.1	16.5	72.5	89.8	16.7	12.7	8.7
VV	-11.7	22.4	16.8	31.9	14.8	89.1	109.8	24.9	21.0	10.7
TM	-7.5	16.6	14.7	19.8	9.6	52.2	52.8	19.0	12.1	8.8
HC	0.8	-0.3	-0.1	-0.7	-1.7	-0.1	-5.2	-0.8	-1.0	-0.4
MC	1.2	0.6	0.7	0.3	-1.7	2.7	10.4	1.4	1.1	-0.2

TR, *Trifolium repens* L.; FRR, *Festuca rubra* subsp. *Rubra*; FA, *Festuca arundinacea;* TFF, *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture; VV, *Vicia villosa*; TM, *Trifolium meneghinianum*; MC, Mechanically cultivated; HC, Herbicide treatment; C, Control; BD, Bulk density; FC, Field capacity; PWP, Permanent wilting point; AWC, Available water content; F, Total porosity; θ, Volumetric water content; Ks, Saturated hydraulic conductivity; AS, Aggregate stability; MWD, Mean weight diameter; SSI, Structural stability index

The differences in BD and F values of cover crop treatments were not found to be significant for the 20-40 cm soil depth in both years of the experiment. The BD values varied between 1.47-1.54 g cm^{-3}, the F values between 41.5-44.5% (Table 7).

Table 7- Effects of treatments on physical soil quality variables at 20-40 cm soil depth

2013										
Treatments	*BD, gr cm^{-3}*	*FC, %*	*PWP, %*	*AWC, %*	*F, %*	*Θ, %*	*Ks, cm h^{-1}*	*AS, %*	*MWD, mm*	*SSI, %*
TR	1.48	15.9	10.0	5.9	44.3	10.6	6.2	15.3	0.39	10.2
FRR	1.50	16.5	10.0	6.5	43.4	10.1	6.3	15.1	0.42	9.6
FA	1.49	16.4	10.2	6.3	43.8	9.9	6.3	15.1	0.41	10.0
TFF	1.48	16.0	10.1	5.9	44.2	9.6	6.1	14.9	0.40	10.0
VV	1.47	15.8	9.9	5.9	44.5	10.9	7.0	15.3	0.40	9.8
TM	1.50	16.6	10.1	6.5	43.4	10.0	6.0	15.3	0.39	10.1
HC	1.50	15.7	9.8	6.0	43.4	9.1	5.9	15.0	0.40	9.8
MC	1.51	15.2	9.6	5.6	43.0	9.4	6.1	15.0	0.41	9.7
C	1.51	16.1	9.8	6.3	43.0	9.3	5.9	14.9	0.40	9.9
2014										
TR	1.49	16.1	10.0	6.1	43.7	11.7	6.1	15.5	0.40	10.4
FRR	1.50	16.4	10.5	5.9	43.4	10.5	6.9	16.1	0.40	10.2
FA	1.52	16.3	10.1	6.2	42.6	10.1	6.5	15.6	0.42	10.0
TFF	1.50	15.7	10.0	5.6	43.4	10.1	6.2	16.1	0.42	10.2
VV	1.50	16.1	10.4	5.7	43.4	11.8	6.9	16.1	0.40	10.0
TM	1.50	15.7	10.5	5.1	43.4	10.1	5.9	15.7	0.40	10.5
HC	1.55	15.5	9.8	5.7	41.5	9.7	6.4	15.9	0.42	10.1
MC	1.52	16.0	10.2	5.7	42.6	10.1	6.7	15.4	0.40	10.0
C	1.54	16.1	10.0	6.2	41.9	9.8	6.2	15.6	0.41	10.3

TR, *Trifolium repens* L.; FRR, *Festuca rubra* subsp. *Rubra*; FA, *Festuca arundinacea;* TFF, *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture; VV, *Vicia villosa;* TM, *Trifolium meneghinianum;* MC, Mechanically cultivated; HC, Herbicide treatment; C, Control; BD, Bulk

density; FC, Field capacity; PWP, Permanent wilting point; AWC, Available water content; F, Total porosity; θ, Volumetric water content; Ks, Saturated hydraulic conductivity; AS, Aggregate stability; MWD, Mean weight diameter; SSI, Structural stability index

3.3.2. Soil field capacity (FC) and the permanent wilting point (PWP) and available water content (AWC)

There were significant increases in field capacity ($P<0.01$), permanent wilting point ($P<0.05$), available water capacity ($P<0.05$) with cover crop treatments at 0-20 cm soil depth in the second year of the experiment (2014) as compared to control (Table 5). As compared to control, the percent changes in available water capacity, field capacity, permanent wilting point are provided in Table 6. Percent increases in AWC values as compared to the control treatment at 0-20 cm soil depth in 2013 varied between 22.6% in *Trifolium meneghinianum* treatment and 26.7% in mixture (*T. repens* (40%)+*F. rubra rubra* (30%)+*F. arundinacea* (30%)) treatment (Table 6). As compared to control, the greatest increase in FC, PWP and AWC values was respectively observed as 22.4%, 16.8% and 31.9% in VV treatment in 2014. Improved organic matter contents also provide better aggregation and aggregate stability, water holding capacity and reduce soil bulk density. Soil organic matter supplementation to soils improves water holding capacity (Candemir & Gülser 2010; Demir & Gülser 2015; Demir 2019; Demir 2020). Cover crops can increase soil moisture due to higher water holding capacity of the soil from increased soil C input and aggregation (Auge´ et al 2001). Demir et al (2019a) reported that highest rises were in the *Vicia villosa* Roth treatment, diminishing the BD by 12.7% while rising the SOM by 63.5%, Ks by 248.7%, AWC by 19.4% and SSI by 9.4% in the 0-20 cm soil depth in the apricot orchard with clay soil.

The differences in FC, PWP and AWC values of cover crop treatments were not found to be significant for the 20-40 cm soil depth in both years of the experiment. The FC values varied between 15.2-16.6%, the PWP values between 9.6-10.5%, the AWC values between 5.1-6.5% (Table 7).

3.3.3. Saturated hydraulic conductivity (Ks) and *volumetric water content (θ)*

There were significant increases ($P<0.01$) in saturated hydraulic conductivity values (Ks) and volumetric water content (θ) with cover crop treatments when compared to values of the control in both years of the experiment (Table 5). As compared to control, the percent changes in Ks and θ are provided in Table 6. The greatest increases in Ks and θ values at 0-20 cm depth in the apple orchard in 2013 were observed in *Vicia villosa* treatments (99.1% and 79.6%, respectively). The greatest increase in saturated hydraulic conductivity and volumetric water content were observed in VV (respectively as 109.8% and 89.1%) and the smallest increase in saturated hydraulic conductivity and volumetric water content was observed in TM (respectively as 52.8% and 52.2%) at the 0-20 cm soil depth in 2014. Volumetric water contents generally increased with decreasing bulk densities of cover crop treatments. Among the physical soil quality variables, the greatest increase was observed in Ks values of soil samples with cover crop treatments. Gülser (2004) found that due to cropping effects, volumetric water content value increased from 18.7% for control soil to 26.5% for the ryegrass treatment.

The differences in Ks and θ values of cover crop treatments were not found to be significant for the 20-40 cm soil depth in both years of the experiment. Ks values ranged from 5.9 cm h^{-1} in control and herbicide treatment to 7.0 cm h^{-1} in *Vicia villosa* (VV) treatment for the 20-40 cm soil depth in 2013. Ks values ranged from 5.9 cm h^{-1} in *Trifolium meneghinianum* (TM) treatment to 6.9 cm h^{-1} in *Vicia villosa* (VV) and Festuca rubra subsp. rubra (FRR) treatments for the 20-40 cm soil depth in 2014 (Table 7). The mean θ values for 20-40 cm soil depth were 9.88% in 2013 and 10.43% in 2014 (Table 7).

3.3.4. Aggregate stability (AS) and mean weight diameter (MWD)

As compared to control, cover crop treatments significantly increased the aggregate stability (AS) and mean weight diameter (MWD) values at 0-20 cm soil depth in both years of the experiments (Table 5). Cover crop treatments increased AS value from 17.2% in the control treatment to 20.7% in *Trifolium repens* L. (TR) treatment in 2013. The greatest AS value (21.5%) was observed in VV and least (17.1%) in HC in 2014. The greatest increases in MWD values at 0-20 cm depth in the apple orchard in both years were observed in *Vicia villosa* treatments (14.7% in the first and 21.0% in the second year) (Table 6). Improved aggregate stability increased crop yield and organic matter returns (Demir & Gülser 2015; Demir & Işık 2019c). Yakupoglu et al (2011) reported that annual *V. lutea* L. (wild) L. and *sphaericus* L. (wild) under Mediterranean climate significantly increased aggregate stability by 73% and 63%, respectively, in surface soil when compared to values of the control plot. According to the studies of Parlak et al (2015), the lowest aggregate stability (31.19%) was determined in control while the highest (66.29%) one in vetch treatmnet in olive orchards. Increasing vegetative cover also increases root development. Close relationships between plant roots and soil erodibility and indicated improved soil strength, shear strength, structural stability and aggregate stability with improved soil properties

(Zhou & Shangguan 2007). However, any root related parameters were not observed in this study. In our study, wet aggregates stability increased in the presence of the cover crops. Several authors (Tisdall & Oades 1979; Tisdall & Oades 1982; Six et al 2004) have reported that the organic substances supplied by roots, i.e. root debris and exudates, may stabilize aggregates directly or indirectly by providing a source of energy for microorganisms in the rhizosphere which may produce stabilizing materials such as mucilaginous polysaccharides. Ramos et al (2010) reported that two cover crop (oat-*Avena sativa* L. and oat- vetch-*Vicia sativa* L.) treatments increased mean MWD values (%15) as compared to control. Changes in the mean weight diameter showed that aggregates were more resistant to physical abrasion under cover crops.

The differences in AS and MWD values of cover crop treatments were not found to be significant for the 20-40 cm soil depth in both years of the experiment. AS values at 20-40 cm soil depth varied between 14.9-16.1% with a mean value of 15.44% (Table 7). The mean MWD values for 20-40 cm soil depth were 0.40 mm in 2013 and 0.41 mm in 2014 (Table 7).

3.3.5. Structural stability index (SSI)

The highest SSI value was obtained from in *Vicia villosa* (VV) *in* 2013 (13.4%) and in 2014 (13.5%) (Table 5). Percent increase in the SSI values with cover crop treatments as compared to control varied between 5.6% in the FRR and 10.7% in VV in 2014 (Table 6). Many studies indicated that cover crops sustained a better soil structure, increased soil total porosity, aeration and water holding capacity and thus decreased bulk density (Steele et al 2012). Similarly, Demir et al (2019a) determined that different cover crops (*Vicia pannonica* Crantz, *Vicia pannonica* Crantz (70%)+*Triticale* (30%) mixture, *Phacelia tanacetifolia* Benth., *Vicia villosa*, and *Fagopyrum esculentum* (Moench.) increased available water capacity, field capacity, permanent wilting point, volumetric water content, total porosity, aggregate stability and saturated hydraulic conductivity values and significant decreases in bulk density values in an apricot orchard with clay soil. Gülser (2004) found that due to cropping effects, SSI values increased from 57.4% for control soil to 63.0% for the bromegrass treatment.

The differences in SSI values of cover crop treatments were not found to be significant for the 20-40 cm soil depth in both years of the experiment. Mean SSI values at 20-40 cm soil depth varied between 9.6-10.5% (Table 7).

3.4. Relationships among the soil quality variables

Significant correlations were observed between EC and OM (0.801^{**}), between EC and BSR (0.753^{*}), between the OM and P (0.816^{**}), between the pH and Na (0.749^{*}), between the total N and K (0.876^{**}) and between the OM and K ($0,802^{**}$). Gülser (2006) reported reduced the bulk density and increased total porosity with increasing the organic carbon contents. The organic matter is the primary binding agent among soil aggregates. Therefore, manure or sludge rich in organic carbon improves aggregate stability. The OM had significant positive correlations with Ks (0.960^{**}), AS (0.915^{**}), FC (0.827^{**}) and significant negative correlations with BD (-0.761^{**}).

3.5. Comparison between different types of cover crop

All cover crop treatments increased organic matter, total N, electrical conductivity, exchangeable K, available P, basal soil respiration, available water content, saturated hydraulic conductivity, total porosity, volumetric water content, aggregate stability, mean weight diameter and structural stability index while decreasing soil pH, exchangeable Na and bulk density values as compared to control without cover crops. However, legume cover crops (*Trifolium repens* L. (TR), *Vicia villosa* (VV) and *Trifolium meneghinianum* (TM)) were found mostly more effective non-legume (*Festuca rubra rubra* L. (FRR) and *Festuca arundinacea* (FA)). Cover crops improve nutrient utilization when the species have root systems that are able to extract and mobilize nutrients from deeper layers and the legumes can add nutrients to the soil by biological fixation (USDA 1996). Therefore, in this study, the improvements in soil quality were more pronounced with legume and grass cover crops mixture (*T. repens* (40%)+*F. rubra rubra* (30%)+*F. arundinacea* (30%)) than with grass cover (*Festuca rubra* subsp. *rubra* and *Festuca arundinacea*) treatments. It is clearly known that legume and grass cover crops have positive effects on soil quality variables, but these effects vary depending on plant species. Thus, a particularly careful selection of suitable cover crops is significant to improve soil quality besides their yields for specific ecological conditions. Cropping systems improve soil structure through several mechanisms such as aggregate enrichment by fine roots and associated fungal hyphae, modified soil-water relationships or stimulation of microbial carbohydrate production

(Tisdall & Oades 1979; Obi 1999; Tisdall & Oades 1982). Cover crops, especially *Vicia villosa* and *Trifolium repens* could be incorporated into cropping systems to improve soil quality and to increase apple yield and to provide sustainable soil management. The improvements were more pronounced with legume covers than with grass covers.

3.6. Biomass yield of cover crops

In general, importantly higher biomass was produced in 2013 than in 2014 (Figure 1). *Trifolium repens* (40%)+*Festuca rubra rubra* (30%)+*Festuca arundinacea* (30%) mixture produced significantly higher fresh biomass (Figure 1a) than the other species in both years of the experiment (respectively as 57790 kg ha^{-1} and 33193 kg ha^{-1}). The lowest cover crop dry biomass (Figure 1b) was obtained from *Trifolium meneghinianum* (2125 kg ha^{-1}) in 2013 and *Vicia villosa* (590 kg ha^{-1}) in 2014.

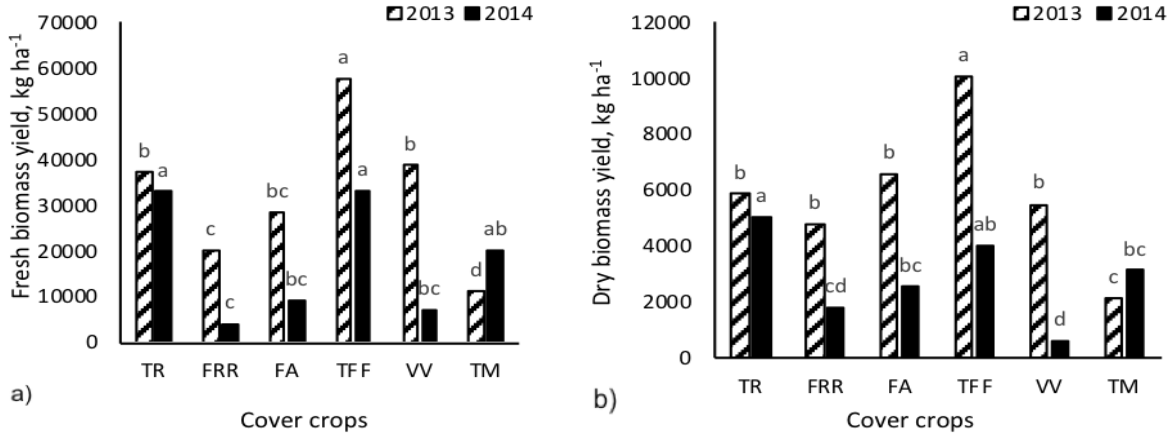

Figure 1- Fresh (a) and dry (b) biomass yield values of cover crops (TR, *Trifolium repens* L.; FRR, *Festuca rubra* subsp. *rubra*; FA, *Festuca arundinacea*; TFF, *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture; VV, *Vicia villosa*; TM, *Trifolium meneghinianum*; MC, Mechanically cultivated; HC, Herbicide treatment; C, Control) (P<0.05)

3.7. Apple yield

Cover crop treatments increased yield in the apple orchard compared to the control in both years of the experiment, and these increases were statistically significant (P<0.05) (Figure 2). Cover crop treatments increased apple yield content from 20.1 ton ha^{-1} in the control to 26.0 ton ha^{-1} in *Trifolium repens* L. (TR) and *Vicia villosa* (VV) treatments in the first year of the experiments. Apple yield in the second year of the experiment were ordered as; MC (12.4 ton ha^{-1}) < HC (14.7 ton ha^{-1}) < C (16.6 ton ha^{-1}) < FRR (18.0 ton ha^{-1}) < FA (19.6 ton ha^{-1}) < TM (20.6 ton ha^{-1}) < TR (21.6 ton ha^{-1}) < TFF = VV (21.8 ton ha^{-1}) (Figure 2). While the highest mean apple yield (23.9 ton ha^{-1}) was obtained from VV, the lowest mean yield (13.7 ton ha^{-1}) was obtained from MC treatment. Perennial cover crops, a mixture of legumes and grasses increased apple yield. Regarding the effect of cover crops on the apple yields, the lowest yield was obtained from the MC plots. Since the plow in the orchards damages the tree roots, mechanical weed control was practiced in the form of mow. Such a case then, caused the weeds to germinate again and thus increased the yield loss. Mullinix & Granatstein (2011) reported that *Trifolium repens* led to improved tree growth, greater fruit yield and lower water use than bare ground in a mature apple orchard. Işık et al (2014) reported that regarding the impacts of cover crops on hazelnut yields, the lowest yield was obtained from control (1439.3 kg ha^{-1}), while the highest yield was determined from *F. Arundinacea* (1546.3 kg ha^{-1}) treatment. Demir et al (2019a) found that cover crop treatments (*Vicia pannonica* Crantz, *Vicia villosa* Roth, a mixture of *Vicia pannonica* Crantz (70%) and *Tritikale* (30%), and *Fagopyrum esculentum and Phacelia tanacetifolia* Benth) generally increased mean yield levels in apricot orchard with clay soil as compared to control without cover crops. Even with the use of cover crops the addition of organic fertilizers is necessary in order to maintain good yields and sufficient tree vigor (Sánchez et al 2007). Present findings comply with the results of earlier studies (Reddy 2003; Harrington et al 2005). Crop yields primarily depend on organic matter contents of soils (Mann et al 2002). Organic matter directly improves physico-chemical and biological quality attributes of the soils, thus improves yield levels (Franzluebbers 2002). However, the amount of increase in crop yield depends upon crops grown. In addition, species of cover crop also have effects on yield increases (Chalk 1998).

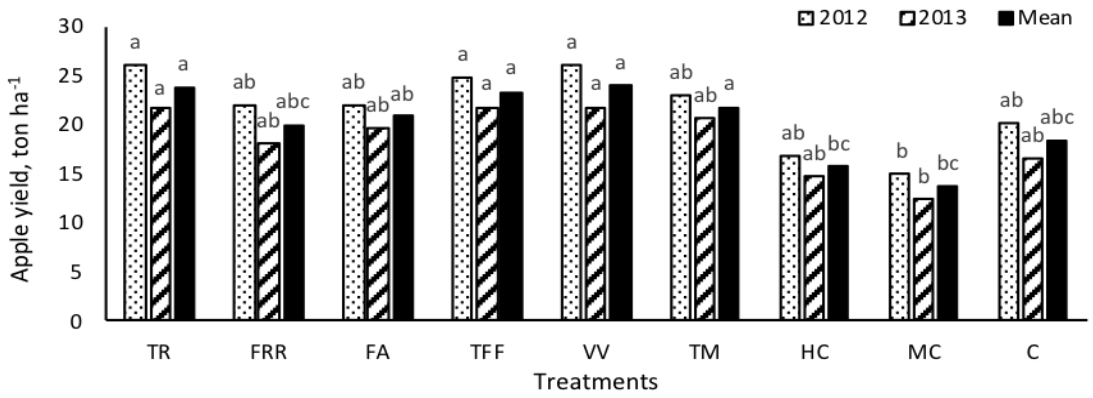

Figure 2- Effects of treatments on apple yield (TR, *Trifolium repens* L.; FRR: *Festuca rubra* subsp. *rubra;* FA, *Festuca arundinacea*; TFF, *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture; VV, *Vicia villosa*, TM, *Trifolium meneghinianum*; MC, Mechanically cultivated; HC, Herbicide treatment; C, Control) (P<0.05)

4. Conclusions

In the present study, cover crop treatments generally improved soil quality variables at 0-20 cm soil depth and increased apple yield compared to the control. The cover crop treatments improved soil quality variables like soil organic matter, basal soil respiration, bulk density, aggregate stability, saturated hydraulic conductivity, available water capacity compared to the soil of a nontreated control. The organic matter is one of the primary soil quality properties and has significant correlations with the other soil quality variables. The improvements were more pronounced with the legume covers (*Trifolium repens* L., *Vicia villosa* and *Trifolium meneghinianum*) than with the grass covers (*Festuca rubra* subsp. *Rubra* and *Festuca arundinacea*). While the highest mean apple yield was obtained from *Vicia villosa* and *Trifolium repens* L. treatments, the lowest mean apple yield was obtained from mechanically cultivated treatment. According to control, the differences in the physical and chemical soil quality variables and basal soil respiration values of all treatments were not found to be significant for the 20-40 cm soil depth in both years of experiments. Mechanically cultivated and herbicide treatments were not found to be significant for the 0-20 and 20-40 cm soil depths as compared to control. However, in coverless (bare) apple fields may result in increased erosion and run-off. In addition, the results of this study are important in the perspective of organic fruit production. It was concluded based on current findings that cover crops, especially *Vicia villosa* and *Trifolium repens* could be incorporated into cropping systems to improve soil quality and to increase apple yield and to provide sustainable soil management. Furthermore, the cover crops could enhance soil, water, and environmental quality by reducing the use of chemical fertilizers.

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Antioxidant Activity and Total Phenolics of Local Apple Cultivars Encountered along the Coastal Zone of Northeastern Anatolia Region of Turkey*

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ABSTRACT
In this study, antioxidant activity and total phenolics in fruit flesh and fruit flesh + skin tissue of local apple cultivars encountered along the coastal zone of Northeastern Anatolia Region (Turkey) and 5 standard cultivars were determined. In local cultivars, antioxidant activities (µmol Trolox equivalent (TE) antioxidant g fresh weight $(fw)^{-1}$) varied between 0.17-1.70 in fruit flesh and between 0.35-1.55 in fruit flesh + skin tissue; in standard cultivars, the values varied between 0.24 ('Royal Gala') and 0.29 ('Granny Smith' and 'Pink Lady') in fruit flesh and between 0.27 ('Jonagold') and 0.61 ('Royal Gala') in fruit flesh + skin tissue. In local cultivars, total phenolics (Gallic acid equivalent (GAE) kg fw^{-1}) varied between 53.26- 00.54 in fruit flesh and between 89.32-406.91 in fruit flesh + skin tissue; the values in standard cultivars varied between 56.30 ('Royal Gala') and 124.64 ('Jonagold') in fruit flesh and between 102.73 ('Summerred') and 198.72 ('Jonagold') in fruit flesh + skin tissue. Present findings revealed that local cultivars generally had 3-4 times greater antioxidant activity and total phenolics than the standard cultivars. Fruit flesh + skin tissue generally had greater antioxidant activity and total phenolics than the fruit flesh alone. However, the local apple cultivar of 'Hemşin Elması' had greater values of both parameters in fruit flesh than in fruit flesh + skin tissue.

Keywords: Apple; Trolox; Gallic acid equivalent; Local cultivar

1. Introduction

For healthy diets, present researches mostly focus on fruits and vegetables rich in phenolics. Phenolic compounds are found in different organs of the plants. They are not nutritious, but play a great role in human health because of their antioxidant characteristics (Margaret et al 2015). Phenolic compounds are structurally composed of simple phenolic molecules bearing one or more hydroxyl groups over an aromatic chain and also cover highly-polymerized components (Balasundram et al 2006; Stratil et al 2007). These compounds are also called as secondary metabolites. They prevent plants from oxidative damages, wounds, pathogen infections and play a role in various physiological processes. Recent epidemiological studies revealed that a diet rich in fruit and vegetables with abundant phenolics recessed the aging process and reduced the risk of cardiovascular diseases, cancer, rheumatoid arthritis, lung diseases, cataracts, Parkinson and Alzheimer diseases (Garcia et al 1997; Middleton et al 2000; Manach et al 2005). Such preventive effects of these compounds are mostly attributed to antioxidative of phytochemicals and vitamins (Szajdek & Borowska 2008). In general, fruits and vegetables have higher phenolic compounds, thus they have greater effect on both human health and

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country economy. Apple fruits are quite rich in phenolic compounds and have been consumed abundantly since the old ages because of positive health impacts. Today, apple is among the mostly produced and consumed fruit species worldwide. In the U.S.A., about 22% fruit phenolic compounds are obtained from apple (Vinson et al 2001). Apples are also processed into apple cider, apple juice, and apple puree. Oxy-reduction characteristics allow antioxidant activity of the phenolics to behave like reducing agents, hydrogen donors and singlet oxygen quenchers. Apple phenolics are also used as metal chelating agent (Rice-Evans et al 1995). Phenolic antioxidants of apples are responsible for majority of fruit antioxidant activities (Lee et al 2003). As compared to fruit flesh or seeds, fruit skin has greater amount of phenolic compounds (Wolfe et al 2003; Tsao et al 2005). It was indicated in previous studies that environmental conditions during fruit growth and development significantly influenced phenolic contents and total antioxidant activity in fruit (Imeh & Khokhar 2002; Lee et al 2003; Vrhovsek et al 2004; Veberic et al 2005). Apples have different phenolic compounds. The quantity and distribution of them vary greatly from one cultivar to another (Khanizadech et al 2008; Bohm et al 2018). Therefore, it is so important to determine phenolics and antioxidant activities of existing and new cultivars.

This study was conducted to determine total phenolics and antioxidant activity of the local apple cultivars prominent with appearance and eating quality in apple genetic sources of Northeastern Anatolia Region of Turkey in comparison with some commercial cultivars.

2. Material and Methods

In this study, 50 local apple cultivars originated from the coastal region of Northeastern Anatolia Region in Turkey were used as plant material. Besides, 5 commercial cultivars (‘Summerred’, ‘Royal Gala’, ‘Jonagold’, ‘Granny Smith’ and ‘Pink Lady’) were used as controls. Local apple cultivars were summer, autumn or winter types available for fresh consumption. They were selected from a collection orchard established with 250 local apple cultivars on M9 rootstocks in 2010 based on their appearance and eating quality. The orchard has stringed irrigation and drip irrigation systems and the trees are cultivated according to the spindle system. The apple orchard where the study was conducted is located in Ankara province at 848 m height 39˚57′ North latitude and 32˚51′ East longitude.

The harvest dates were determined according to fruit color, flesh firmness and soluble solids content. The fruits harvested from local and standard cultivars were washed through distilled water and whole fruit were kept at -20 ºC in a regular freezer for further analyses of total phenolics and antioxidant activity. Summer, autumn or winter cultivars were kept in these conditions for approximately 4, 2 and 1 month, respectively.

Sample preparation for analyses: 10 fruits were collected from 3 trees for each sample. Fruits were initially thawed at room temperature. Then, 10 g samples were taken from homogeneously from all 3 sides of the fruit flesh and fruit flesh + skin tissue. They were placed into 50 mL centrifuge tubes and supplemented with 20 mL 80% acetone. Samples were mixed roughly in a homogenizer for 5 minutes, centrifuged at +5 ºC and 12000 rpm for 20 minutes and filtered through Whatman Grade 1 filter paper into another tubes. Then, 5 mL of resultant filtrate was placed into 100 mL flask and acetone was evaporated by a rotary evaporator at +40 ºC. Following the drying process, samples were supplemented with 5 mL 0.01% hydrochloric acid (HCl, M= 36.45 g mol^{-1}) and manually shaken. For 200 mL 0.01% HCl solution, 54 µL 37% HCl was added to 60 ml distilled water and final volume was completed to 200 mL. Entire liquid in flask was drawn into a syringe, passed through 0.45 micronic filter and placed into two 2.5 ml Eppendorf tubes. The tubes were then preserved at -20 ºC until further analysis.

Antioxidant activity: Antioxidant activity of the samples was determined in accordance with TEAC (TE Antioxidant Capacity) method (Re et al 1999).

Solutions: 12.25 mM potassium persulphate ($K_2O_8S_2$) (M= 270.32 g mol^{-1}) solution; for 100 mL solution, 0.331 g $K_2O_8S_2$ was dissolved in 50 mL double deionized water (ddH_2O) and final volume was completed to 100 mL. ABTS radical solution; for 10 mL solution, 0.0384 g ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) (M= 548.7 g mol^{-1}) was placed into a dark-color bottle and supplemented with 2 mL 12.25 mM potassium persulphate solution. The final volume was completed to 10 mL with ddH_2O, kept at room temperature for 12-16 hours and preserved in a fridge at +4 ºC, then used in analysis. The life span of this solution is only two days. PBS (Phosphate Buffered Saline) solution; for 1.8 mM monopotassium phosphate (KH_2PO_4) (M= 136.08 g mol^{-1}), 0.24 g KH_2PO_4 was dissolved in 500 mL ddH_2O and supplemented with 8 g NaCl to get 137 mM sodium chlorine (NaCl) (M= 58.44 g mol^{-1}),

supplemented then with 0.2 g KCl to get 2.7 mM potassium chlorine (KCl) (M= 74.55 g mol^{-1}) and finally supplemented with 1.4 g Na_2HPO_4 to get 10 mM disodium phosphate (Na_2HPO_4) (M= 141.96 g mol^{-1}). Final value of the solution was completed to 1 liter. Solution pH was adjusted to 7.4 with 0.1 M HCl. The solution was autoclaved at 121 ºC and 1 atm pressure for 20 minutes and preserved under room conditions. For dilution of ABTS radical solution with PBS, Analytik Jena Specord 200 (Analytik Jena, Germany) model spectrophotometer was set as 734 nm wave length and 1 mL ABTS was mixed with sufficient quantity of PBS as to read 700 nm (±1) in spectrophotometer. This absorbance was used in antioxidant activity measurements of the samples.

Sample dilution and measurement: Spectrophotometer was initially set to zero against air, then against PBS, 990 µL absorbent was taken and the value assumed to be minute-zero (700±1 nm) was read. The cuvette was removed from the device and 10 µL fruit sample which was taken from 20 ºC before 10 minutes, thawed at room temperature and mixed in vortex tube mixer, was added to available absorbent (990 µL), waited for 6 minutes and a new reading was performed in spectrophotometer. Since the % inhibition ratio of this initial measurement value should not exceed 35%, 10 µL samples was taken into another Eppendorf tube and diluted with ddH_2O at 1/2, 1/3, 1/5 or 1/10 ratios. Diluted fruit sample (10 µL) was again mixed with 990 µL absorbent and reading was renewed. Following the reduction of inhibition below 35%, 20 µL and 30 µL diluted samples were added to 980 µL and 970 µL absorbents to complete the sample volume of the cuvettes to 1000 µL and 3 measurements were performed. The % inhibition ratio of the samples was calculated with the aid of the following equation:

$$\% \text{ Inhibition} = \left\{\frac{\text{Spectrophotometer reading at minute 0} - \text{reading at 6th minute}}{\text{reading at minute 0}}\right\} \text{X } 100$$

Before the analyses, 2.5 mM Trolox stock solution was taken into 4 flasks in 2, 4, 6 and 8 mL and the flasks were then completed to final volume with PBS solution to get standard solutions. From these solutions, 10 µL was taken and added to 1 mL radical solutions in micro cuvettes to prepare Trolox-containing solutions at 5, 10, 15 and 20 µmol concentrations. The spectrophotometric treatments applied to the samples (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) were also applied to Trolox standards, mean inhibition values were calculated and graphed against Trolox concentrations. Linear regression analysis was applied to resultant data to get Trolox standard curve and the equation defining this curve (Figure 1). Results were expressed as TEAC value. This value was obtained as the ratio of the slope of sample percent inhibition curve to the slope of Trolox standard curve. Resultant slope value was multiplied by dilution factor to get antioxidant activity of the samples (Tahmaz & Söylemezoğlu 2017). Results were expressed in µmol TE g fw^{-1} (µmol TE g fw^{-1}) for both fruit flesh and fruit flesh + skin tissue.

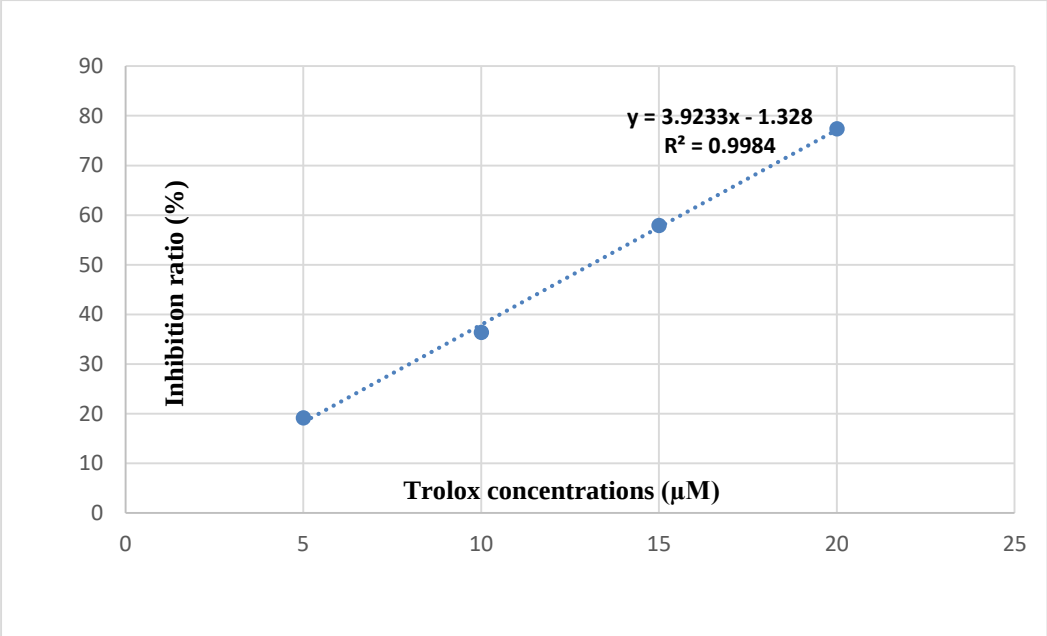

Figure 1- Trolox standard curve

Total phenolics: Total phenolics were determined in accordance with Folin-Ciocalteu method (Singleton & Rossi 1965). Samples were thawed at room temperature, mixed in a vortex tube mixer and placed into Eppendorf tubes. Then, 100 µL sample was taken and placed into 10 mL flasks, supplemented with 8.4 mL distilled water and 500 µL folin (1 L = 1.24 kg) and the flask was intermittently shaken in hand during 3 minutes of waiting period. Resultant mixture was supplemented with 1000 µL 20% sodium carbonate (Na_2CO_3) solution (for 20% 250 mL Na_2CO_3 solution, 50 g Na_2CO_3 (M= 105.99 g mol^{-1})) was dissolved in distilled water. The mixture was then instantly kept at dark conditions for 1 hour. Blanks were prepared before spectrophotometric readings of the samples. For blanks, distilled water was used instead of fruit juice. As defined above, mixture of 100 µL distilled water + 8.4 mL distilled water + 500 µL folin were mixed for 3 minutes, kept at room temperature, supplemented with 1000 µL Na_2CO_3 and kept at dark instantly for 1 hour. Before reading, 5 mL blank mixture was drawn into syringe, filtered through 0.45 micronic filter and 2 mL filtrate was placed into the cuvette. Spectrophotometer was set to zero against air, then set to 765 nm wave length. The cuvette with blank mixture was placed into the device and reading was performed. Then the cuvette with 2 mL of sample was placed into the device and reading was performed. The cuvette with blank mixture was placed into the device after each sample reading to make the device ready for the subsequent sample readings.

For calculations, gallic acid solutions were prepared at different concentrations 50, 100, 150, 250, 500 and 750 mg L^{-1} (R^2= 0.9999). The spectrophotometric treatments were applied to gallic acid standards and then absorbance values were calculated according to gallic acid concentrations. Linear regression analysis was applied to data to get gallic acid standard curve and the equation defining this curve (Figure 2). The R^2 equation best fitting to sample concentrations was selected and each reading was calculated with the appropriate equation to improve the accuracy of the results. The value obtained from the equations of the standard curves was multiplied by a dilution factor. Results were expressed in mg GAE kg fw^{-1} for both fruit flesh and fruit flesh + skin tissue (Tahmaz & Söylemezoğlu 2017).

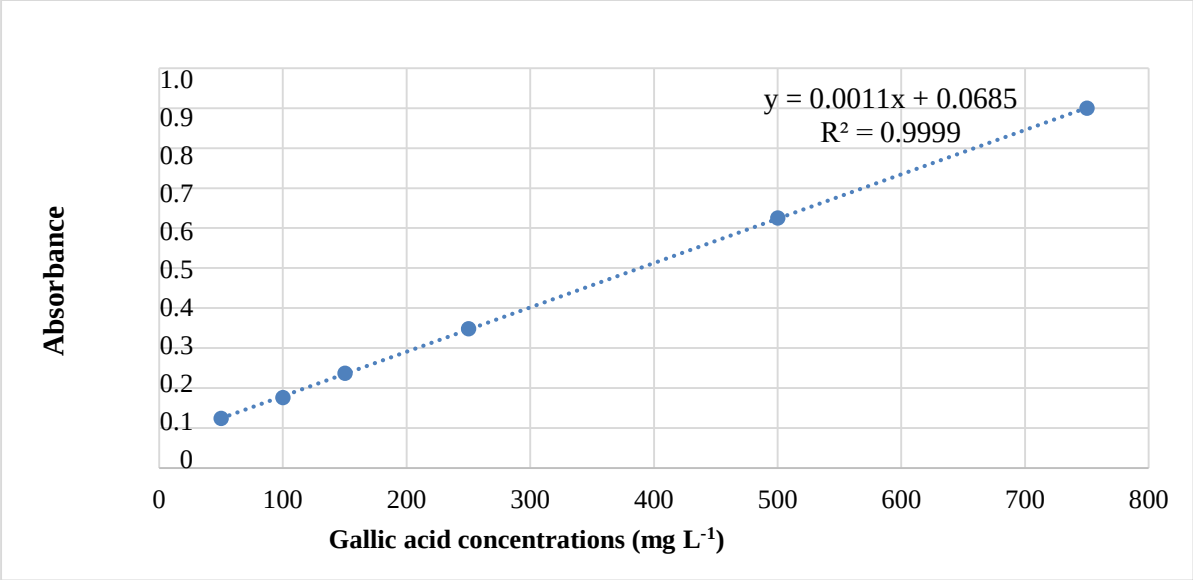

Figure 2- Gallic acid standard curve

Each experiment was conducted in randomized plots design with 3 replications. Experimental data were subjected to variance analysis and *F*-test ($P \leq 0.05$) with Minitab software (MINITAB Inc. version 17). Significant means were compared with Duncan's multiple range test at 5% significance level.

3. Results and Discussion

The differences in antioxidant activity and total phenolics in fruit flesh and fruit flesh + skin tissue of the cultivars was found to be significantly different ($P \leq 0.001$) (Table 1).

Table 1- Antioxidant activity and total phenolics in fruit tissues of the local and standard apple cultivars

Code	*Name*	*Antioxidant activity (µmol TE g fw^{-1})*		*Total phenolics (mg GAE kg fw^{-1})*	
		Fruit flesh	*Flesh + skin*	*Fruit flesh*	*Flesh + skin*
1	Misket	0.17±0.00 Y*	0.72±0.01 QR	107.20±2.24 Z	177.05±1.07 UV
21	Ekşi Elma	0.71±0.00 HI	0.78±0.00 O	200.00±1.76 I	209.47±1.05 OP
23	Kava Elması	0.38±0.01 Q	0.76±0.01 OP	98.72±0.56 a*	154.32±0.80 XY
29	Ham Elma	0.29±0.01 UV	0.62±0.00 T	139.47±1.45 ST	106.90±2.89 f
30	Hollanda Elması	0.41±0.01 OP	0.81±0.01 MN	119.94±0.84 X	205.55±0.40 PQ
37	Karpuz Elma	0.40±0.00 PQ	0.59±0.01 TU	106.15±1.14 Z	176.14±0.52 V
38	Amasya Elması	0.32±0.00 ST	0.35±0.01 a	65.54±0.54 g	90.69±0.70 h
41	Aşısız Elma	0.56±0.00 L	0.70±0.02 R	133.41±0.95 V	290.37±1.57 F
44	Ekşi Elma	0.33±0.00 RST	0.67±0.00 S	156.21±0.76 P	191.75±0.40 S
46	Çıngıraklı Elma	0.27±0.00 VW	0.59±0.01 TU	67.83±1.54 g	134.78±1.72 a
50	Yazlık Elma	0.35±0.00 R	0.87±0.01 JK	145.23±1.84 QR	149.02±1.90 Z
53	Kuzbahçe Elma	0.52±0.01 M	0.89±0.01 J	177.20±0.66 M	248.12±1.00 J
55	Mahmut Elma	0.28±0.00 V	0.60±0.01 T	74.77±0.95 f	89.32±2.05 h
57	Bal Elma	0.64±0.01 J	0.82±0.02 M	67.65±0.66 g	233.88±0.80 K
63	Keş Elma	0.95±0.02 D	1.23±0.01 C	173.71±0.80 MN	347.71±1.70 D
68	Ekşi Sınap	0.78±0.01 G	1.05±0.00 G	228.72±0.41 G	235.68±2.74 K
73	Soğan Elması	0.73±0.02 H	0.87±0.01 JK	197.50±0.52 IJ	275.69±0.26 H
93	Unknown	0.31±0.00 TU	0.92±0.00 I	186.45±0.39 L	281.44±0.66 G
97	Yamaçlar Ekşi	0.35±0.00 R	0.61±0.01 T	133.73±1.19 V	218.88±0.26 LM
99	Sulu Elma	0.59±0.00 K	0.70±0.00 R	142.65±0.84 RS	151.29±0.84 YZ
100	Sabuncular Sivri	0.25±0.00 WX	0.48±0.01 WXY	127.50±0.26 W	128.26±0.81 b
103	Arap Kızı	0.93±0.03 D	1.17±0.01 E	157.20±3.20 P	213.41±1.84 NO
104	Ancerlinin Elması	0.70±0.00 I	0.66±0.01 S	132.97±0.95 V	245.53±1.86 J
107	Emrullahın Elması	0.34±0.01 RS	0.77±0.02 O	92.21±1.60 c	165.83±1.09 W
111	Unknown	0.27±0.00 VW	0.57±0.01 UV	169.79±0.69 O	156.44±0.66 X
120	Hemşin Elması	0.88±0.01 E	0.73±0.01 PQR	190.69±0.70 K	155.99±0.54 X
129	Çüçkuş Elması	0.47±0.00 N	0.73±0.01 QR	141.00±1.87 ST	201.59±2.58 QR
131	Unknown	0.38±0.01 Q	0.72±0.01 QR	94.18±0.54 b	274.64±0.54 H
140	Elif Elması	0.46±0.00 N	0.86±0.01 JK	204.93±1.06 H	211.15±1.14 O
141	Laz Elması (Of)	1.11±0.00 B	1.55±0.03 A	292.20±0.66 B	390.85±0.98 B
149	Tatlı Elma	0.43±0.00 O	0.89±0.00 J	284.63±1.71 C	360.40±2.39 C
150	Unknown	0.33±0.01 RST	0.73±0.00 PQR	127.52±0.95 W	245.10±0.79 J
151	Bar Elması	0.71±0.01 HI	0.83±0.02 LM	134.78±2.33 UV	221.89±1.79 L
152	Demir İyidere Çizgili	1.10±0.00 B	1.10±0.02 F	300.54±1.18 A	360.84±0.84 C
162	Yumuşak Tongar	1.70±0.00 A	1.20±0.00 D	280.99±1.61 D	406.91±1.45 A
172	Fışfış Elma	0.81±0.01 F	0.85±0.00 KL	171.74±1.24 NO	256.01±1.50 I
175	Unknown	0.62±0.01 J	1.15±0.01 E	138.26±3.06 TU	247.65±1.97 J
180	Arap Kızı (Trabzon)	0.34±0.00 RS	0.50±0.00 W	147.67±1.49 Q	112.05±3.03 d
185	Batum Elması	0.86±0.01 E	0.74±0.03 PQ	234.18±1.84 F	316.91±1.31 E
194	Ferik Elması	0.43±0.01 O	1.20±0.00 D	257.80±1.45 E	403.11±1.54 A
204	Kırmızı Ekşi Elma	1.00±0.00 C	0.98±0.01 H	194.03±1.24 JK	236.59±0.69 K
208	Unknown	0.54±0.01 LM	0.46±0.01 YZ	83.42±0.80 e	110.68±1.98 e
209	Necati Kızılkaya Elması	0.34±0.00 RS	1.27±0.01 B	116.29±1.06 X	216.29±1.06 MN
210	Sarı Bağ Elması	0.59±0.00 K	0.79±0.02 NO	157.98±2.14 P	237.81±2.38 K
211	Unknown	0.35±0.00 R	0.49±0.01 WX	93.58±0.32 b	220.40±0.79 LM
212	Unknown	0.52±0.01 M	0.76±0.00 OP	132.35±0.40 V	289.63±1.45 F
213	Unknown	0.48±0.01 N	0.56±0.01 V	112.50±0.46 Y	182.81±0.56 T
214	Ünye Elması	0.29±0.00 UV	0.78±0.00 O	53.26±0.55 h	194.18±0.41 S
		Commercial cultivars			
	Summerred	0.27±0.01 VW	0.47±0.00 XYZ	84.14±0.99 e	102.73±0.50 g
	Royal Gala	0.24±0.00 X	0.61±0.01 T	56.30±1.98 h	174.32±2.09 V
	Jonagold	0.27±0.00 VW	0.27±0.00 b	124.64±0.67 W	198.72±1.35 R
	Granny Smith	0.29±0.00 UV	0.55±0.01 V	119.18±0.54 X	180.98±1.54 TU
	Pink Lady	0.29±0.00 UV	0.45±0.00 Z	89.48±1.93 d	121.44±2.66 c

*, Significant differences were indicated with capital letters ("A"…), then small letters ("a"…) after "Z". Mean separation within columns followed by the same letter are not significantly different at P≤0.05 by Duncan's multiple range test

Antioxidant activities of majority of local apple cultivars were 1.2-4.2 times greater in flesh + skin than in flesh. Total phenolics were 1.2-3.6 times greater in flesh + skin than in flesh (Table 1). In previous studies comparing fruit

skin and fruit flesh, antioxidant activity of fruit skin was reported as 1.5-9.2 times greater than the fruit flesh and total phenolics of fruit skin was reported as 1.2-6.0 times greater than the fruit flesh (Özgen & Tokbaş 2007; Drogoudi et al 2008; Yuri et al 2009; Vieira et al 2011; Wang et al 2015). Present findings for majority of local apple cultivars comply with those earlier ones. However, antioxidant activity of 6 local cultivars (104, 120, 162, 185, 204, 208) was greater in fruit flesh compared with flesh + skin tissue and antioxidant activity of 4 local cultivars (21, 38, 152, 172) in flesh + skin was close or equal to antioxidant activity of fruit flesh. Total phenolics of 4 genotypes (29, 111, 120, 180) were greater in fruit flesh than in flesh + skin and total phenolics of 5 local cultivars (21, 50, 68, 99, 100) in flesh + skin was close or equal to total phenolics of fruit flesh. However, it was reported in literature that apple fruit flesh generally had lower antioxidant activity and total phenolics than the whole fruit with fruit skin or only fruit skin tissues. Present findings were different from those earlier reports since several local cultivars were included in this study and there were quite high genetic diversity among these cultivars, thus, such a diversity might have resulted in having unexpected antioxidant activity and total phenolics for these cultivars. Especially the local cultivar 120 was quite remarkable with its greater antioxidant activity and total phenolics in fruit flesh than in flesh + skin tissue. In majority of local cultivars, antioxidant activity and total phenolics of fruit flesh and flesh + skin tissues had greater than the values of standard cultivars (Table 1). As compared to the standard cultivars, 87.5% of local cultivars had greater antioxidant activity in fruit flesh and 77.1% had greater antioxidant activity in fruit flesh + skin tissue. The local cultivar 'Yumuşak Tongar' had a greater antioxidant activity in flesh compared with flesh + skin, while its antioxidant activity in flesh was higher 6 times in average compared with those from commercial cultivars. The local cultivars of 141, 152 and 204 had 3.4-4.2 times greater antioxidant activity in fruit flesh than the standard cultivars. In fruit flesh + skin tissue, the local cultivars of 63, 141, 162 and 209 had 2-6 times greater antioxidant activity than the standard cultivars. Again, as compared to the standard cultivars, 68.8% of local cultivars had greater total phenolics in fruit flesh and 60.4% had greater total phenolics in fruit flesh + skin tissue. The local cultivars of 152, 141, 149 and 162 had 2-5 times greater total phenolics in fruit flesh than the standard cultivars. In fruit flesh + skin tissue, the local cultivars of 162, 194, 141 and 149 had 2-4 times greater total phenolics than the standard cultivars. As μmol TE g fw^{-1}, antioxidant activity in fruit flesh of local cultivars varied between 0.17 (cultivar 1) and 1.70 (cultivar 162) and antioxidant activity in fruit flesh of standard cultivars varied between 0.24 ('Royal Gala') and 0.29 ('Pink Lady' and 'Granny Smith'); antioxidant activity in flesh + skin tissue of local cultivars varied between 0.35 (cultivar 38) and 1.55 (cultivar 141) and antioxidant activity in flesh + skin tissue of standard cultivars varied between 0.27 ('Jonagold') and 0.61 ('Royal Gala'). As mg GAE kg fw^{-1}, total phenolics in fruit flesh of local cultivars varied between 53.26 (cultivar 214) and 300.54 (cultivar 152) and total phenolics in fruit flesh of standard cultivars varied between 56.30 ('Royal Gala') and 124.64 ('Jonagold'); total phenolics in flesh + skin tissue of local cultivars varied between 89.32 (cultivar 55) and 406.91 (cultivar 162) and total phenolics in flesh + skin tissue of standard cultivars varied between 102.73 ('Summerred') and 198.72 ('Jonagold'). Present findings for 'Jonagold' apple cultivar partially comply with the findings of Lachman et al (2006). Although researchers reported quite greater total phenolics for Jonagold apples than the present values (1216.43±12.64 mg kg fw^{-1}), complying with the present findings, they reported greater total phenolics for 'Jonagold' apples than for the other standard cultivars. In present study, the local cultivars of 141, 152 and 162 had high antioxidant activity and total phenolics both in fruit flesh and flesh + skin tissue. There are great differences in antioxidant activity and total phenolics of the apple cultivars in different studies since different methods were employed in analyses. Albayrak et al (2010) classified the methods used for antioxidants of the plants in two groups as of electron transfer (ET)-based and hydrogen atom transfer (HAT) reactions-based methods. Özgen & Tokbaş (2007) used TEAC method and reported antioxidant capacity of 'Amasya' apple as 19.8 μmol TE g fw^{-1} in skin and as 5.0 μmol TE g fw^{-1} in fruit flesh. Vieira et al (2011) investigated antioxidant capacity of 11 Brazilian apple cultivars with TEAC method and reported antioxidant activities of the apple cultivars varied as between 3.8 ('Golden Delicious') and 9.6 ('Epagri-F_5P_{283}') μmol TEAC g fw^{-1} in fruit flesh and as between 12.25 ('Golden Delicious') and 41.4 ('Catarina') μmol TEAC g fw^{-1} in fruit skin. Wang et al (2015) also used TEAC method and reported antioxidant capacity of 'Gale Gala' cultivar as 29.9 TE μmol g fw^{-1} in fruit skin and as 3.6 TE μmol g fw^{-1} in fruit flesh. Although the same method was employed, those reports of the previous researchers were both different from each other and different from the present ones. Such differences were mainly attributed to differences in genotypes and dilution procedures used in preparation of the samples. A similar case is also valid for total phenolics. Present findings on total phenolics comply with the findings of Markowski et al (2007) reporting average total phenolics of 'Champion', 'Jonagold', 'Idared' and 'Topaz' apple cultivars as 821 mg kg^{-1}, findings of Kevers et al (2011) reporting total phenolics in whole fruit of 'Gala' apple cultivar as 2250 mg GAE kg fw^{-1}, findings of Wang et al (2015) reporting total phenolics of 'Gale Gala' apple cultivar as 1641.8 mg kg fw^{-1} in skin tissue and as 160.3 mg kg fw^{-1} in fruit flesh. Such differences between the total phenolics of the previous studies and differences from the present ones again mainly resulted from the differences in dilution procedures.

Present findings revealed a great variation in antioxidant activity and total phenolics of high-table value local apple cultivars encountered along the coastal zones of Northeaster Anatolia Region of Turkey. Majority of local cultivars had greater antioxidant activity and total phenolics both in fruit flesh and flesh + skin tissue than the standard cultivars. With regard to these parameters of the local cultivars, the ones with greater values in fruit flesh than in flesh + skin tissue were remarkable. Further research is recommended to determine the other biochemical characteristics of the local cultivars prominent with their antioxidant activity and total phenolics.

4. Conclusions

Present study revealed a great variation in antioxidant activity and total phenolics of local apple cultivars encountered along the coastal zones of Northeaster Anatolia Region of Turkey. Majority of local cultivars had greater antioxidant activity and total phenolics in both flesh and flesh + skin tissues compared with commercial cultivars. With regard to these parameters of the local cultivars, the ones with greater values in flesh than in flesh + skin tissues were remarkable. Further research is needed to determine the other biochemical characteristics of the local cultivars prominent with their antioxidant activity and total phenolics. Recognizing the potential antioxidants in local cultivars enables researchers to use these unique genetic materials in national apple breeding programs.

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The Effect of Sodium Silicate and Methyl Jasmonate on Pigments and Antioxidant Activity of Tomato (*Solanum lycopersicum* L.) Under Salinity Stress

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ABSTRACT
The present study aimed to investigate the effects of sodium silicate (Si) and methyl jasmonate (MeJA) on the pigments and antioxidant activity of tomato, under salinity stress. For this purpose, completely randomized factorial design with three factors including three levels of salinity (0, 4 and 6 dS m^{-1}), Si (0, 4 and 8 mM) and MeJA (0, 5 and 7.5 µM), and three replications was used. The present study displayed that the increase in salinity level reduced chlorophyll index, fluorescence, and vitamin C; however, the catalase (CAT) and ascorbate peroxidase (APX) activities increased. MeJA and Si enhanced the chlorophyll index and vitamin C at different salinity levels, respectively. CAT and APX decreased when the salinized plants were treated with MeJA and Si. MeJA and Si may act to mitigate the adverse effect of salinity stress by reducing the H_2O_2 production. Finally, it can be concluded that MeJA and Si partially offset the adverse impacts of salinity stress.

Keywords: Methyl jasmonate, Salinity stress, Sodium silicate, Tomato

1. Introduction

Nowadays, soil and water salinity are the main obstacles and limitation factors for agriculture development all around the world (Behzadifar et al 2013). Approximately 33 M ha of all agricultural lands (55%) in Iran are affected by soil and water salinity (Haghighi & Pessarakli 2013). Salinity is the major environmental stress that adversely affects plant metabolism and growth. Yield reductions caused by salinity occur on an estimated 50% of all irrigated land worldwide (Panhwar et al 2017). Plants employ various methods and physiological mechanisms to adapt to salinity stress so as to mitigate its adverse impacts (Enteshari & Jafari 2013; Sheikh Beig Goharrizi et al 2016). The destructive effects of salinity on tomato physiology and biochemistry have been subjected to extensive research (Cuartero & Fernández-Muñoz 1998; Maggio et al 2007). Jasmonate is one of the most important plant growth regulators which can mitigate the adverse effect of environmental stresses. Methyl jasmonate (MeJA) influences the production of proteins and enzymes through gene regulation (Reinbothe et al 1994; Hao et al 2015; Losvik et al 2018). It, also, affects the production of antioxidants which improves plant resistance to environmental stresses (Akbari et al 2018; Boroomand et al 2018). Furthermore, jasmonate plays a crucial role in defense mechanisms against abiotic stresses such as drought, salinity, and low temperature (Yoon et al 2009; Qiu et al 2014). Salinity stress can significantly disrupt the physiological and biochemical activity of tomato. Methyl Jasmonate (MeJA) alleviates the harmful effects of salinity in tomato plants by inducing biochemical and physiological resistance mechanisms (Manan et al 2016). Under salinity condition, plants produce free radicals such as

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superoxide anion, radical hydroxyl, and peroxides (Enteshari & Jafari 2013). Silica can reduce the concentration of malondialdehyde under salinity stress, which reflects the role of silica in alleviating lipoid peroxidation (Soylemezoglu et al 2009). Silicon may enhance the antioxidant defense mechanisms through increasing the antioxidant enzymes activities and non-enzymatic antioxidants contents. It may also involve in osmotic adjustment and increase photosynthetic enzymatic activities (Zhu & Gong 2014). The probable mechanisms for the better growth of the plants exposed to silica under salinity stress may be related to preserving nutrient balance, developing the photosynthetic rate, increasing water uptake and maintaining water in leaves (Kardoni et al 2013). Silica improved the soybean growth through increasing in chlorophyll and gibberellic acid contents under salinity stress (Lee et al 2010). In different parts of Iran, tomato production is limited by a variety of stresses such as salt, drought, cold and heat. To the best of our knowledge, no study to date has studied the effects of simultaneous use of MeJA and sodium silicate (Si) on plants under salinity stress. The present study aimed to investigate the effects of MeJA and Si on pigments and antioxidant activity of tomato under salinity stress.

2. Material and Methods

2.1 Plant material and experimental conditions

The present study was carried out at a commercial greenhouse in Jahrom, Fars province, Iran, during 2017-2018. The greenhouse conditions were as follows: area of 300 m^2, thermostat heating system, fan and pad cooling system, plastic cover, the relative humidity of 67 ± 3%, and day and night temperatures of 26 ± 3 and 17 ± 2 °C, respectively. The seeds of tomato 'Dafnis' (hybrid F1 made in India with 99% purity and 94% germination) were sown on a seed planting tray. The plants were transferred to pots at 5 to 6 leaf stage. The pots were filled by 8 kg of sand with an in-row and inter-row spacing of 35 and 75 cm, respectively. The results of soil and water analyses were presented in Table 1. A completely randomized factorial design with three factors including three levels of salinity (0, 4 and 6 dS m^{-1}), Si (0, 4 and 8 mM) and MeJA (0, 5 and 7.5 µM), and three replications was used. Two weeks after transplanting, water salinity treatments were manually applied, until the end of the plants' lifespan. The salinity solution was composed of sodium and calcium chloride salts (2:1 ratio). Both Si and MeJA were applied in three times at one, 7 and 15 weeks after transplanting. According to Table 2, the plants were fertigated. Also, chelated iron fertilizer (350 g) and 0.04 kg of a mix of trace elements (containing 1.45% B, 3.2% Cu, 7.5% Fe, 8.15% Mn, 4.6% Mo, and 4.5% Zn) were dissolved in 500 L of water and used for plant fertigation. At the end of the experiment, the following characters were measured, vitamin C, chlorophyll index, chlorophyll fluorescence, lycopene content, catalase (CAT) and ascorbate peroxidase (APX) activities.

Table 1- The results of water and soil analyses

Water analyses									
CO_3^{2-} meq/L	HCO_3^- meq/L	Cl^- meq/L	SO_4^{2-} meq/L	Total nions meq/L	Ca^{2+} meq/L	Mg^{2+} meq/L	Na^+ meq/L	K^+ meq/L	Total cations meq/L
0.00	0.85	0.80	0.82	2.47	1.25	0.55	0.86	0.01	2.67
EC µS/cm	TDS mg/L	pH	SSP	SAR	TL mg/L	TA mg/L			
280.00	171.38	7.35	32.21	0.91	90.00	42.50			

Soil analyses							
EC µS/cm	pH	TNV	OC (%)	OM (%)	TN (%)	P (mg kg^{-1})	K^+ (mg kg^{-1})
798	7.25	68.0	0.31	0.53	0.03	3.28	0.79
Clay (%)	Silt (%)	Sand (%)	Texture	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
13.5	9.20	77.3	Sand	0.20	5.30	3.60	0.80

Table 2- Fertigation program

	Fertigation time (weeks after sowing)	Type and composition of fertilizer (mg/ dm^3) and manufacturing country					
		Calcium nitrate $Ca(NO_3)_2$	Potassium nitrate KNO_3	Ammonium nitrate $(NH_4)(NO_3)$	Mono Ammonium Phosphate – MAP- $NH_4H_2PO_4$	Petassium sulfate K_2SO_4	Magnesium sulfate MgSO4
Autumn planting	1-4	15	16	1.5	5.5	-	12.5
	5-8	15	16		5.5	-	12.5
	9-10	15	16		5.5	-	12.5
	11-12	10	11		5.5	17	12.5
	13-14	10	6		5.5	27	12.5
	15-18	10	3.5		5.5	16.5	12.5
Total consumed fertilizer		75	66.5	1.5	33	60.5	75

2.2. Vitamin C

The vitamin C content was measured using the titration method (Suntornsuk et al 2002). The sample extract (10 mL), distilled water (20 mL) and 2 mL of 1% starch solution were mixed in a 250-mL Erlenmeyer. Then, the content of each Erlenmeyer was titrated with the iodide solution in potassium iodide (KI) until the blue-black color was observed. Vitamin C content (mg per 100 g sample) was obtained from the following equation:

Vitamin C content = volume of solution used for each sample × 0.88 (1)

2.3. Chlorophyll index and fluorescence

In order to measure the chlorophyll index, four upper developed leaves of each seedling were subjected to a Minolta SPAD-502 chlorophyll meter and the average of three seedlings was recorded for each experimental unit.

The chlorophyll fluorescence was measured by a chlorophyll fluorometer (model OS1-FL, Opti-Sciences, Tyngsboro, MA, USA). The quantum yield of photosystem II (ΦPSII) were determined under light conditions by the following equation (Maxwell & Johnson 2000):

$$\Phi\ PSII \ = \frac{F_v}{F_m} \quad (2)$$

Variable fluorescence (F_v) was calculated as F_m-F_0, where, F_0 and F_m stand for minimum and maximum chlorophyll fluorescence, respectively.

2.4. Lycopene content

The lycopene content was measured by a spectrophotometer (T80, UV/VIS PG Instruments Ltd) by the standard method. The measurement was performed at 503 nm in hexane solvent (Choudhary et al 2009). The amount of full-trans lycopene was calculated using the following equation and the specific extinction coefficient (Choudhari & Ananthanarayan 2007).

$$\text{Lycopene} \quad (\text{mg}) \ = \frac{A \times dil \times ml \times 10}{E_{1cm}} \quad (3)$$

Where A, dil, ml, and E_{1cm} were the solution absorbance in a 1-cm cuvette, dilution factor, final volume, and specific extinction coefficient of the sample, respectively. Lycopene extraction efficiency was calculated in mg per 100 g sample using the following equation:

$$\text{Yield} \quad (\text{mg}/100 \quad \text{g}) \ = \left(\frac{C \times V}{M} \right) \times 100 \quad (4)$$

Where C, V, and M were the extracted lycopene content (mg), the extracted oleoresin volume (g), and the weight of the sample skin or dried waste of tomato, respectively.

2.5. Antioxidant activity

For determination of antioxidant activity, samples were ground in liquid nitrogen and homogenized in the reaction solution contained 50 mM phosphate buffer (pH 7.8, 0.1 mM Na_2EDTA, 1.5% (m/v) PVPP and 0.1% (v/v) Triton X100). Then it was centrifuged at 12000g for 10 min at 4 °C. The supernatant was collected for determination of CAT and APX activities. The CAT activity was measured by calculating H_2O_2 absorption decrease at 240 nm. One enzymatic unit of catalase is the amount of enzyme that can decompose 1 mM H_2O_2 in one minute (Mazorra et al 2002). Ascorbate peroxidase enzyme activity was calculated by the decrease in absorbance of ascorbate within one min at 290 nm. One enzymatic unit of APX is the amount of enzyme that oxidizes 1 mM ascorbate in 1 min (Chen et al 2009). Enzyme activity was recorded in terms of enzymatic unit per total protein (mg) content.

2.6. Statistical analysis

The study was set up as a completely randomized design, with a factorial arrangement and three replications. The normality of the data was evaluated by the Kolmogorov–Smirnov test with the SPSS 25.0 software (SPSS Inc, Chicago,

IL, USA). Analysis of variance was performed on the data using Statistical Analysis Sofware (Version 9.1 for Windows; SAS Institute, Cary, NC) and the means were compared by the Tukey test (P<0.05).

3. Results and Discussion

3.1. Analysis of variance

The ANOVA demonstrated that there were significant differences among the three levels of salinity for vitamin C content (P<0.01), chlorophyll index (P<0.05) and chlorophyll fluorescence (P<0.01) characters (Table 3). The simple effect of Si was only significant for chlorophyll fluorescence. Also, the results revealed that the double interactions between MeJA with salinity and MeJA with Si were significant for chlorophyll index (P<0.05 and P<0.01, respectively). In addition, ANOVA showed that the triple interactions among the three factors were significant for lycopene content and CAT and APX activities. According to Wickens and Keppel (2004), when the interaction of factors is significant, then less attention is paid to the main effects and then the main focus will be on the interaction effects.

Table 3- ANOVA of studied traits in tomato treated by Si and MeJA under salinity stress

S.O.V.	df	Mean Squares (M.S.)					
		Vitamin C	Chlorophyll index	Chlorophyll fluorescence	Lycopene content	Catalase activity	Peroxidase activity
Salinity (A)	2	8.81**	40.89*	0.012**	4.32	4899.47	18.80
Na_2SiO_3 (B)	2	1.45	6.29	0.001*	5.50	868.09	10.80
MeJA (C)	2	1.15	0.13	0.0004	1.27	759.56	8.39
A × B	4	2.61*	20.95	0.0006	5.28	4969.84	14.22
A × C	4	0.59	26.00*	0.0003	0.93	4128.58	10.91
B × C	4	1.57	114.57**	0.0005	4.58	2968.55	12.72
A × B × C	8	1.87	4.64	0.0005	7.51*	6845.18**	65.23*
Error	54	0. 9	9.1	0.0004	3.02	2253.21	26.82
C.V. (%)		8.9	5.83	2.53	29.02	32.5	28.3

(*, **; significant at 0.05 and 0.01 levels, respectively)

3.2. Vitamin C

The results demonstrated that the highest vitamin C content was observed in the interaction of 0 dS m^{-1} salinity with 4 mM Si (Figure 1). Also, the results showed that at lower levels of salinity (0 and 4 dS m^{-1}), 4 mM Si was more effective to increase vitamin C compared to the other Si levels, while at 6 dS m^{-1} of salinity the highest vitamin C content was obtained at 6 mM Si. The mean values of vitamin C content in this study were within the range normally in tomato reported by Fontes et al (2004). Stamatakis et al (2003) stated that the amount of vitamin C in the plants was increased due to the application of Si to the nutrient solution. Marodin et al (2016) reported that the amount of vitamin C was increased with increasing Si doses, however, at the highest level of Si, it was decreased. The role of Si in metabolic pathways involved in the biosynthesis of vitamin C is unclear yet.

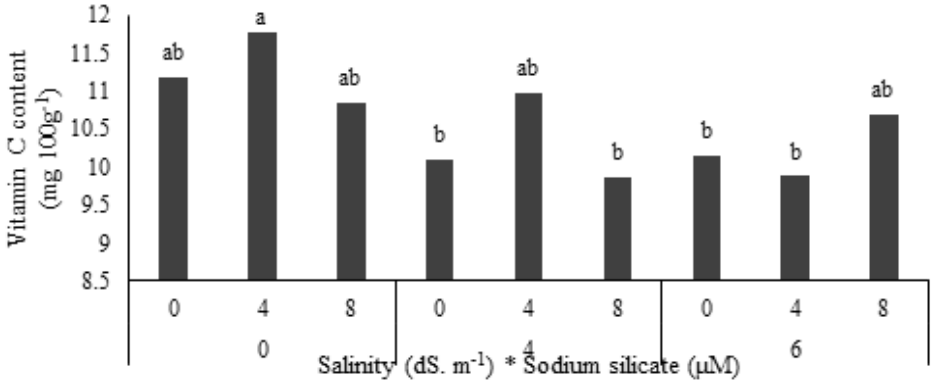

Figure 1- Effect of the interaction between salinity and Si factors on vitamin C content of tomato

3.3. Chlorophyll index and fluorescence

According to the results of ANOVA two interactions of the factors including salinity with MeJA and Si with MeJA were significant for chlorophyll index. The interaction between salinity and MeJA showed that with increasing the salinity level from 0 to 6 dS m^{-1}, the chlorophyll index was decreased, however, at 4 and 6 dS m^{-1} salinity levels, the MeJA enhanced the character (Figure 2a). In addition, the lowest and the highest chlorophyll index were obtained from the interactions of 0 and 4 dS m^{-1} salinity levels with 7.5 µM MeJA, respectively.

The interaction between Si and MeJA revealed that at 0 mM Si, with increasing in MeJA, the chlorophyll index was increased and then decreased, respectively (Figure 2b), whereas the opposite trend was observed for 4 mM Si with the MeJA levels. Low concentrations of jasmonic acid in *Chlorella vulgaris* significantly increased the chlorophyll *a* and b contents (Czerpak et al 2006). Our results suggested that the effect of MeJA on chlorophyll index was depending on its concentration. Similar results have already been reported (Czerpak et al 2006; Ueda & Saniewski 2006; Fugate et al 2018). It has been documented that MeJA involved in the expression of a set of genes encoding key enzymes that responsible for chlorophyll biosynthesis via the formation of 5-aminolevulinic acid (Wang et al 2019; Wu et al 2019). MeJA hinders the degradation of chlorophyll and the loss of photosynthesis through activating antioxidant enzymes in the chloroplast, thereby enhancing the growth and activity of the plant (Babst et al 2005).

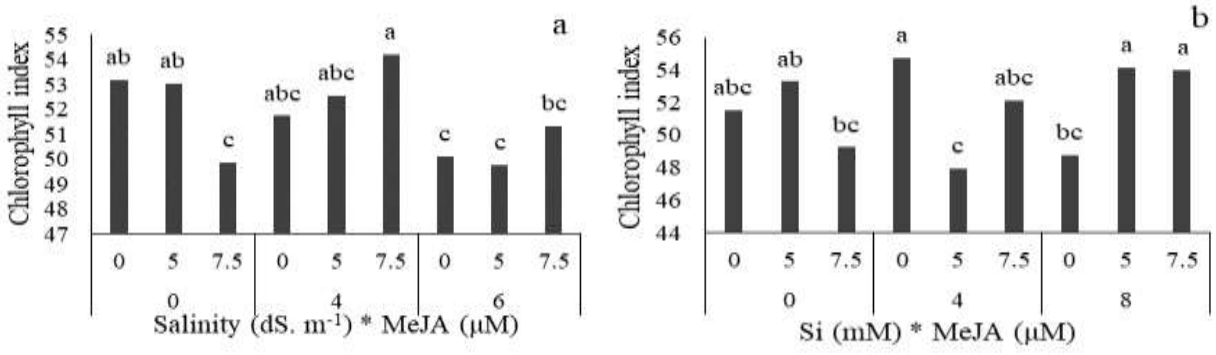

Figure 2- Effect of the interaction between salinity with MeJA (a) and Si with MeJA (b) on chlorophyll index of tomato

The results of ANOVA revealed that the main effects of salinity and Si were significant for chlorophyll fluorescence, while there were no significant effect for the main effect of MeJA and the interactions of the factors. The results also uncovered that the increase in the salinity level resulted in the loss of chlorophyll fluorescence so that the lowest chlorophyll fluorescence was observed at 6 dS m^{-1} salinity level (0.771; Figure 3a). Similar to salinity levels with increasing in Si levels the chlorophyll fluorescence was also decreased so that the lowest chlorophyll fluorescence was obtained at 8 mM Si (0.775), although it was not significant with 4 mM Si (Figure 3b). Despite that our results showed a reduction in the chlorophyll fluorescence using Si application, Al-aghabary et al (2005) reported that photochemical efficiency of PSII (F_v/F_m) was enhanced by Si in tomato under salinity stress. Morales et al (1992) indicated that salinity stress had no significant effect on PSII. However in this study salinity stress significantly decreased the chlorophyll fluorescence. Environmental stresses such as salinity, drought, and heat cause damage to PSII (Rochaix 2011; Mosavi et al 2018).

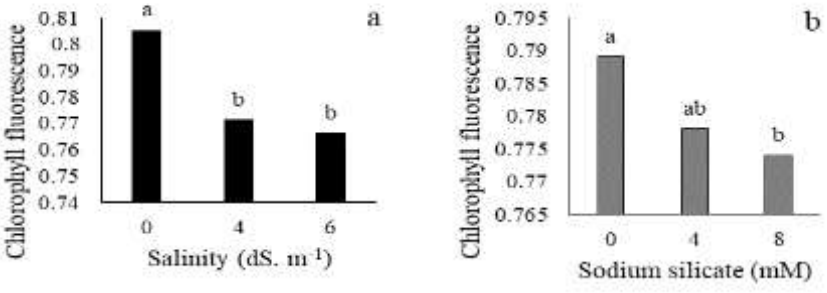

Figure 3- Effect of salinity (a) and Si (b) on chlorophyll fluorescence of tomato

3.4. Lycopene content

The interaction between the three factors was significant for lycopene content. The results revealed that the highest and lowest lycopene contents were obtained in control (7.98 mg L^{-1}) and 0 mM Si and 7.5 µM MeJA at 4 dS m^{-1} salinity (4.51 mg L^{-1}) treatments, respectively (Table 4). According to the results, the average values of lycopene content at 0 dS m^{-1} salinity with the other factors was more than the other treatments. The salinity stress restricted lycopene content in tomato fruit (Stamatakis et al 2003). Pascale et al (2001) reported that at higher levels of salinity (more than 4 dS m^{-1}) the lycopene contents were declined. In the present study a similar result was observed. Based on the results, Si and MeJA had no significant effect on lycopene content. In contrast with our finding Stamatakis et al (2003) and Marodin et al (2016) reported that Si significantly enhanced lycopene content in tomato. Fruit appearance in tomato is directly related to lycopene content, which the red color of the plant is due to the ratio of lycopene to beta-carotene (Catalkaya & Kahveci 2019).

Table 4- Effects of the interaction among salinity, Si and MeJA factors on three measured characters of tomato

Salinity (dS. m^{-1})	Na_2SiO_3 (mM)	MeJA (mM)	Lycopene (mg L^{-1})	Catalase activity (unit / mg protein)	Peroxidase activity (unit / mg protein)
		0	7.98 a	67.58 c-f	9.77 ab
	0	5	7.66 a-c	70.22 c-f	12.91 ab
		7.5	5.94 b-d	60.83 d-f	10.32 ab
		0	6.93 a-d	150.59 ab	10.78 ab
0	4	5	6.16 b-d	75.63 b-f	7.40 ab
		7.5	6.08 b-d	68.53 c-f	7.89 ab
		0	5.14 b-d	98.98 a-f	5.66 b
	8	5	5.02 cd	53.3 d-f	9.91 ab
		7.5	6.28 b-d	109.98 a-e	10.59 ab
		0	5.09 cd	156.85 a	10.33 ab
	0	5	5.62 b-d	31.2 f	10.78 ab
		7.5	4.51 d	98.14 a-f	10.41 ab
		0	6.38 b-d	90.52 a-f	10.59 ab
4	4	5	5.88 b-d	107.45 a-f	10.33 ab
		7.5	6.44 b-d	52.45 d-f	9.81 ab
		0	4.76 d	79.53 a-f	14.912 a
	8	5	6.32 b-d	143.82 a-c	10.32 ab
		7.5	4.99 cd	75.63 b-f	8.61 ab
		0	7.4 a-c	75.21 b-f	15.32 a
	0	5	5.11 cd	117.6 a-d	8.54 ab
		7.5	6.26 b-d	107.78 a-f	13.16 ab
		0	4.76 d	31.3 f	13.49 ab
6	4	5	6.32 b-d	76.14 b-f	10.87 ab
		7.5	4.99 cd	32.91 ef	10.33 ab
		0	5.1 cd	31.3 f	8.61 ab
	8	5	5.71 b-d	43.15 d-f	13.32 ab
		7.5	6.09 b-d	82.06 a-f	8.54 ab

3.5. CAT and APX activities

The results indicated that the interaction among the three factors were significant for both enzymes. Also, results showed that the highest CAT and APX activity (156.85 and 15.32 unit/mg protein, respectively) were achieved at 4 and 6 dS m^{-1} salinity with 0 mM of Si and 0 µM MeJA, respectively (Table 4). According to the results, the CAT activity was increased by salinity stress, however, the average values of the enzyme at moderate salinity level was more than the other levels. The APX activity was increased by increasing salinity levels from 0 to 6 dS m^{-1}. The results of the present study revealed that salinity stress increased CAT and APX activities. Environmental stresses such as salinity and drought led to an increase in the antioxidant enzyme activity. There are many reports which indicated that the salinity stress enhanced POD and APX activities in different plants such as cucumber (Xie et al 2008), tomato (Srinieng et al 2015), Kentucky bluegrass (Puyang et al 2015), wheatgrass (Sheikh-Mohamadi et al 2017, 2018). CAT and APX are two main hydrogen peroxide-scavenging enzymes in the plants, which transformed the overproduction of H_2O_2 induced by salinity stress to H_2O and O_2 (Ashraf et al 2019). Salinity stress led to a reduction in the activity of these enzymes, but their activities were restored in the presence of the Si and MeJA. It seems that Si and MeJA mitigated the adverse effects of salinity stress by reducing the H_2O_2 production. Si can reduce the malondialdehyde concentration under salinity stress, which can be attributed to its antioxidant role in the plant defense system (Kim et al 2017).

4. Conclusion

The present study displayed that the increase in salinity level reduced chlorophyll index and fluorescence while vitamin C, CAT and APX activities were increased. MeJA and Si enhanced the chlorophyll index and vitamin C at different salinity levels, respectively. However, CAT and APX decreased when the salinized plants were treated with MeJA and Si. MeJA and Si may act to mitigate the adverse effects of salinity stress by reducing the H_2O_2 production. Finally, it can be concluded that MeJA and Si partially offset the adverse impacts of salinity stress.

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Modeling and Design a Special Type of Passive Solar Greenhouse in Cold Climate by TRNSYS

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ABSTRACT
To improve the thermal performance, storage and saving heat solar energy of conventional greenhouse, a passive solar greenhouse was built which its north wall was made of soil. The bottom part of the north, south, west and east walls were sloping and constructed below ground surface. The indoor air temperature was measured during January and February. To optimize the size of greenhouse in cold climate condition a TRNSYS model was created and validated using experimental data. According to the results obtained, Total Incident Solar Radiation (TISR) in the north wall was 484 MJ during January and February and there was the possibility of cultivation in it. More specifically, the variation of TISR during 60 days varied from 190 to 3811 kJ h^{-1} m^{-2}. The indoor air temperature of the greenhouse varied from -4.3 to 42.4 °C while the outdoor temperature fluctuated between -13.8 to 10.6 °C. In addition, the differential temperature between modeled and measured data at climate conditions of snowy, rainy, cloudy and sunny days were 2.3, 0.2, 0.2, and 2.6 °C during daytime and -1.8, -2, 0.3 and 1 °C at nighttime, respectively. The obtained coefficient of determination (R^2) was 95.95% for measured and modeled data.

Keywords: Passive solar greenhouse; Renewable energy; TRNSYS; Solar radiation

1. Introduction

By increasing fossil fuel price and its effect on greenhouse gases (GHG), researchers are attempting to develop energy saving technologies (Erdem et al 2016). In cold climate, reducing energy consumption for greenhouse heating is an important parameter for greenhouse existence (Mathala et al 2002). A lot of research efforts have been made to improve energy consuming of active and passive greenhouse types (Alkilani et al 2011). There are several methods for this aim such as thermal insulation, solar energy, earth to air heat exchanger, geothermal energy, and different heat storage system (Beshada et al 2006; Mashonjowa et al 2013; Patil et al 2013; Sethi et al 2013; Zhang et al 2015; Bin et al 2016; Jieyu et al 2017; Wei et al 2017). Each of these methods has been reported to be efficient in a specific climate condition.

For instance, Beshada et al (2006) investigated a passive solar greenhouse using thermal blanket for reducing heat loss during winter condition in southern Manitoba. The greenhouse had an insulated solid north wall to store solar energy in the daytime and afterward to release saved thermal energy in the nighttime. In addition, they used a thermal blanket which covered the glazed surface (single layer plastic) in the nighttime to minimize the heat loss. In another research, Bin et al (2016) conducted a study to assess thermal performance improvement of conventional single span

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greenhouses in different condition of half-removable back wall and fully-removable back wall. The thermal environment of the greenhouses was recorded and compared with a typical single span polyethylene hoop greenhouse. They claimed that the both two investigated greenhouse types showed that could be better alternatives to the conventional greenhouse (Bin et al 2016). Lately, Jieyu et al (2017) investigated on a solar greenhouse which was equipped with economical energy storage device for managing greenhouse stored energy. Wall storage actively managed with energy-store/retrieve funs and safety energy which is a solar collector and fully thermally isolated heat tank. In another study, a solar greenhouse was designed to use thermal storage of a water storage tank for heating. The water tank was heated using solar energy. The experimental greenhouse was 272 m^2 area which was equipped with 46 m^2 solar collecting plates. Their results showed that an additional 14 m^2 solar collector was needed to maintain greenhouse night air temperatures above 12 °C whereas outdoor average nighttime temperature was less than 7.8 °C (Wei et al 2017).

In regard to this fact, experimental works need a lot of budget therefore simulation model is an easy way to reduce costs and as well as reducing human errors. There are a lot of simulation researches to model greenhouse structure and inside temperature managing. For instance, Zhang et al (2015) proposed developing and investigation of soil heat storage for the greenhouse and compared experimental and simulation results. Attar et al (2014) investigated solar water heating system and influencing parameters on storage system.

Amongst all different modeling software, TRNSYS (TRaNsient System Simulation) is complete and extensible simulation software for the transient simulation of multi-zone buildings like greenhouses. It is employed to validate energy concepts of systems, such as to design and simulate hot water system of building, air conditioner equipment, control strategy and renewable energy systems. TRNSYS could easily connect graphical components described by mathematical model of a system in the Simulation Studio (Vadiee & Martin 2013; Attar et al 2014). This simulation software provides a lot of choices to find better analyzing of energy systems of agriculture building. In recent years, several studies have been done by agriculture researchers by using TRNSYS for design the greenhouse structure, energy management, and analyzing solar heating system and different energy resources. A lot of researchers have successfully evaluated feasibility of predicting and modeling greenhouse air temperature using TRNSYS to minimize the energy consumption using solar energy (Chung et al 1998; Voulgaraki & Papadakis 2008; Vadiee & Martin 2012; Asdrubali et al 2012; Carlini et al 2012; Chargui et al 2012; Marucci et al 2013; Mashonjowa et al 2013; Patil et al 2013; Vadiee & Martin 2014; Attar et al 2014; Ishigami et al 2014; Zhang et al 2015).

Candy et al (2012) designed a greenhouse in remote region in Humla, Nepal and used TRNSYS for modeling the greenhouse. The result showed good agreement between measured data obtained from created greenhouse and those of modeled by TRNSYS. One year later, Patil et al (2013) assessed solar heating of a greenhouse with seasonal storage. They focused on comparing results obtained from TRNSYS and EQUEST on controlling environment temperature, thermal storage, and solar thermal heat of the greenhouse. In another study a semi-solar greenhouse was studied by Taki et al (2016). In this research, the inside environment parameters were considered which were inside air temperature below screen, inside air temperature above screen, crop temperature, inside soil temperature, cover temperature, and thermal screen temperature (T_{sc}). In addition, they investigated dynamic heat and mass transfer model to find temperature in different points and crop evapotranspiration of the semi-solar greenhouse. Lately, Carlini et al (2012) studied on thermal behavior of greenhouse and thermal exchange rate in a greenhouse by using TRNSYS.

In present study, a passive solar greenhouse constructed 1m below the surface of the ground and subsequently the greenhouse was modeled by TRNSYS. The north wall in present work was constituted by excavated soil. In this research, we are investigated on modeling indoor temperature and TISR on five different points (soil, 2, 1.5, 1, and 0.5m height) on walls, roof, and floor. Validation was done using measured indoor air temperature in different days (snowy, cloudy, rainy and sunny) and their comparison with those obtained from TRNSYS model was done as well.

2. Material and Methods

The paper presented is in large part based on the operational characteristics of greenhouses in Tabriz; a region with the cold climate for greenhouses in northwestern of Iran. Information has been obtained from the software program TRNSYS. This design (shown in Figures 1 and 2) is intended to be built on a flat surface in a cold climate with minimum winter temperatures of more than -13 °C. To conform this design for Tabriz, a number of changes were necessary related to mathematic basic, materials, size, heating, cooling, ventilation, and glazing.

2.1. Mathematical description

Energy and mass balance equations were used in TRNSYS in order to calculate the temperature of the proposed thermal zones. The sensible energy balance for an arbitrary thermal zone (i) is defined by Equation 1:

$$\dot{Q}_i = \dot{Q}_{surf,I} + \dot{Q}_{inf,I} + \dot{Q}_{vent,I} + \dot{Q}_{gc,I} + \dot{Q}_{cplg,i} \quad (1)$$

Where; $\dot{Q}_i$, is the net heat gain; $\dot{Q}_{surf\,i}$, is the total heat gain or loss from the surface (including walls, roofs and floor); $\dot{Q}_{inf\ i}$, is the infiltration gains (air flow from outside only); $\dot{Q}_{vent,i}$, is the ventilation heat gain from the user defined source; $\dot{Q}_{gc\ i}$, is the internal convective heat gain by crops, people and other equipment; and $\dot{Q}_{cplg\ i}$, is the convective heat gain.

C_p, is fluid specific heat (kJ kg^{-1} K^{-1}); $\dot{V}$, is rate of attic infiltration of outside air (m^3 h^{-1}); ρ, is density of outside air (kg m^{-3}); T_{Zone}, is zone temperature (°C); $T_{ventilation}$, is temperature of ventilation air (°C).

$$\dot{Q}_{inf\ i} = \dot{V} \,.\, \rho.\, C_p\, (T_{outside,i} - T_{air}) \quad (2)$$

$$\dot{Q}_{vent\ i} = \dot{V} \,.\, \rho.\, C_p\, (T_{ventilation,i} - T_{air}) \quad (3)$$

$$\dot{Q}_{cplg\ i} = \dot{V} \,.\, \rho.\, C_p\, (T_{zone,i} - T_{air}) \quad (4)$$

The energy balance of the greenhouse consists of heat source and heat sinks in the greenhouse (equation5) due to different heat transfer phenomena (TRNSYS 17 Multizone Building modeling).

Heat storage = Heat gains (Heat source) + Heat losses (Heat sinks) (5)

In present greenhouse, the walls were created by soil and the south side was covered by polyethylene film to reduce heat losses (Figure 1). The soil surface temperature is higher than the indoor air temperature during the winter nights (Wei et al 2017). This is due to the heat storage of the absorbed solar radiation by soil during the day and its thermal capacity (Joudi & Farhan 2015). Since, solar radiation is not available during the night, heat stored in the soil bed transferred to the surface and is released indoor. In fact, on winter's days, the solar radiation absorbed by the soil surface could divided in two parts, one for heating the air in the greenhouse and other being transferred to the deeper soil (Chen & Liu 2006).

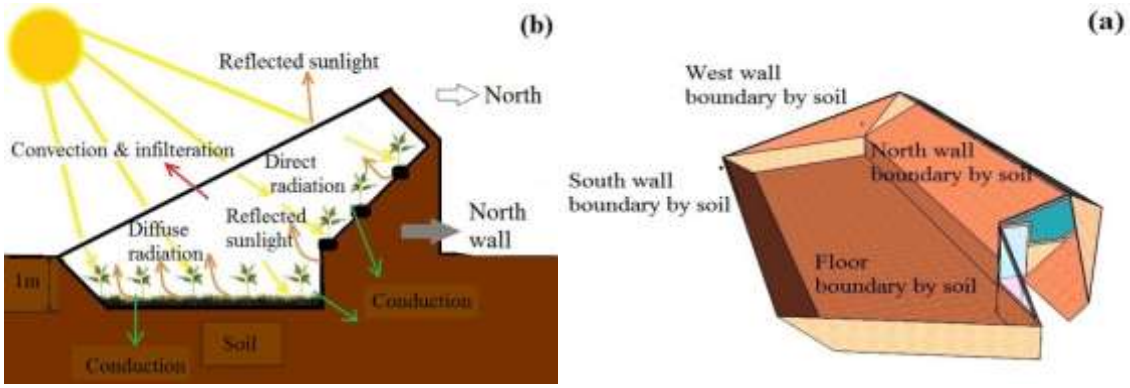

Figure 1- Schematic views as 3D (a) and 2D (b) of greenhouse parts

2.2. Design and modeling

Three software tools were used to construct the building models: sketch UP which is a 3-D drawing tool, TRNBUILD which is an interface to add the physical properties of the building to the geometry definition, and TRNSYS which is an

energy systems simulation software package. To produce an accurate model the following three steps need to be taken into account. At first, a three dimensional model of the building was drown in SKETCHUP, then the 3-D model was imported to TRNBUILD to add the thermal properties of the greenhouse and create the greenhouse information file (BUI) and at last the BUI was imported to TRNSYS for simulation the model to obtain different parameters especially temperature under various air and greenhouse conditions. In this study, a dynamic simulation model was developed to find greenhouse thermal performance using the TYNSYS simulation platform (Candy et al 2012). For this aim, the greenhouse geometry was imported into TRNSYS and became the basis of a greenhouse (type 56) (Figure 2 and 3).

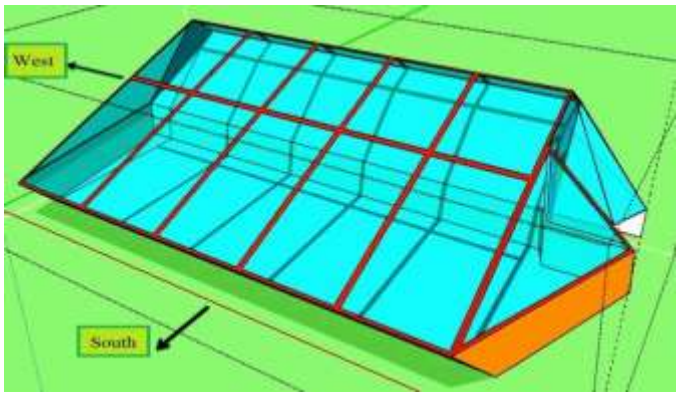

Figure 2- The sketchup of 3-D model of passive solar greenhouse

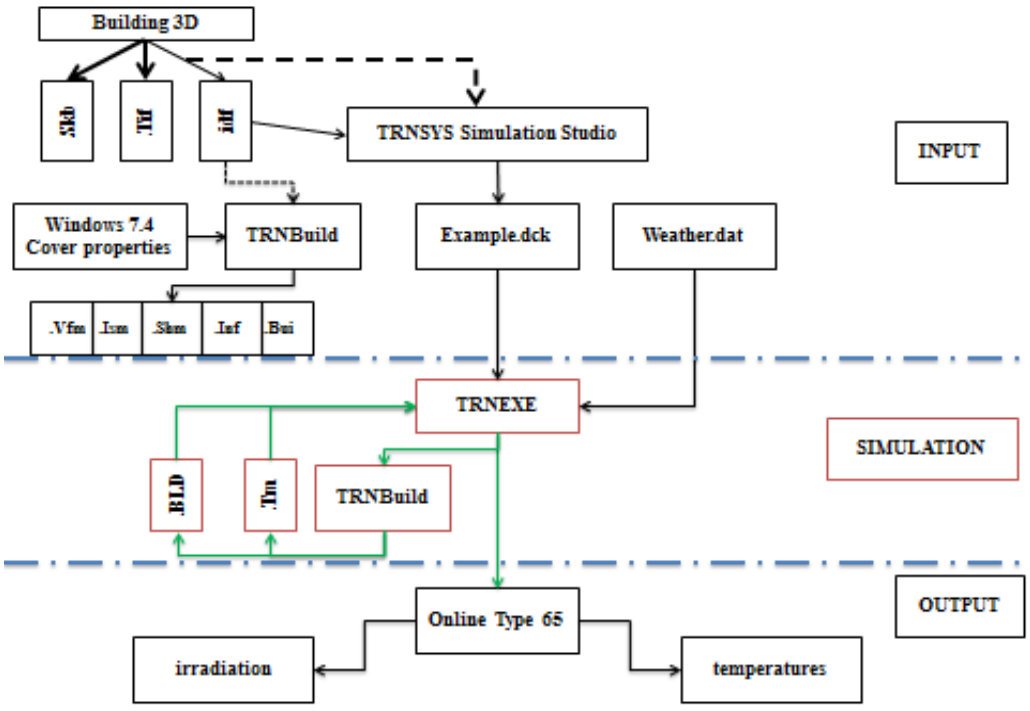

Figure 3- Diagram of dynamic building simulation in TRNSYS

In the present survey, the greenhouse was classified into two zones: above and below the surface ground for simulation purposes, section below is consisting of H-0-0, S- 0-140, W-90-90, N-180-90, N-180-140, N-180-40, E-270-90, E-290-45 walls that constructed by soil where the walls of S-0-20, E-290-90, W-110-70 and W-75-60 covered by polyethylene film. Cover thickness was 0.2 mm and convection heat transfer coefficient was 2.44 W m^{-2} K^{-1}. All surfaces (walls, floor, and roof) of greenhouse is defined in Table 1. The software window 7 (Berkeley lab, CA) was used to calculate transmittance properties of the cover (TRNSYS 17 Manual, 2017).

Table 1- Specification of different surfaces of modeled greenhouse

Surface	*Type*	*Area(m^2)*	*Category*	*Condition*
1	Ground floor	87.60	Boundary	H-0-0
2	Soil wall	22.87	Boundary	S-0-140
3	Soil wall	5.81	Boundary	W-90-90
4	Soil wall	19.00	Boundary	N-180-90
5	Soil wall	46.83	Boundary	N-180-140
6	Soil wall	18.00	Boundary	N-180-40
7	Soil wall	5.67	Boundary	E-270-90
8	Soil wall	9.33	Boundary	E-290-45
9	Poly ethylene	121.68	External	S-0-20
10	Poly ethylene	5.44	External	E-290-90
11	Poly ethylene	2.00	External	W-110-70
12	Poly ethylene	10.80	External	W-75-60

To simulation the thermal behavior, TYPE56 requires a several building data like geometrical data, wall construction data and some other data which influence the building such as radiation, ambient temperature, humidity, and building schedules. At first, the data were collected and then defined for the TRNSYS simulation. Figure 2 shows a schematic flow diagram of present thermal building simulation with TRNSYS.

2.3. Construction of passive solar greenhouse

To assessment and validation of simulated model, a solar greenhouse was built for cold climate (Figure 4a). All the used bars were woody because of its thermal insulation property. The greenhouse structure had well-insulated by soil walls and a floor 6.00* 17.40 m^2 and glazing on the south wall climate (Figure 4b).

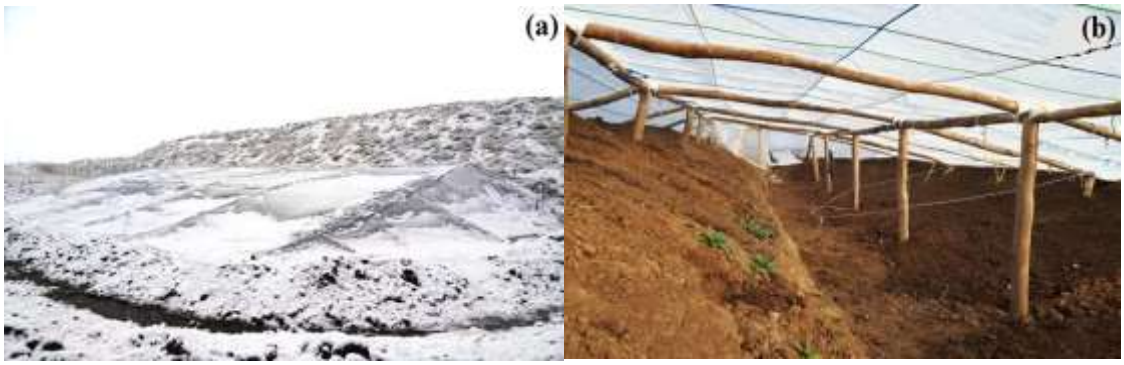

Figure 4- Outside (a) and inside (b) of greenhouse in winter

The north wall was created by the excavated soil of floor while in the passive solar greenhouse in last research was built by light weight concrete block, heavy weight concrete, wood, and common brick (Figure 4b).

The north wall size contained three parts of 5.11, 18.00, 46.86, and 19.00 m^2 (sum 88.97 m^2) which 18 m^2 of them was placed below ground surface. The bottom part of the south, west, and east wall were sloping and constructed below ground surface. The overall roof area of each three sides (south, east and west) was 168.48 m^2. Also, the area under cultivation was 168.55 m^2 that was consisted of ground floor and the bottom part of the south, west, and east. The highest indoor point of the greenhouse was 3.20 m at the center of greenhouse floor (Figure 4b).

Thermal conductivity coefficient and thermal resistance of employed polyethylene were 0.4796 W m^{-1} K^{-1} and 0.41 m^2 K W^{-1}, respectively. The door and ventilation flaps have been installed on the front corners of the greenhouse in east and west side (Figure 4a, b).

3. Results and Discussion

The indoor air temperature and solar radiation data was obtained by TRNSYS simulation from 1 January till 1 March (1440 hours). Prediction model was created using the average air temperature and solar radiation (of snowy, rainy, cloudy, and sunny days) collected by TRNSYS. To validate TRNSYS model, these data were subsequently compared with the measured average temperature in snowy, rainy, cloudy, and sunny days (Figure 5).

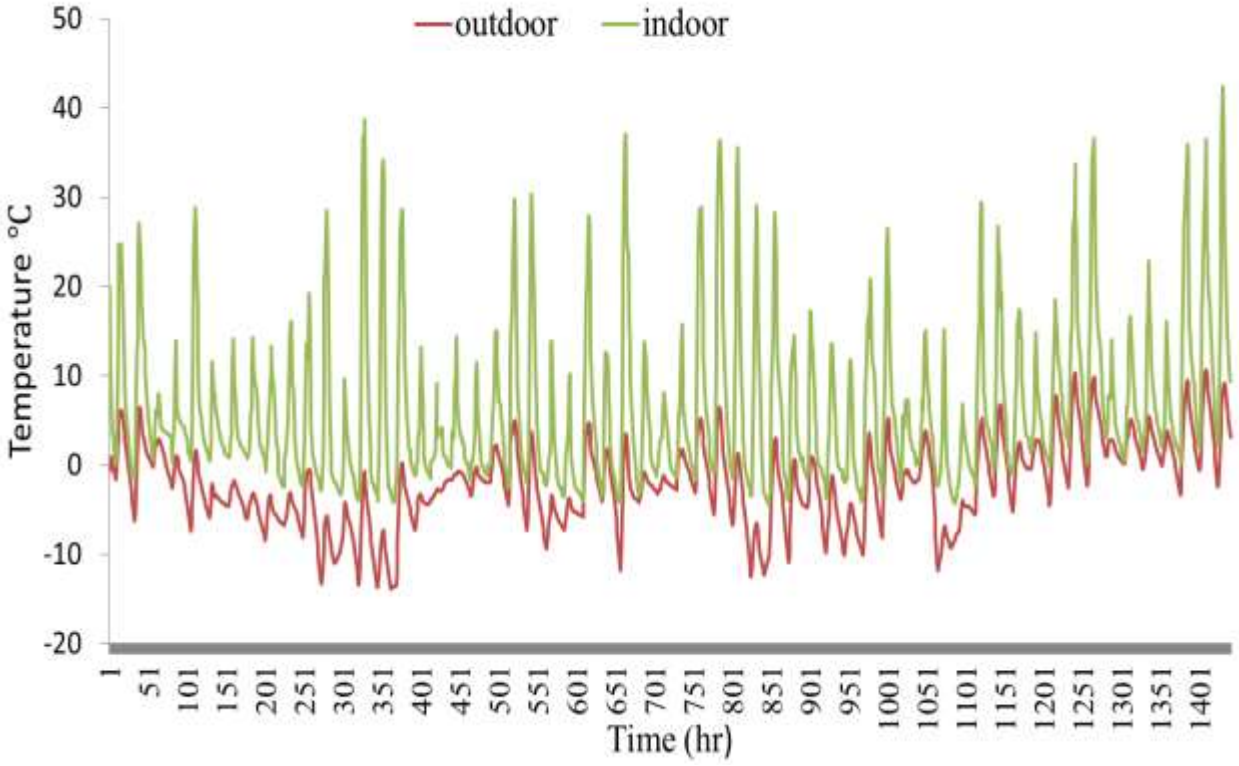

Figure 5- The outdoor (red line) and indoor (green line) temperatures in simulation studio of TRNSYS software

3.1. Modeling results greenhouse during January and February months

The results of modeled greenhouse air temperatures and TISR are well investigated from 1 January to 1 March (1440 hours). The indoor air temperature (green line) and the outdoor air temperature (red line) showed in Figure 5. The results presented in this figure show that the greenhouse air temperature varied from -4.3 to 32.40 °C while the outdoor temperature fluctuated between -13.8 to 10.6 °C. More specifically, minimum indoor temperature was recorded on freeze day (4 February), but the lowest outdoor temperature occurred on 15 January (-13.8 °C). In a research conducted by Beshada et al (2006) who used thermal blanket to cover greenhouse at night, the results obtained showed that in the coldest day in February, lowest recorded nighttime temperature in the inside greenhouse was -4.9 °C while the outdoor greenhouse temperature was -29.2 °C. In another study, Wei et al (2017) who used solar water heating method using solar energy in a water storage tank for heating the greenhouse during the night. In this system, the air temperature greenhouse increased 3.7 °C at night. The inside-outside temperature different ranged from 14.3 to 21 °C (Bashada et al 2006; Wei et al 2017).

Also, results of solar radiation on walls showed a variation of 190 to 3811 kJ h^{-1} m^{-2} in TISR values in the coldest days during January and February months. In snow day, TISR were between 190 and 760 kJ h^{-1} m^{-2}. In better word, the TISR values obtained were 623.231, 480.273, 567.521, and 278.058 MJ for south, floor, east, and west walls in January and February months, respectively (Figure 6).

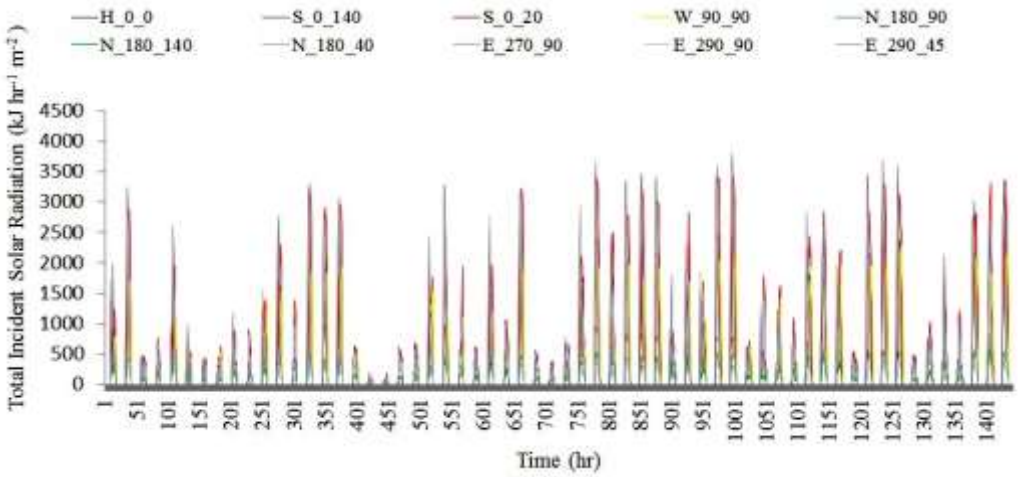

Figure 6- Total incident solar radiation on walls for 1440 hours from 1 January to 1 March

Temperature levels measured at different points in the greenhouse showed that the minimum and maximum temperatures were observed around the vicinity of the northern wall which was 9±1 and 15±1 °C higher than the outdoor air temperature (Figure 7). The maximum temperature recorded in N, I, M, and H points in snowy, cloudy, and rainy days (Figure 7). In the study of Bin et al (2016) report their greenhouse could maintain indoor temperature above 8.2 °C by removable back walls when minimum of temperature in polyethylene greenhouse was 2.9 °C. The maximum difference of indoor and outdoor in single span greenhouses was 9 °C whereas the systems of half- removable back wall and fully- removable back wall was 6.8 and 6.1 °C, respectively. Recently, Jieyu et al (2017) built a greenhouse including north wall which could storage heat energy during day. They employed this saved energy using fans for heating the greenhouse during the nights. However, the air temperature in greenhouse ranged from 1.18 to 12.56 °C while whiteout storage heat ranged from -3.9 to 12.56 °C (Bin et al 2016; Jieyu et al 2017). In the present study, indoor and outdoor temperatures of the greenhouse were below zero for 168 and 982 hours, respectively.

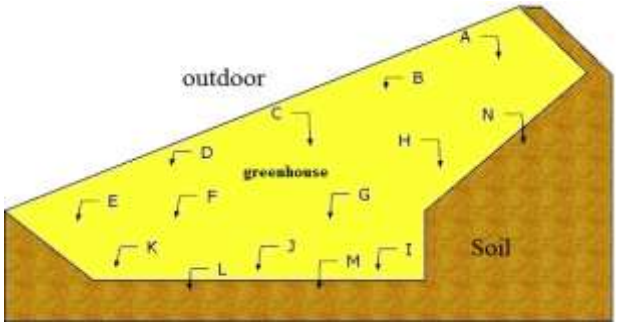

Figure 7- Points defined for measuring of temperature in greenhouse

For more examination of the solar radiation effects on the greenhouse walls, the TISR value were recorded during four different days with different amounts of solar radiation (Figure 8). In terms of climate condition, during snow days, south wall (S-0-20) showed highest TISR (1630 kJ hr^{-1} m^{-2}) between 9:30 and 16:45. At the same time, results of present TRNSYS model showed that the maximum air temperature was 15.15 °C at 16:00. While outdoor air temperature showed 6.95 °C reduction (Figure 8a), while on sunny day (31 January) the inside air temperatures showed a high increment. Although solar radiation was increased by air temperature increasing, however, as expected solar radiation extremely decreased after 16:00. The largest indoor-outdoor air temperature difference was recorded by 28.84 °C at 15:30. Also, amount of TISR at concurrent air temperature was 2974 kJ hr^{-1} m^{-2} which was observed in the east wall (Figure 8b). The results of air temperature at cloudy air condition are shown in Figure 8c which reveal a constant temperature during four hours from 12:30 to 16:30. In addition, the maximum TISR in cloudy day was highly decreased by 1057 kJ hr^{-1} m^{-2} which was recorded on south wall. Overall, the minimum TISR values were observed in cloudy and

rainy days and on the top of that these days had the lowest observed different radiation and temperature between all the days (Figure 8c, d).

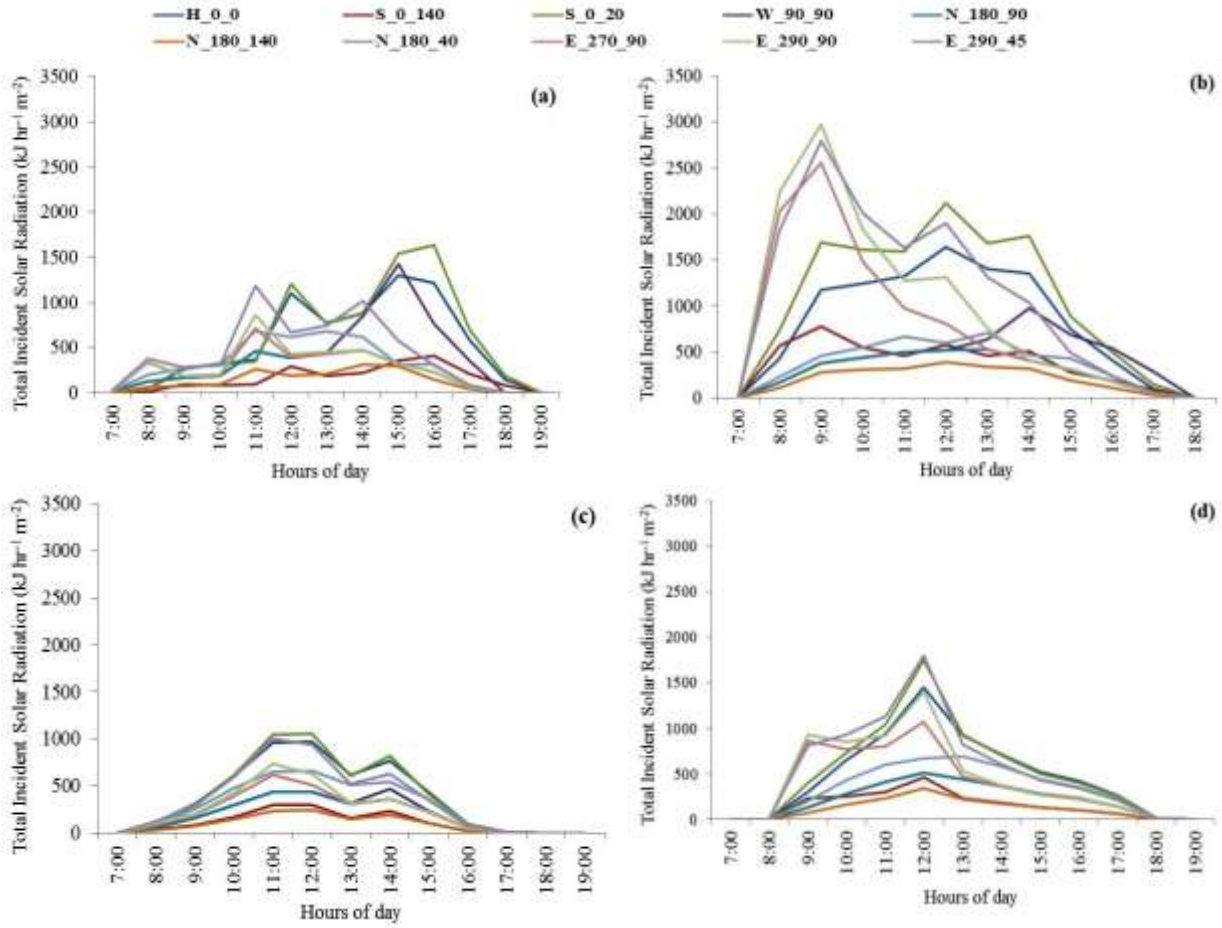

Figure 8- TISR at air condition of: (a) snow day, (b) sunny day, (c) cloudy day, and (d) rainy day

In regarding to investigate heating greenhouse during cold climate, Candy et al (2012) investigated on a solar greenhouse and their findings showed that the lowest temperature was around 2 to 3 °C which was happened at 7:00 am. More specifically, the average air temperature of indoor greenhouse was 3.5 °C between sunset and sunrise, which indicated on this fact that their greenhouse could led to a 1.2 °C higher air temperature than the average outdoor. During daytimes, the average air temperature inside the greenhouse was 14.3 °C while the average outdoor was 7.4 °C (Candy et al 2012). In another research, Zhang et al (2015) investigated on greenhouse heating during cold climate and their results showed that the maximum temperature was monitored in the floor greenhouse in cloudy day and maximum temperature was recorded in the greenhouse ceiling in sunny day. The indoor air temperature varied from -5 to 10 °C while outdoor air temperatures were -4 to 29.2 °C. In addition, they reported that the average indoor-outdoor air temperature difference during nights were 2.4 and -13.1 °C (Zhang et al 2015).

3.2. Validation

Figure 9 shows average temperatures measured and modeled in different conditions such as snow, cloudy, rainy, and sunny days. Results between average measured temperature and modeled data displayed that minimum measured temperature in snowy day was -2.2 °C while minimum modeled data was -4.39 °C and average maximum measured temperature and modeled data were +15 °C at 16:00, while the difference between the measured and modeled data in snowy, rainy, and sunny days were 0.2, 0.3, and 0.2 °C, respectively (Figure 9).

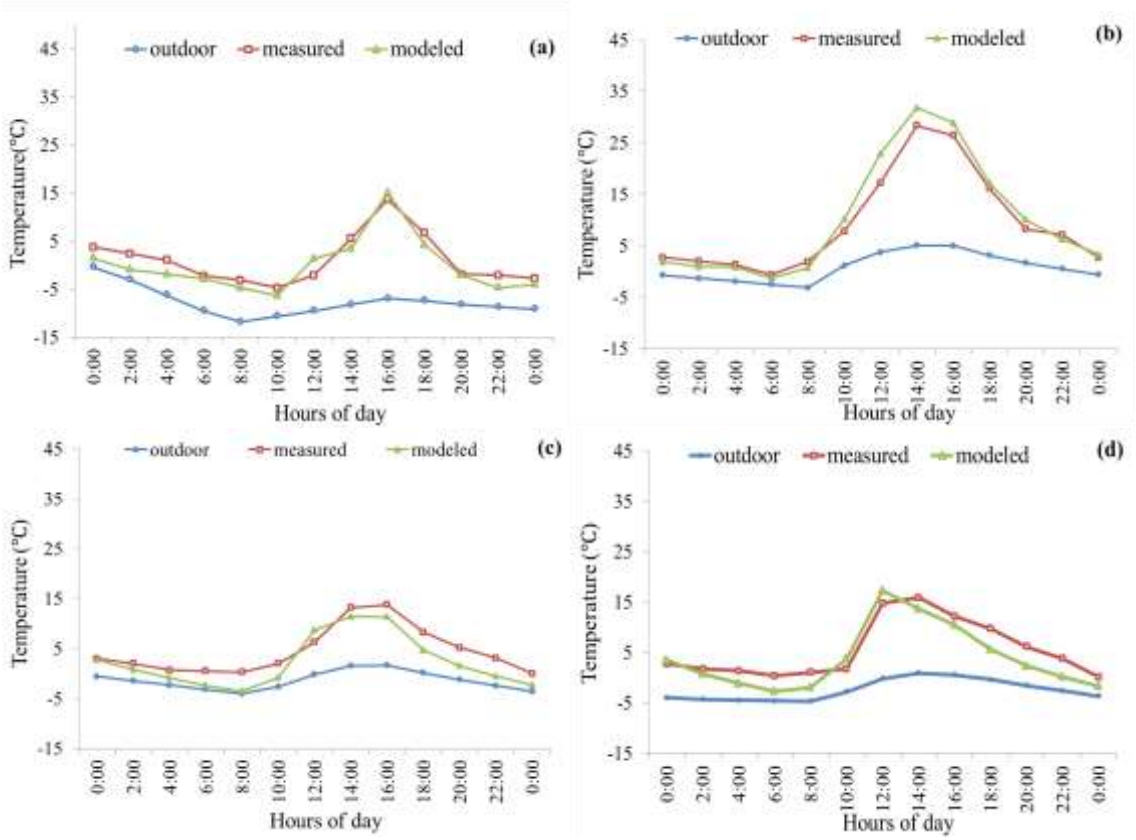

Figure 9- Average air temperature for modeled and experimental data at air condition of: snowy (a), sunny (b), cloudy(c), and rainy (d) day

Analysis of regression is shown in Figure 10. Results showed a significant linear regression between the values of measured and simulated model. The coefficient of determination (R^2) indicated 95.95% between values obtained from measured and modeled data.

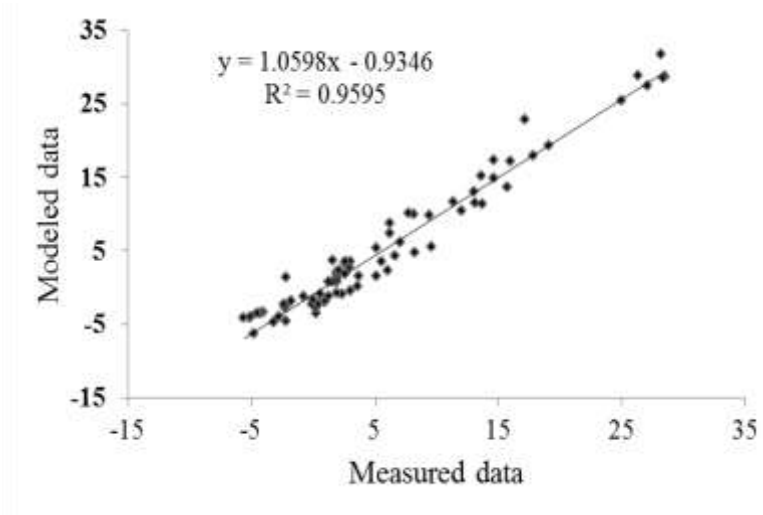

Figure 10- Relationship between measured and modeled data for indoor temperature of greenhouse

4. Conclusions

In this work, a passive solar greenhouse was built in depth 1m underground and analyzed based on two parameters of outdoor-indoor air temperature and TISR values during two cold months (January and February) and also a TRNSYS model was built to optimize greenhouse characteristics. The following conclusions could be derived based on the findings obtained in the present study:

- In modeled passive solar greenhouse, north wall was built vertical rather than Radiation line and made by excavated soil. A part of south, north, west, and east walls is placed in 1m underground that this method prevented from heat loss. The Area under cultivation was 168.55 m^2 and the polyethylene cover area was 168.48 m^2. In this design, TISR in the north wall was 484 MJ during January and February and there was the possibility of cultivation in it.

- Results of present TRNSYS model showed slight difference between average measured and simulation greenhouse temperatures which were up to -2 in rainy night and 2.6 °C in sunny day. This means that present TRNSYS model could predict indoor air temperature condition as well.

- Insolation of north wall and the lower part of west, east, and south walls slightly increased the indoor air temperature which could be due to the fact that the temperature was below zero for 168 hours while the outdoor air temperature was 982 hours below zero.

- The obtained coefficient of determination (R^2) was 95.95% for measured and modeled data that the present model is suitable for simulation of TISR and the indoor air temperature.

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Postharvest Salicylic Acid Treatment Influences Some Quality Attributes in Air-Stored Pomegranate Fruit

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ABSTRACT
Popularity of pomegranate fruit has increased in recent years because of its health benefit content, economic value and medicinal characteristics. Since pomegranate fruit is perishable species, prolonging storage life, keeping fruit quality during storage and marketing period with minimum quality and quantity loss after harvest are essential. Influence of salicylic acid treatments on some quality properties in 'Hicaznar' cultivar fruit were investigated in the current study. After harvest at commercial maturity, fruit were exposed to salicylic acid (SA) treatments. Controls (C1) were untreated. The other groups were dipped into a solution containing 0.01% Tween 20 (C2), 0.01% Tween 20+2 mM SA (SA1), and 0.01% Tween 20+4 mM SA (SA2). Then fruit were stored at 5±1 °C temperature, 85-90% relative humidity for 120 days. Changes in fruit skin and aril color, soluble solids content, titratable acidity, weight loss and chilling injury rate total phenolic content, antioxidant activity were followed at 60 days intervals. Neither SA1 nor SA2 affected total phenolic content and antioxidant activity levels of fruit. But, both treatments helped to maintain *C** values in arils and skin, titratable acidity and soluble solids content. Since SA2 treatment significantly reduced chilling injury symptoms during cold storage period of 120 days, it could be considered as promising postharvest technology.

Keywords: *Punica granatum* L.; Chilling injury; Antioxidant; Phenolics

1. Introduction

Pomegranate (*Punica granatum* L.) has been a traditional fruit cultivated for many years. Recent studies revealed the positive effects of pomegranate fruit on human health, especially because of its high antioxidant activity (Wang et al 2018). It is also rich in sugar, pectin, vitamin C, amino acids, mineral substances, fiber and phenolic compounds (Aviram et al 2000). Because of these properties, it has been considered as one of the functional foods in the world in recent years.

Pomegranate fruit have a non-climacteric respiration pattern (Kader 2006). Even if they have long storage period at the proper temperature, chilling injury, fungal decay and weight loss which are major limiting factors for long storage period, can cause fruit losses at an important rates. Chilling injury rates and low temperature sensitivity during cold storage mostly depend on cultivar. According to Kader et al (1984), 'Wonderful' cultivar fruit show chilling injury when it is stored at temperature lower than 5 °C for longer periods and a storage temperature of 7 °C is recommended. Köksal (1989) reported that 'Gök Bahçe' cultivar retained its quality for 3 months when it was stored at 5 °C. 'Hicaznar' fruit can be stored under cold storage conditions for 4 months (Candir et al 2018) and under controlled atmospheric conditions for 5 months (Koyuncu et al 2019) at 6 °C temperature.

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Besides storage temperature, modified atmosphere packaging (Selcuk & Erkan 2015), chitosan coating (Candir et al 2018), putrescine, salicylic acid (Koyuncu et al 2019), heat (Mirdehghan et al 2007), oxalic acid (Sayyari et al 2010) and methyl jasmonate (Sayyari et al 2011) treatments were used to reduce physiological disorders and extend storage period in pomegranate fruit of different cultivars. The effect of these treatments mostly depended on concentration of treatment material, cultivar and storage temperature.

Salicylic acid (SA) is a naturally synthesized phenolic compound effecting plant growth and development (Shaarawi et al 2016). Raskin (1992) recognizes it as a plant hormone. It is considered as 'Generally Recognized As Safe' (GRAS) (Sayyari et al 2009). Due to its positive effects on plant stress tolerance, SA might increase fruit resistance to stress conditions such as low temperature, inappropriate atmospheric conditions etc. after harvest (Asghari & Aghdam 2010; Sayyari et al 2017). Sayyari et al (2009; 2017) mentioned that this compound had a significant effect on preventing chilling injury symptoms such as aril and skin browning, on reducing ascorbic acid loss in fruit of 'Malas Saveh' and 'Mollar de Elche' pomegranate cultivars. Wang et al (2006) reported that SA treatments decreased chilling injury symptoms in peach fruit. Srivastava & Dwivedi (2000) for banana fruit and Zhang et al (2003) for kiwifruit revealed that SA maintained flesh firmness by retarding ethylene production. According to Chan & Tian (2006), it reduced the fungal decays in sweet cherry.

'Hicaznar' is the most famous pomegranate cultivar in Turkey with its sour-sweet taste and attractive dark red skin color. It can be stored up to late March at 6 °C temperature. There are few information concerning the effect of SA treatment on some fruit quality characteristics of 'Hicaznar' pomegranate cultivar during regular air cold storage conditions. It has been known that ecology has an important effect on fruit quality and keeping quality during postharvest period and storage. There are not any data on storage potential of pomegranates grown in Mersin province of Turkey. In the current research, effect of SA treatments at various concentrations on some fruit quality parameters in fruit of 'Hicaznar' cultivar grown in Mersin-Tarsus area was investigated during cold storage period of 120 days.

2. Material and Methods

2.1. Fruit samples

'Hicaznar' pomegranate fruit were harvested from commercial orchard in Mersin-Tarsus (36º55'57"N 34º51'45"E), Turkey at commercial harvest maturity. Immediately after harvest, fruit were transported to Ankara University Faculty of Agriculture Department of Horticulture Postharvest Physiology Laboratory by a cooled truck.

2.2. SA treatments and storage conditions

After selecting uniform fruit in respect of free from disease and pests, and mechanical damages, they were divided into 4 groups. Control (C1) group were stored without any treatment. The other groups were dipped into a solution containing 0.01% Tween 20 (Merck 9005-64-5) (C2), 0.01% Tween 20+2 mM SA (Merck 69-72-7) (SA1), 0.01% Tween 20+4 mM SA (SA2) for 10 min at 20 °C, respectively. After allowing them to naturally dry under laboratory conditions, they were stored in air at 5±1 °C temperature and 85-90% relative humidity for 120 days. A storage temperature of 5 °C was selected in order to observe whether effect of different SA concentrations especially on chilling injury rate. Changes in some fruit quality parameters mentioned below were determined at two months intervals during storage period.

2.3. Assessments

Skin color was measured at three different points on equatorial surface of fruit. Aril color was determined at three points after cutting fruit from equatorial part with a colorimeter (CR-200 Minolta, Japan). Data were presented as hue ($h°$), chroma (C^*) and L^* (McGuire 1992).

Soluble solids content (SSC) was read by a digital abbe refractometer (Leica 10480, Germany) in fruit juices obtained after squeezing and filtering of arils with a Whatman filter paper.

After joining pomegranate juice of 3 mL with 50 mL distilled water, solution was titrated with 0.1 N NaOH (Merck, 106462) until pH= 8.1 with an automatic titrator (Mettler Toledo DL 50 Graphix, USA) for titratable acidity (TA) measurements and the results were expressed as citric acid%.

For weight loss determinations, same fruit at all analysis dates were weighed with a scale (Mettler Toledo, USA) and this parameter was presented as percentage in respect of initial weight.

Chilling injury (CI) symptoms in fruit such as skin browning and pitting, aril and membrane browning were individually evaluated by naked eye. Fruit showing injury symptoms were rated to the total fruit number. The results were presented as percentage.

For the extraction of total phenolic content (TPC), arils of 5 g was taken from each replicate and homogenized with 10 mL acetone (80%) (Merck, 100014) containing 0.2% formic acid (Merck, 100264) for 2 min at 13500 rpm in a homogenizer (Janke & Kunkel, Ultraturrax 725). Then extracts were centrifuged (Sigma 3K30) at 14 000 rpm for 20 min at 0 °C. This process was performed 2 times as in Selcuk & Erkan (2015). Total phenolic content was determined according to the method of Spanos & Wrolstad (1990) with slight modifications. A hundred µL extract, 900 µL double deionized water and 5 mL diluted Folin-Ciocalteu reagent (Sigma, F-9252) were vortexed in a 10 mL test tube after incubating at room temperature for 3 min. After adding of 4 mL saturated sodium carbonate (75 g L^{-1}) (Merck, 106392) and keeping it under room temperature for 90 min, the absorbance value of final mixture were read by a spectrophotometer (Shimadzu) at 765 nm wavelength. The results were expressed as mg of gallic acid equivalent per 100 g of fresh weight (mg GAE 100 g^{-1} fw).

The antioxidant activity was determined according to Benvenuti et al (2004) and Sánchez-Moreno et al (1999) with some modifications. A six hundred µL 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma, D9132) solution was added in 5 test tubes and 20, 40, 60, 80 and 100 µL of diluted pomegranate juice were added to these tubes, respectively. They were completed to 6 mL with methanol (Sigma, 34860) and throughly mixed with vortex. Then they were incubated for 15 min in the dark. Absorbance values at 517 nm of these solutions were determined via spectrophotometer (Shimadzu). The % inhibition values corresponding to each sample volume were calculated and after plotting them against sample volumes, the curve was drawn for each sample by linear regression analysis. Using the regression equation and dilution factor, EC_{50} (efficient concentration) values were calculated. This value refers to the concentration of the antioxidant substance that inhibits 50% of the DPPH radical. The lowest EC_{50} value means highest antioxidant activity (Olugbami et al 2015).

2.4. Statistical analysis

This study was set according to completely randomized experimental design with 4 replications and randomly selected 4 fruit were included in each replication. Analysis of Variance (ANOVA) was performed on the data by Minitab software (MINITAB 17 Inc., trial version) at $P \leq 0.05$ error level. Means were compared by Tukey's test by MSTAT-C (Michigan State University, MI, USA) software and differences among means at $P \leq 0.05$ error level were marked with letters. ArcSin transformations were applied to data of weight loss and chilling injury rate which were calculated as percentage.

3. Results and Discussion

3.1. Skin and aril color

Like other fruit species, fruit skin color is accepted as the most attractive property in pomegranate fruit, as well. In this experiment fruit skin L^* and $h°$ values mostly changed by the storage period (P= 0.000) (Table 1). After a storage period of 120 days, the lowest L^* (44.77) and $h°$ (28.22°) values were measured on the fruit skin. Similar trend were also observed in skin C^* values and compared to the harvest time (47.62), the lowest C^* (42.41) value were determined at the end of storage period. Similar changes in these parameters were also reported by Selcuk & Erkan (2015) and Koyuncu et al (2019) for the fruit of the same cultivar. In the current study, significant effect of postharvest salicylic acid treatments was considerable for only skin C^* values (Table 1). It was observed that SA2 treatment included fruit having the highest C^* values ($P \leq 0.05$) (Table 2). As generally known that C^* values present saturation level of a color and the higher C^* values refers the better saturated and the more attractive color. Similarly, Koyuncu et al (2019) determined higher C^* values in SA treated fruit compared to the controls. But the same researchers also mentioned that putrescine treatments were more successful to maintain higher C^* values in fruit of 'Hicaznar' cultivar than salicylic acid at the end of the controlled atmosphere storage of 6 months.

Table 1- Results of variance analyzes

Assessments	*Factors*		
	SP[1]	*T*[1]	*SP x T*[1]
Skin *L**	0.000***[2]	0.151 ns[3]	0.625 ns
Skin *C**	0.000***	0.010**	0.365 ns
Skin *h°*	0.000***	0.706 ns	0.991 ns
Aril *L**	0.016*	0.066 ns	0.428 ns
Aril *C**	0.000***	0.007**	0.331 ns
Aril *h°*	0.000***	0.431 ns	0.892 ns
SSC (%)	0.000***	0.000***	0.000***
TA (citric acid %)	0.000***	0.003**	0.059 ns
Weight loss (% and angle)	0.000***	0.000***	0.001***
Chilling injury (% and angle)	0.000***	0.000***	0.000***
Total phenolics content (mg GAE 100 g^{-1} fw)	0.000***	0.956 ns	0.996 ns
Antioxidant activity (mL g DPPH)	0.000***	0.516 ns	0.337 ns

[1]SP, storage period (0^{th}, 60^{th}, 120^{th} days); T, treatments (C1, C2, SA1, SA2); SP x T, Storage period x treatments interactions; [2] *, P≤0.05; **, P≤0.01; ***, P≤0.001; [3]ns, non-significant

*L**, *h°* and *C** values of arils decreased depending on storage periods (P≤0.05) (Table 1) and the lowest values were determined at the end of the storage period (Table 2). Similar results have been previously reported for 'Hicaznar' cultivar (Selcuk & Erkan 2015; Candir et al 2018; Koyuncu et al 2019). On the other hand, treatments were significantly affected these data in our study (P= 0.000) (Table 1). Fruit in C1 group had lowest aril *C** values than the others. Within C2, SA1 and SA2 groups which had similar values, fruit treated with SA2 (22.83) showed significantly higher values (Table 2). On the other hand, Vatanparast et al (2012) did not find significant effect of SA treatment on *C** values of arils in 'Malas Yazdi' pomegranate cultivar. This result could show that the effect of salicylic acid treatments on aril color may be depended on cultivar and/or ecology characteristics during fruit growth.

Table 2- The effect of storage period (SP) and treatments (Ts) on skin and aril color in 'Hicaznar' pomegranate fruit

Factors	*Assessments*					
	*Skin L**	*Skin C**	*Skin h°*	*Aril L**	*Aril C**	*Aril h°*
SP						
0	53.46±0.48 a[1]	47.62±0.19 a[1]	33.74±0.74 a[1]	26.74±0.26 a[1]	24.49±0.36 a[1]	24.51±0.40 a[1]
60	46.65±0.62 b	44.00±0.51 b	28.57±0.55 b	27.29±0.49 a	21.54±0.38 b	19.50±0.73 a
120	44.77±0.69 c	42.41±0.58 c	28.22±0.54 b	25.06±0.80 b	20.52±0.37 c	19.95±0.47 b
Ts						
C1	47.33±1.52	44.67±0.84 b[2]	29.70±1.17	25.06±0.49	21.03±0.86 b[2]	21.07±0.95
C2	49.06±1.19	43.70±0.89 b	30.81±0.90	27.02±0.63	22.15±0.59 a	22.26±1.03
SA1	47.63±1.33	44.29±0.75 b	29.77±1.08	27.27±0.26	22.72±0.48 a	21.21±0.80
SA2	49.16±1.14	46.06±0.72 a	30.42±0.99	26.11±1.05	22.83±0.51 a	20.76±0.88
SP x Ts						
0 x C1	53.46±1.08	47.62±0.44	33.74±1.65	26.74±0.59	24.49±0.80	24.51±0.91
0 x C2	53.46±1.08	47.62±0.44	33.74±1.65	26.74±0.59	24.49±0.80	24.51±0.91
0 x SA1	53.46±1.08	47.62±0.44	33.74±1.65	26.74±0.59	24.49±0.80	24.51±0.91
0 x SA2	53.46±1.08	47.62±0.44	33.74±1.65	26.74±0.59	24.49±0.80	24.51±0.91
60 x C1	45.98±1.62	44.16±0.96	27.74±1.41	25.24±0.24	19.82±0.84	18.75±1.67
60 x C2	47.96±0.78	42.58±0.47	29.40±0.91	28.69±1.40	21.47±0.40	21.53±2.21
60 x SA1	45.38±0.80	43.34±0.30	27.95±1.30	27.78±0.31	22.48±0.26	19.22±0.70
60 x SA2	47.28±1.56	45.93±1.39	29.19±0.99	27.48±0.72	22.39±0.63	18.52±0.76
120 x C1	42.55±1.01	42.25±1.32	27.64±1.47	23.22±0.40	18.79±0.80	19.94±0.54
120 x C2	45.75±1.85	40.89±0.50	29.29±1.08	25.65±0.71	20.49±0.45	20.74±1.84
120 x SA1	44.05±0.70	41.90±0.36	27.62±0.76	27.30±0.34	21.20±0.14	19.89±0.55
120 x SA2	46.73±1.16	44.62±1.45	28.33±1.21	24.09±3.00	21.60±0.60	19.25±0.37

[1] Letters represent the differences between average values belonging each storage period at P≤0.05 error level; [2] Letters represent the differences between average values belonging each treatment at P≤0.05 error level

3.2. Soluble solids content

Storage period and salicylic acid treatments interactively affected SSC of fruit (P= 0.000) (Table 1). This parameter tended to decrease by cold storage period and reached lowest values at the end of storage period (120th day) in all treatments. Similar results were reported in air-stored 'Mollar' and CA-stored 'Hicaznar' fruit (Artes et al 1998; Koyuncu et al 2019). Fruit in SA1 (17.15%) and SA2 (17.15%) groups showed significantly highest SSC on the 120th day of storage period (Table 3). It means that these treatments helped to keep SSC level in fruit during 120 days. However in some other studies with 'Malas Saveh' and 'Shishe-kab' cultivars, SA treatments were not effective on SSC of fruit (Sayyari et al 2009; Moradinezhad & Khayyat 2014).

3.3. Titratable acidity content

TA is one of the major quality characteristics of pomegranate fruit and citric acid is the dominant organic acid in fruit of 'Hicaznar' cultivar (Selcuk & Erkan 2015). In the current study, significant losses in TA levels were observed during storage period, and the lowest mean value was measured at the end of storage period in fruit of 'Hicaznar' cultivar ($P \leq 0.05$) (Table 1 and 3). According to Selcuk & Erkan (2015), decrease in TA during storage period of pomegranate fruit resulted from fruit respiration because organic acids are mostly consumed components throughout respiration pathway. Our results are in accordance with those obtained by Shaarawi et al (2016) in fruit of 'Wonderful'. In the current study, SA1 and SA2 treatments were effective on keeping TA content of fruit during cold storage period of 120 days (Table 3). Koyuncu et al (2019) stated that SA treatment is effective for maintaining the TA level in fruit of 'Hicaznar' cultivar during controlled atmosphere storage of 6 months. However, Sayyari et al (2009) mentioned opposite results in fruit of 'Malas Saveh' cultivar during cold storage.

3.4. Weight loss

Weight loss in pomegranate fruit during storage is mostly due to the fact that a high porosity of the fruit skin (Elyatem & Kader 1984). In our study, storage period and salicylic acid treatments interactively effected weight loss in fruit of 'Hicaznar' (P= 0.000) (Table 1). In all groups, this parameter increased through storage period and significantly highest values were determined at the end of cold storage period of 120 days compared to the harvest time. At the end of the 120th day, while fruit in C1 (4.55%) and SA1 (4.21%) had higher weight loss values, fruit in C2 (2.70%) and SA2 (3.59%) groups showed lower weight loss levels (Table 3). It seems that SA2 also helped to prevent weight loss in fruit compared to untreated fruit (C1). Similar finding was reported by Koyuncu et al (2019). According to the researchers, 2 mM SA treatment decreased weight loss at a rate of 2.76% in fruit of 'Hicaznar' cultivar during controlled atmosphere storage of 6 months. On the other hand, Moradinezhad & Khayyat (2014) reported that 2 mM SA treatment did not have a significant effect on weight loss levels of 'Shishe-Kab' fruit during cold storage period. This can be resulted from differences in fruit skin structure with regard to porosity in different cultivars.

3.5. Chilling injury

It is conversant that chilling injury (CI) in pomegranate fruit is defined by membrane breakdown resulting in loss of tissue completeness companied by skin browning (Sayyari et al 2017). In fruit of different pomegranate cultivars, such as 'Wonderful' or 'Malas Saveh', CI symptoms like browning of skin, arils and membrane were observed, when they were stored at temperatures lower than 5 or 6 °C depending on the cultivars (Defilippi et al 2006; Sayyari et al 2009). In our study, the significant effect of storage periods x treatments interactions on CI was determined (P= 0.000) (Table 1) and CI was firstly observed at the end of the cold storage period (Table 3). In control groups (C1 and C2, 62.50%), more than half of the fruit showed CI symptoms and fruit loss was the highest in these groups. Fruit in SA2 group were more resistant to the storage temperature and had the lowest CI rate as 12.50%. It seems that 4 mM SA treatment helped to improve fruit tolerance to low temperature. Similarly, in some studies (Moradinezhad & Khayyat 2014; Sayyari et al 2011 and 2017), it was indicated that postharvest SA treatments reduced CI in fruit of 'Shishe-kab' and 'Mollar' cultivars. Koyuncu et al (2019) observed any CI symptoms in SA-treated 'Hicaznar' fruit during controlled atmosphere storage of 6 months because they stored fruit at 6 °C and this temperature was 1 °C higher than our storage temperature. Current results showed once more the importance of storage temperature in pomegranate fruit and lower temperature of 1 °C could increase fruit loss because of CI.

Table 3- The effect of storage period (SP) and treatments (Ts) on some quality assessments in 'Hicaznar' pomegranate fruit

Factors	*Assessments*					
	SSC[1] (%)	*TA[1] (citric acid %)*	*WL[1] (%)*	*CI[1] (%)*	*TPC[1] (mg GAE 100 g^{-1})*	*AA[1] (mL g^{-1} DPPH)*
SP (days)						
0	17.50±0.00	1.96±0.00 a[2]	0.00±0.00	0.00±0.00	230.51±1.25 c[2]	55.61±0.59 c[2]
60	17.44±0.01	1.69±0.00 b	1.97±0.17	0.00±0.00	260.78±1.27 a	59.84±1.33 b
120	17.01±0.04	1.49±0.00 c	3.76±0.23	42.19±6.34	250.22±1.35 b	68.68±1.80 a
Ts						
C1	17.24±0.09	1.69±0.05 c[3]	2.34±0.66	20.83±9.15	246.55±3.95	61.05±1.61
C2	17.28±0.08	1.71±0.06 bc	1.29±0.40	20.83±9.15	247.03±3.99	62.76±2.73
SA1	17.36±0.04	1.74±0.05 a	2.20±0.61	10.42±4.82	247.84±3.94	62.06±2.53
SA2	17.38±0.04	1.72±0.05 ab	1.79±0.52	4.17±2.81	247.25±4.38	59.65±1.89
SP x Ts						
0 x C1	17.50±0.00 a, a[4]	1.92±0.00	0.00±0.00 (0.00±0.00)[5] c, a[4]	0.00±0.00 (0.00±0.00)[5] b, a[4]	230.28±2.51	55.40±0.37
0 x C2	17.50±0.00 a, a	1.92±0.00	0.00±0.00 (0.00±0.00) c, a	0.00±0.00 (0.00±0.00) b, a	230.95±3.65	55.73±1.42
0 x SA1	17.50±0.00 a, a	1.92±0.00	0.00±0.00 (0.00±0.00) c, a	0.00±0.00 (0.00±0.00) b, a	231.57±1.50	55.92±0.89
0 x SA2	17.50±0.00 a, a	1.92±0.00	0.00±0.00 (0.00±0.00) c, a	0.00±0.00 (0.00±0.00) b, a	229.23±2.87	55.40±2.01
60 x C1	17.40±0.04 b, a	1.69±0.01	2.48±0.16 (9.05±0.29) b, a	0.00±0.00 (0.00±0.00) b, a	260.53±0.86	61.37±0.65
60 x C2	17.45±0.02 a, a	1.66±0.01	1.18±0.20 (6.19±0.56) b, c	0.00±0.00 (0.00±0.00) b, a	260.32±3.55	58.98±3.45
120 x C1	16.82±0.02 c, c	1.47±0.00	4.55±0.19 (12.31±0.25) a, a	62.50±7.22 (52.50±4.33) a, a	248.85±3.09	66.38±2.79
120 x C2	16.90±0.04 b, b	1.48±0.00	2.70±0.24 (9.44±0.45) a, c	62.50±7.22 (52.50±4.33) a, a	249.82±1.17	73.57±2.80
120 x SA1	17.15±0.02 b, a	1.52±0.02	4.21±0.23 (11.83±0.34) a, a	31.25±6.25 (33.75±3.75) a, b	251.25±2.95	71.25±2.87
120 x SA2	17.17±0.02 b, a	1.51±0.02	3.59±0.32 (10.90±0.48) a, b	12.50±7.22 (15.00±8.66) a, c	250.95±3.95	63.53±4.48

[1]SSC, Soluble solids content; TA, Titratable acidity; WL, Weight loss; CI, Chilling Injury; TPC, Total phenolic content; AA, Antioxidant activity; [2] Letters represent the differences between average values belonging each storage period at P≤0.05 error level; [3] Letters represent the differences between average values belonging each treatment at P≤0.05 error level; [4] The first letters represent the differences between storage periods for each treatment and the second letters represent the differences between treatments for each storage period at P≤0.05 error level; [5] Arcsin transformation values of percentage data

3.6. Total phenolic content and antioxidant activity

TPC and AA are responsible for the most part of health benefits in pomegranate arils. So, maintaining of these parameters during postharvest period is important for not only consumers but also retailers. In the current study, it was revealed that the major effective factor on keeping of these parameters after harvest was storage period and none of postharvest salicylic acid treatments showed significant effect on these parameters (P= 0.000) (Table 1 and 3). Our results are in agreement with Sayyari et al (2011) in 'Mollar de Elche' cultivar and Koyuncu et al (2019) who mentioned no differences among controls and SA-treated 'Hicaznar' fruit in respect of total phenolic content. In contrast, Dokhanieh et al (2016) stated that 250 µM SA treatment delayed decrease in total phenolics during storage period in 'Malase Yazd' fresh cut pomegranates. In the current study, either TPC or AA content in fruit increased during storage period and generally, the higher values were determined at the end of the whole storage period when compared to harvest time. It is known that increase in phenolic compounds of fruit during cold storage was arisen from different stress conditions (Selcuk & Erkan 2015). It can be said that low temperature at 5 °C, as a stress condition; caused increase in these two parameters at the end of storage period. Similar results were obtained in pomegranate cultivars such as 'Hicaznar' (Selcuk & Erkan 2015; Candir et al 2018; Koyuncu et al 2019), 'Wonderful' (Arendse et al 2014), 'Malase Saveh' (Babalar et al 2018). However, the effect of stress conditions on TPC could change based on cultivars. Thus, significant reductions in TPC were

determined in some studies during whole cold storage period in 'Mollar de Elche' (Sayyari et al 2011; Sayyari et al 2017), and 'Malase Yazd' (Dokhaineh et al 2016) cultivars.

4. Conclusions

This study highlights effects of SA treatments at different concentrations on some postharvest quality parameters, TPC and AA in fruit of 'Hicaznar' pomegranate cultivar during cold storage period. SA treatments helped to maintain C^* values in arils and skin, TA and SSC content, and especially 4 mM concentration reduced CI symptoms during cold storage period of 120 days. Conversely, this treatment has not been effective on TPC and AA. Overall, 0.01% Tween 20+4 mM SA dipping for 10 min at 20 °C could be a promising treatment for fruit of 'Hicaznar' cultivar grown in Mersin-Tarsus region in respect of keeping most of quality parameters.

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Domestic Tractor Market Share Estimation by Time Series Analysis Method

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ABSTRACT
This study is aimed to examine the domestic tractor market share with time series analysis to forecast the future probabilities with the help of the models which are created according to the Box-Jenkins method. The study was planned for year 2010, based on 5 different domestic tractor brands which are separately estimated for the domestic tractor market and included the total tractor numbers. It has been observed that the projected value of tractor companies whose market shares are estimated by time series analysis and the total tractor number are very close to their actual values.

Keywords: Box-Jenkins; Tractor market share estimation; Time series analysis; Forecast

1. Introduction

Predicting the future is an indispensable element of a socio-economic development. It is possible for private or public organizations to maintain or improve their future status only if they are able to predict events and find appropriate solutions with good planning. One of the methods used to predict the future is the time series. A time series consists of observation values sorted by time for any variable. Time series analysis is used to create a forecast model for the future periods using the previous period data of the variable to be predicted. Analysis of the time series of the model variable related to the model development is based on determining the main trend and its characteristics. It is used in the estimation phase considering that the model, whose accuracy is accepted under various constraints, will show the same tendency and similar characteristics in the future periods. Predicting the future is an indispensable element of a socio-economic development. It is possible for private or public organizations to maintain or improve their future status only if they are able to predict events and find appropriate solutions with good planning**.** The benefits of predicting the future can be given as 1. Help the company predict the future, 2. Responds to customers' needs, 3. No reduce your sales, 4. Reduces stocking costs, 5. Capture opportunities, 6. Allows you to plan cash flow, 7. It allows us to give the right and timely reproduction decisions, 8. Helps you to make your premium planning fair, 9. Improves your business processes.

Alon & Sadowski (2001) used the Box-Jenkins method in the time series analysis of US integrated retail sales. Burger et al (2001) used time series analysis and done a work on the estimation of travel demands of the tourists. Zhou et al (2002) used the time series analysis method to estimate hourly and daily water demand. Chu & Zhang (2003), using the monthly retail sales data for the years 1985-1999 from the US population administration, conducted studies on the estimation of sales with linear and non-linear models. Yeşil (2007) examined how demand forecasts, which are required

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as input of the process, are affected by the use of traditional and current estimation methods when using the production planning model in stock. As a result of the results obtained, the sector and the most effective forecasting model for the selected sample were evaluated. The time series analysis technique indicated that the estimation values that best represent the actual sales data were achieved. Bek (2008) examined the basic concepts used in time series analysis and ARMA, ARIMA, seasonal Box-Jenkins models and seasonal autoregressive models SAR (P). In order to make the best model choice, it has examined different models. ARIMA (0,1,7) (1,0,1) model was proposed as the best model. Berberoğlu (2010) used time series methods to model milk yields. It is concluded that ARIMA model models the milk yield better and predicts the actual values. Karaman (2010) aimed to determine the control day milk yields by time series method and to determine the number of control days providing the most accurate estimates. The researcher, with the results he obtained, argued that time series approach could be useful in predicting milk yield. In his study, Oğhan (2010) applied Holt Exponential Straightening and Box-Jenkins methods to one of the univariate time series analysis methods for cow's milk prices and made estimates for the series. The researcher stated that time series analyzes were guiding the decision maker in order to direct the producers and determine the policies. Özek (2010) examined the graphs used to determine an initial model for a time series. The researcher stated that ACF and PACF graphs are very useful in determining a suitable model for linear time series. Öztaş (2012) argued that time series are one of the most suitable methods in their short term estimations. Çelik (2013) analyzed the time series of traffic accidents in Turkey and determining the most suitable time series model aimed to estimate of the number of accidents in the future. Özer & İlkdoğan (2013) examined the cotton prices in the world with the ARIMA model, the Box-Jenkins method. Ali (2015) aimed to estimate unemployment and inflation and to determine the relationship between unemployment and inflation. The 5-year data of PM10 air pollutant from air quality measurement station were investigated by Turgut & Temiz (2015). Kapucuoğlu (2016) used analytic network process (ANP) to predict the Turkey tractor market share. In the comparison with the actual market shares of the tractor companies, which are estimated to be "new" and "second hand", it is observed that the estimated values are quite close to the real values on the basis of sector leadership. However, it is necessary not to ignore the sector conditions of the relevant years in the studies to be done.

According to the above researches, time series analysis and modeling has many business and social applications. It is extensively used to forecast company sales, product demand, stock market trends, and agricultural production. Box-Jenkins method is a superior method in determining the structure of a time series, observing the most effective use of observations between them and providing statistical tests in the model determination stages. People face problems of decision making in everyday life. Although some decisions are very simple; many require analysis because of the interactions between factors that influence the decision. Time series method is to make future estimations based on observation of the past. The observation of the past can be done by means of statistical data, in other words, with time series. The tendency is determined by using time series and the consumption of goods and services foreseen in production in previous years. And predictions are made on the assumption that future demand will develop in the same way. Agricultural machinery marketers, producers, tractor companies and all other sales companies want to know how much they will sell in the future.

The aim of this study is to estimate the local tractor market share by means of Time Series Analysis which is one of the quantitative (numerical) estimation techniques. This study is aimed to investigate the local market share of the local tractor by time series analysis and to make future estimations with the help of the models which are formed according to the Box-Jenkins method. The market share of the five local firms which have the largest share in Turkey's tractor market is estimated. In this study, tractor sales values of 5 tractor companies are tried to be estimated, not tractor production. In addition, the total number of tractors including all tractor brands is estimated and the usability of time series method has been investigated in the determination of tractor sales values of tractor companies in the future.

2. Material and Methods

2.1 Material

In this study, we used the monthly sales value data of 5 domestic tractor companies between the years 2005 and 2009. In order to expand the study, the total tractor numbers of all tractor brands were also included between the years 1991-2009. The number of tractor numbers is the sales values of 5 tractor companies included their own manufactured tractors as well as the ones which were purchased from abroad. Box-Jenkins method was used to analyze the time series methods. The data were further analyzed using SPSS 20 statistical package program. Table 1 gives information about five tractor companies. Company names are named as A, B, C, D, and E.

Table 1- Introductory information of five tractor companies

Properties / Tractor types	*A*	*B*	*C*	*D*	*E*
Power range (HP)	35-120	50-113	48-400	48-380	50-115
Gear option (units)	4	7	4	13	6
Area of use (vineyard, garden, field)	2	3	3	2	3
Case type (rops, sun visor, cabin)	3	3	2	3	2
Number of sellers (units)	53	143	63	103	94
Annual production capacity (pcs)	10000	15000	5200	50000	45000
Color option (pcs)	1	1	6	1	1

2.2. Methods

Sales numbers of 2010 were estimated by using the time series analysis method and SPSS statistical package program by using the tractor sales units of the period 2005-2009 for 5 different tractor brands. A similar study was conducted for the total number of tractors belonging to the period of 1991-2009. The total number of tractors estimated by time series analysis was obtained and these estimation values were compared with the actual total tractor numbers. Box-Jenkins method was used in the research. Box-Jenkins method, also known as ARIMA modeling approach; is a statistical method used for predicting single variable time series. The Box-Jenkins method has three models. These are the autoregressive process, the moving average (MA) and the moving autoregressive (ARMA) processes. The ARMA process becomes an integrated motion autoregressive (ARIMA) process when a difference is required for a non-stationary series. The following steps are followed to determine the model with the Box-Jenkins method. 1. Graphs of time (graphs of time series) of the observations are plotted. 2. Data are analyzed and the stability requirement is sought by looking at the ACF (autocorrelation values) and PACF (partial autocorrelation values) graphs. 3. Stability is obtained by taking sufficient difference in non-stationary data. 4. Unit root test is performed and ACF and PACF graphs are checked to see if stability is achieved. 5. After the series has stabilized and truncated, the period of the series is determined by looking at the obtained autocorrelation values. 6. After the period is determined, ACF and PACF graphs are taken into consideration to decide the model (for the determination of AR and MA model ranges). According to the information obtained from these graphs, the degrees of AR and MA models are entered into SPSS program and multiple models are created separately. 7. The meaningful ones are selected from these models created by the help of SPSS program and the meaningless ones are eliminated. 8. The model with the lowest BIC (Schwartz Bayesian information criterion) value is chosen as the most suitable model among the sig <0.05 models. 9. Estimates are made when the condition of the model is sufficient. 10. In the last step, ACF and PACF graphs of errors are generated to see if any errors in the model are white noise. If the errors are white noise, the estimation is determined to be reliable.

3. Results and Discussion

3.1. Findings related to time series methods of tractor sales and model estimation

Sales figures of A, B, C, D and E tractor brands were plotted. Declines and increases in the graphs were observed. Declines and increases in the graphs show that there is a trend, meaning that the series is non-stationary. It is necessary to look at the ACF (autocorrelation values) and PACF (partial autocorrelation values) graphs of the data and also the unit root test (ADF) results to see that there is a trend (Figures 1,2,3,4, and 5).

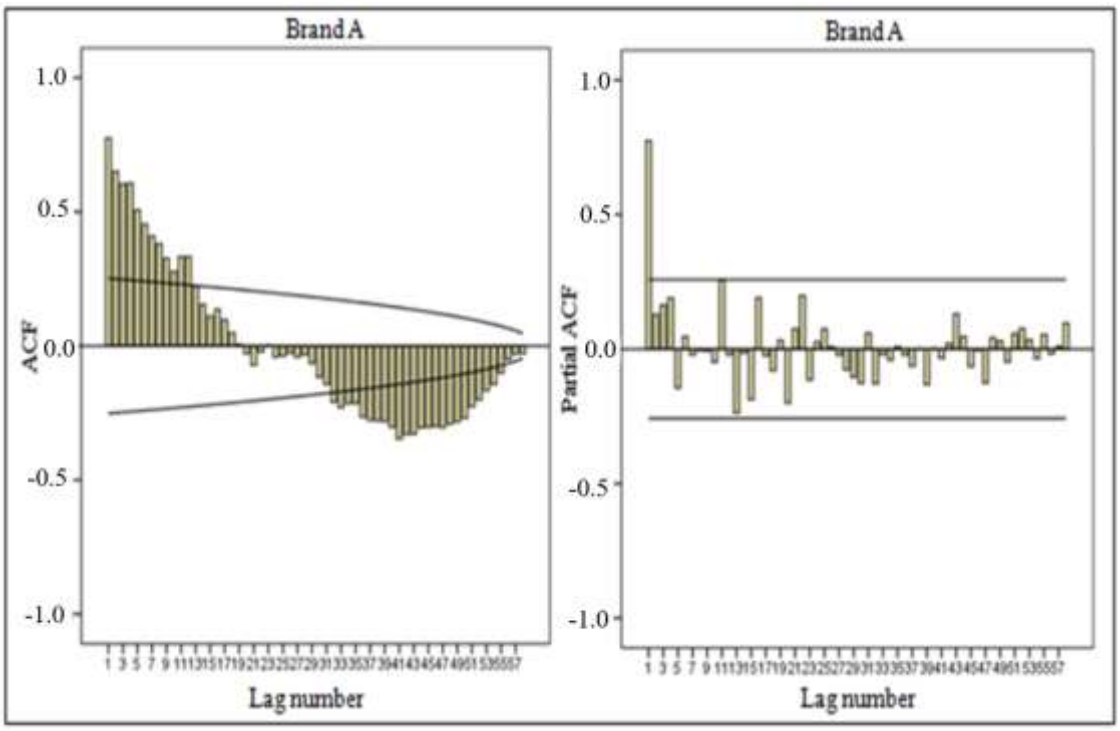

Figure 1- ACF and PACF graphs of brand A

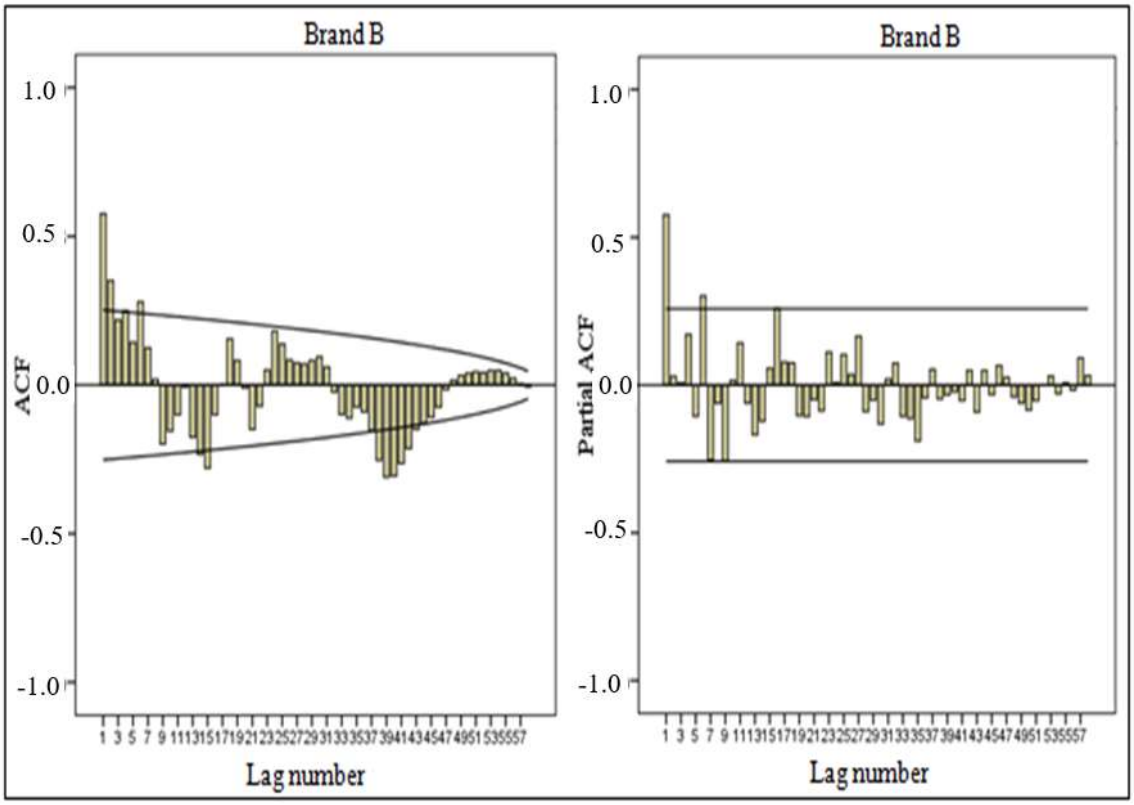

Figure 2- ACF and PACF graphs of brand B

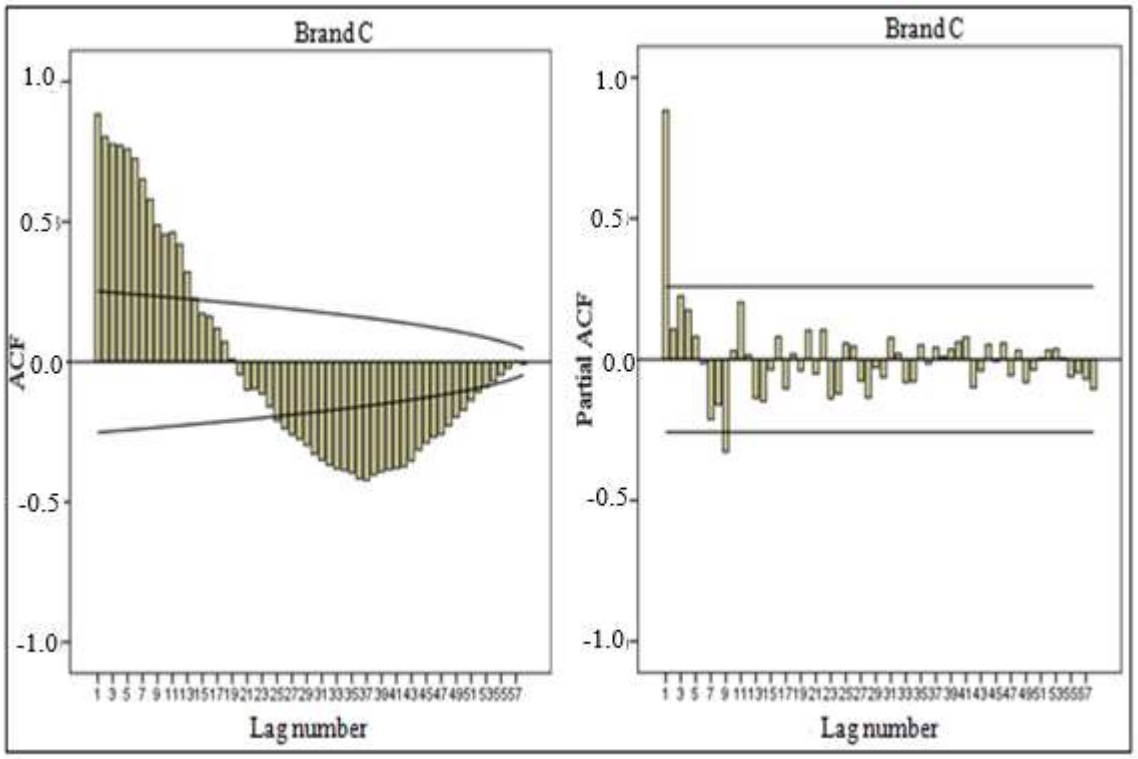

Figure 3- ACF and PACF graphs of brand C

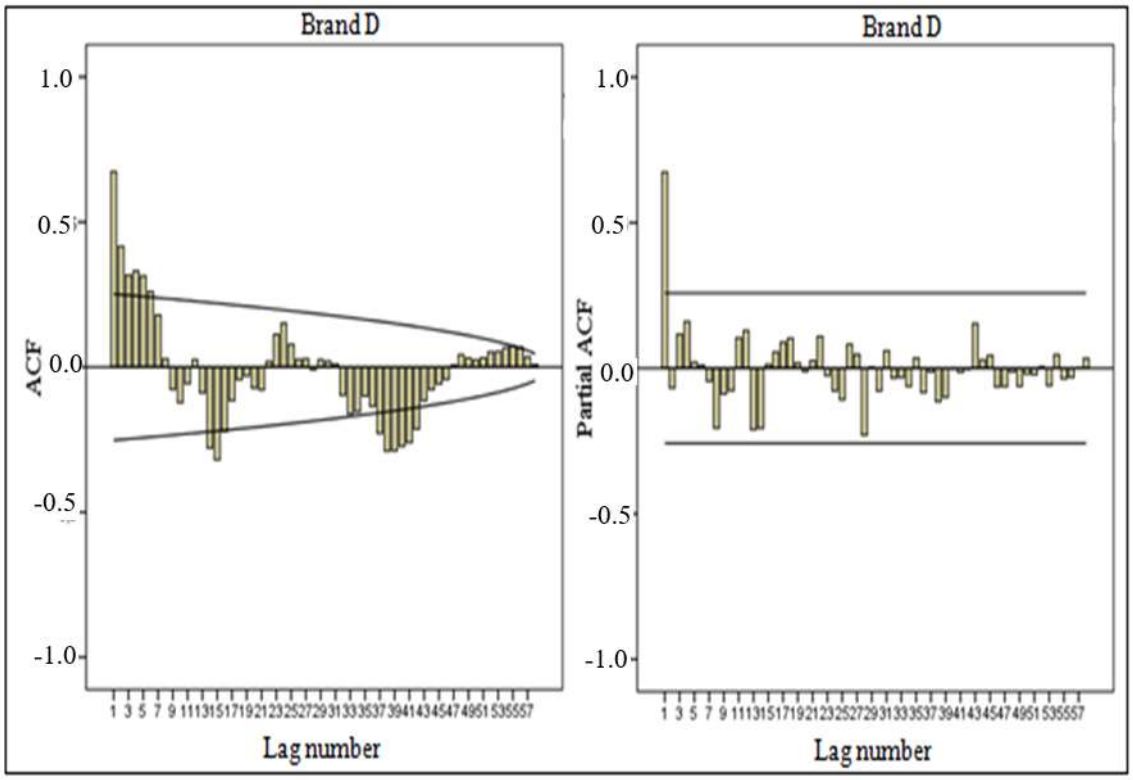

Figure 4- ACF and PACF graphs of the D brand

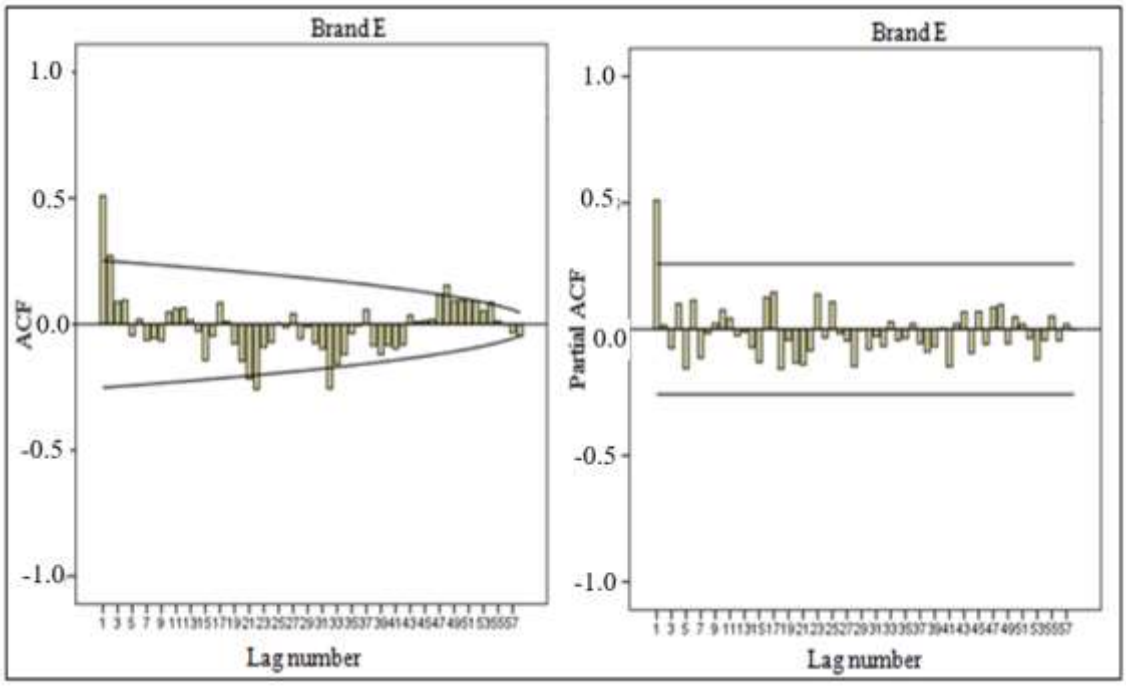

Figure 5- ACF and PACF graphs of the E brand

It was found that for brands A, B, C and D, there are trends in the data since many delays exceed the confidence limits; it can be said that the series is non-stationary. In order to continue the analysis, the data should be truncated. In order to ensure the stability of the series, the first order difference was performed by using SPSS package program. For the E brand, it is understood that there is no trend in the series, ie the series is stationary. When we look at the difference between the A, B, C and D brands, the ACF and PACF graphs show that the delays are within the confidence limits. Therefore, it can be said that the data is free from the trend (Figures 6, 7, 8, and 9).

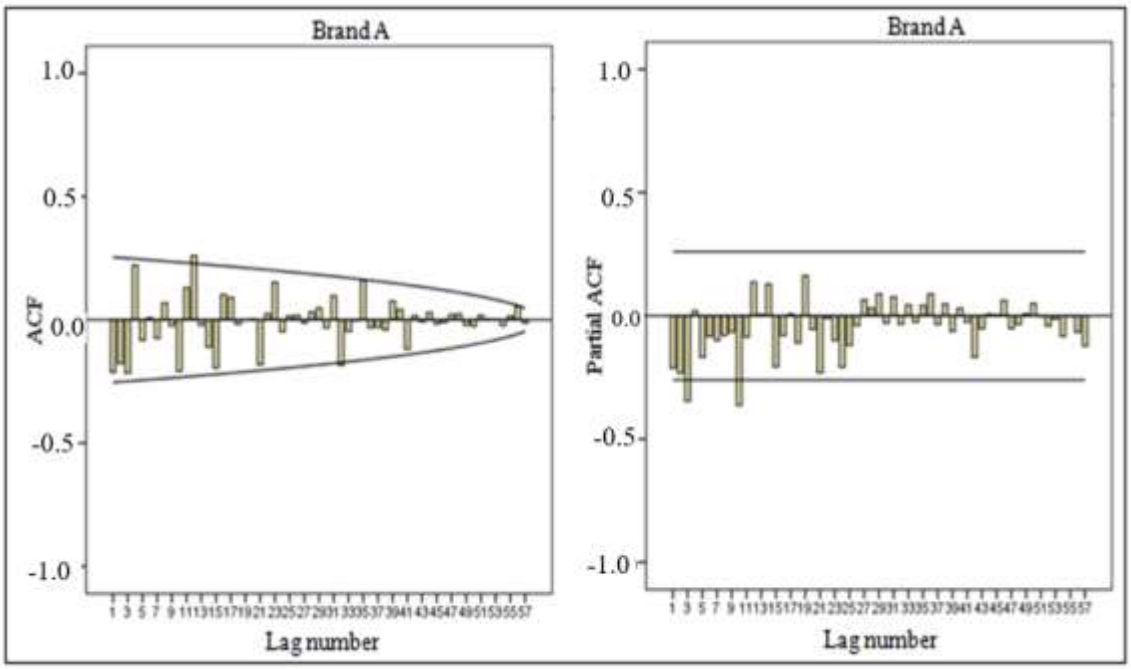

Figure 6- Difference-treated ACF and PACF graphs of brand A

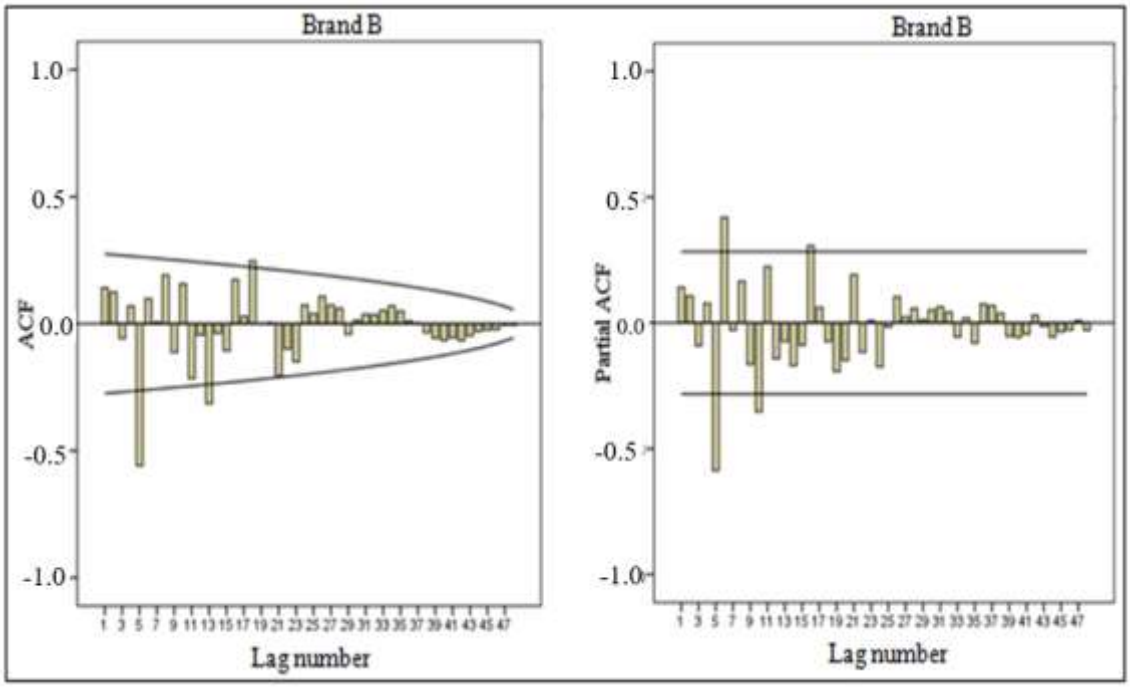

Figure 7- Difference-treated ACF and PACF graphs of brand B

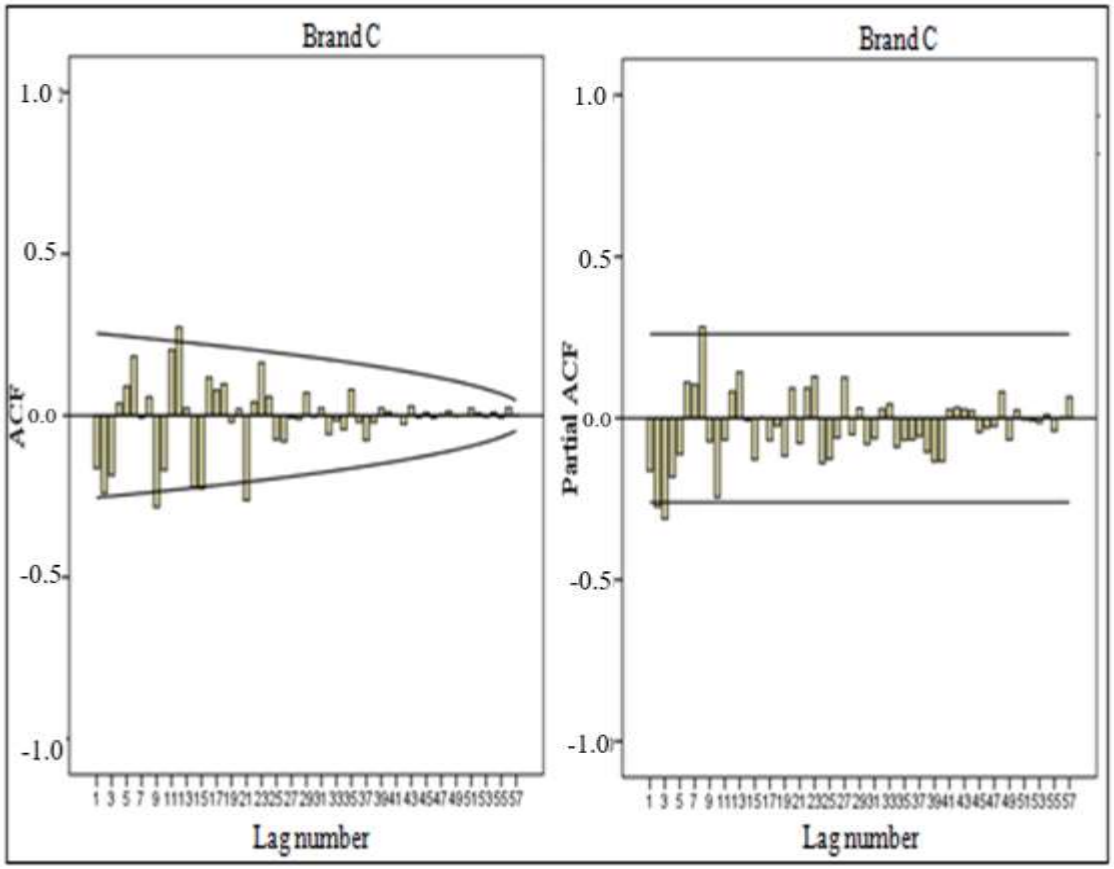

Figure 8- Difference-treated ACF and PACF graphs of brand C

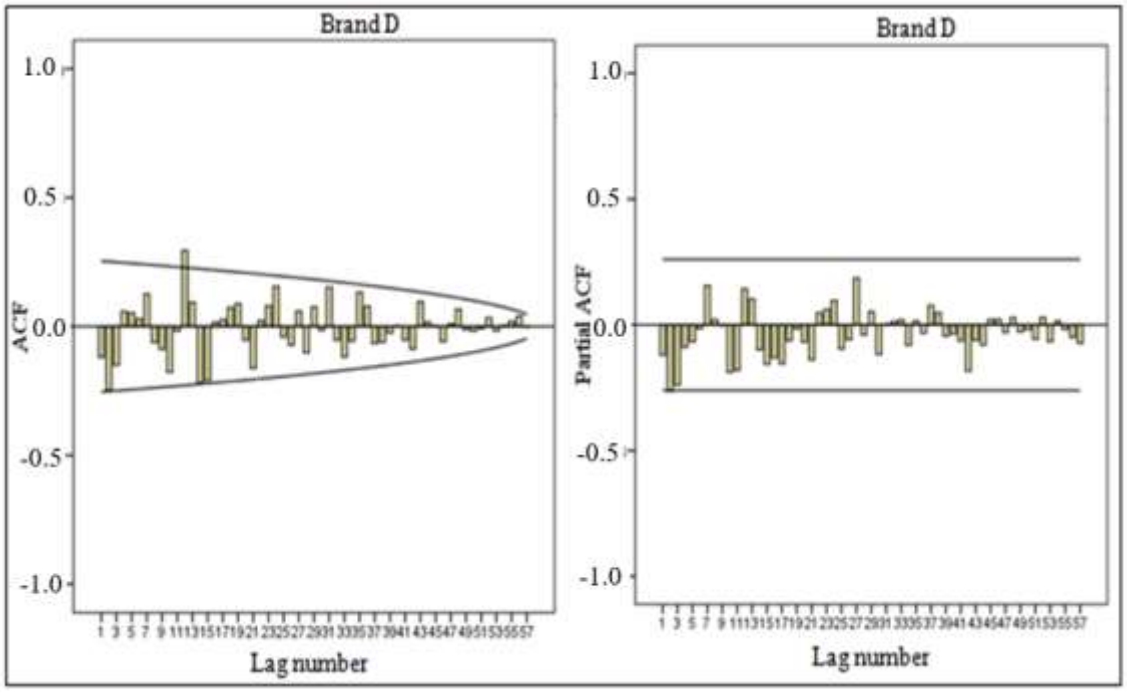

Figure 9- Difference-treated ACF and PACF graphs of brand D

The ADF test (Unit Root Test) was applied to determine whether the difference was stable or not (Berberoğlu 2010). In other words, it has been investigated by the extended Dickey Fuller (ADF) test whether the change in the number of tractor sales has a unit root. According to the results of Unit Root Test obtained in Tables 2, the ADF test statistic is greater than the critical value. From these results, it is concluded that the series is free of trend; in other words, it is understood that the series is not rooted in the unit. Also, the probability value (P) is less than 0.05 (P<0.05) and the series is stationary. Therefore, the series has been successfully transformed into a stable state with first order difference.

Table 2- Unit Root Test for brands A, B,C,D,E (ADF tests)

Augmented Dickey-Fuller Unit Root Test on D(A,B,C,D,E)				
Null Hypothesis:D (A, B,C,D,E) has a unit root Exogenous: Constant Lag Length: 0-4-2-0-0 (Automatic - based on SIC, maxlag=10)				
		Tractor Brands	*t-statistics*	*P -probability*
Augmented Dickey-Fuller Test Statistic		A	-9.474	<0.001
		B	-5.760	<0.001
		C	-7.504	<0.001
		D	-8.451	<0.001
		E	-4.061	0.002
Critical values	1%level	A	-3.548	<0.001
		B	-3.557	<0.001
		C	-3.552	<0.001
		D	-3.548	<0.001
		E	-3.546	0.002
	5%level	A	-2.912	<0.001
		B	-2.916	<0.001
		C	-2.914	<0.001
		D	-2.912	<0.001
		E	-2.911	0.002
	10%level	A	-2.594	<0.001
		B	-2.596	<0.001
		C	-2.595	<0.001
		D	-2.594	<0.001
		E	-2.593	0.002

Table 3, 4, 5,6 and 7 shows the ARIMA model parameters for the brand A-B-C-D-E. The degrees of AR and MA models obtained from the ACF and PACF graphs are entered into the statistic program (SPSS). And then, the model parameters are obtained with BIC values. According to the test results, Ho (absence hypothesis) is rejected because (sig <0.001) <(α = 0.05). Therefore, it can be said that the decision model is meaningful.

Table 3- ARIMA model parameters for brand A

Brand				*Estimate*	*SE*	*t*	*Sig.*
A-brand_1	A brand	Constant		0.138	2.431	0.057	0.955
		Difference		1			
		AR, Seasonal	Lag 1	-0.729	0.090	-8.114	<0.001
		Seasonal Difference		1			

Table 4- ARIMA model parameters for brand B

Brand				*Estimate*	*SE*	*t*	*Sig.*
B-brand_1	B brand	Constant		0.109	4.474	0.024	0.981
		Difference		1			
		AR, Seasonal	Lag 1	-0.321	0.130	-2.463	0.017
		Seasonal difference		1			

Table 5- ARIMA model parameters for brand C

Brand				*Estimate*	*SE*	*t*	*Sig.*
C-brand_1	C brand	Constant		-5.020	12.260	-0.409	0.684
		Difference		1			
		AR, Seasonal	Lag 1	-0.422	0.132	-3.200	0.002
		Seasonal difference		1			

Table 6- ARIMA model parameters for brand D

Brand				*Estimate*	*SE*	*t*	*Sig.*
D-brand_1	D brand	Constant		3.063	32.158	0.095	0.924
		Difference		1			
		AR, Seasonal	Lag 1	-0.566	0.111	-5.081	<0.001
		Seasonal difference		1			

Table 7- ARIMA model parameters for brand E

Brand				*Estimate*	*SE*	*t*	*Sig.*
E-brand_1	E brand	Constant		156.866	13.095	11.980	<0.001
		AR	Lag 1	0.544	0.114	4.783	<0.001

The comparison of the estimated tractor sale values for the tractor brands A, B, C, D and E and the actual tractor sale values are given in Figures 10, 11, 12, 13 and 14. When the graphs in the figures are considered, it is seen that the predicted values of the decided model for all tractor brands are in line with the actual values. This shows that the model was successful.

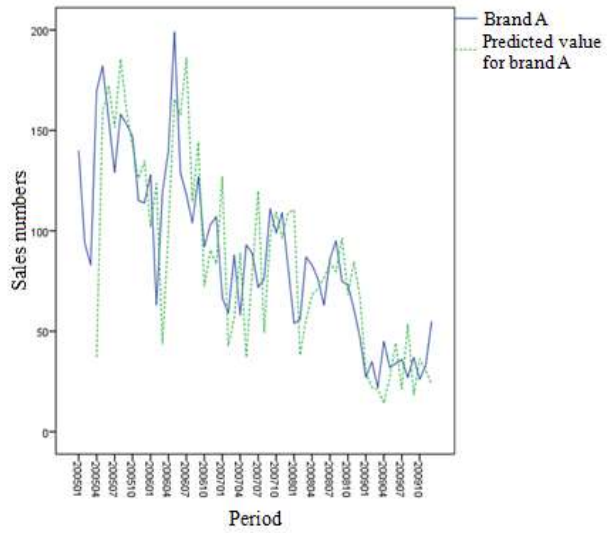

Figure 10- Comparison of actual values with prediction for tractor A brand

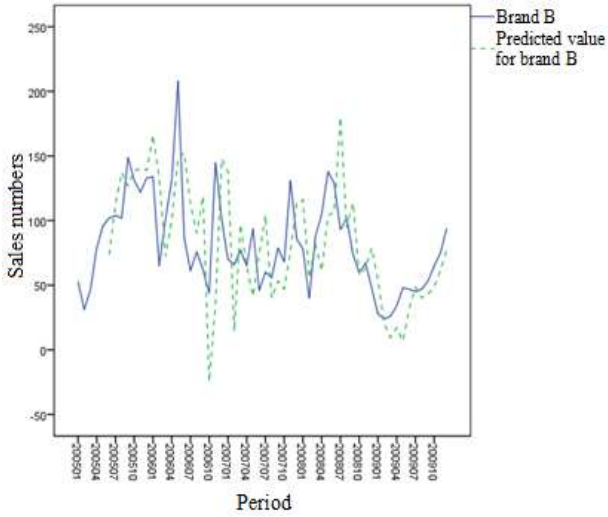

Figure 11- Comparison of actual values with prediction for tractor B brand

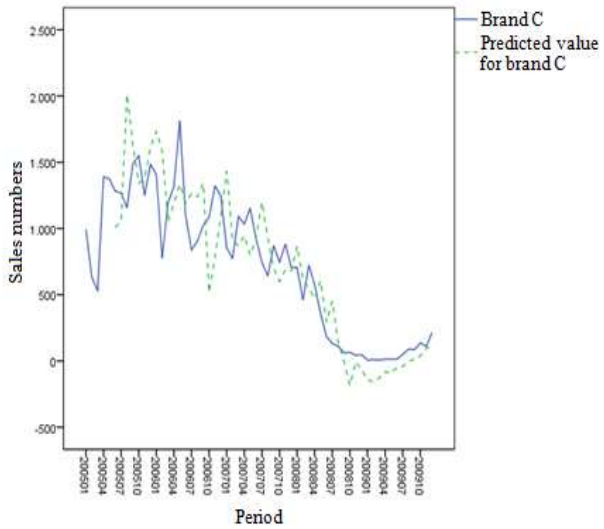

Figure 12- Comparison of actual values with prediction for tractor C brand

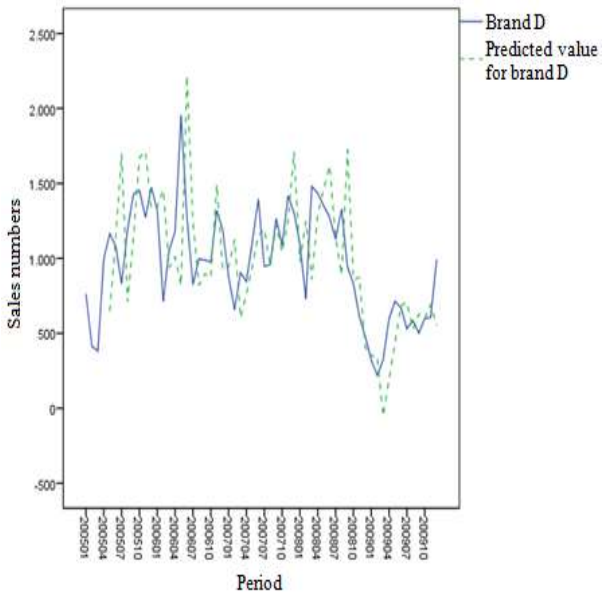

Figure 13- Comparison of actual values with prediction for tractor D brand

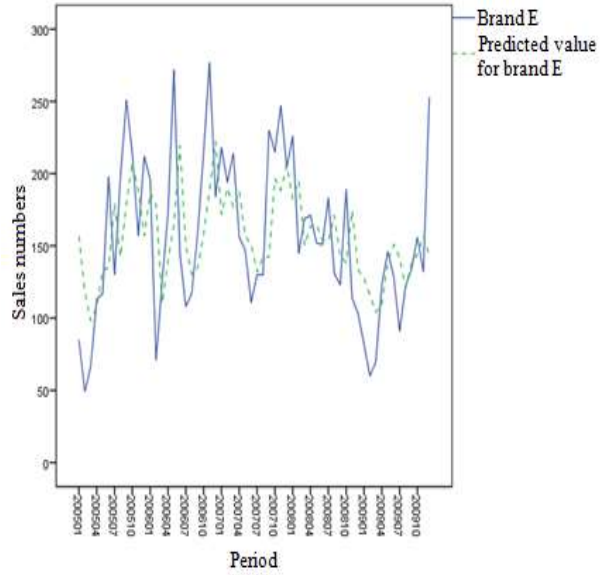

Figure 14- Comparison of actual values with prediction for tractor E brand

For A, B, C, D and E tractor brands, the actual value of the tractor sales and the estimated lower limits of the tractor sales of these brands and the comparison of the estimated upper limits are examined, it was seen that there was a harmony between the lower limit and upper limit values determined by the model and the actual values. With these results, it is understood that the decided model was successful.

Before going into the estimations, the model's errors should be white noise. To be white noise of the errors indicates that the model's estimates are reliable. The ACF and PACF graphs of the errors should be plotted to see if any errors of the model have occurred. These graphs are obtained from SPSS outputs after the model is decided. For the brands A-B-C-D-E the model's errors were determined white noise due to the fact that the majority of the errors are within the limits according to the ACF graph and PACF graphs (Figures 15,16,17,18, and 19).

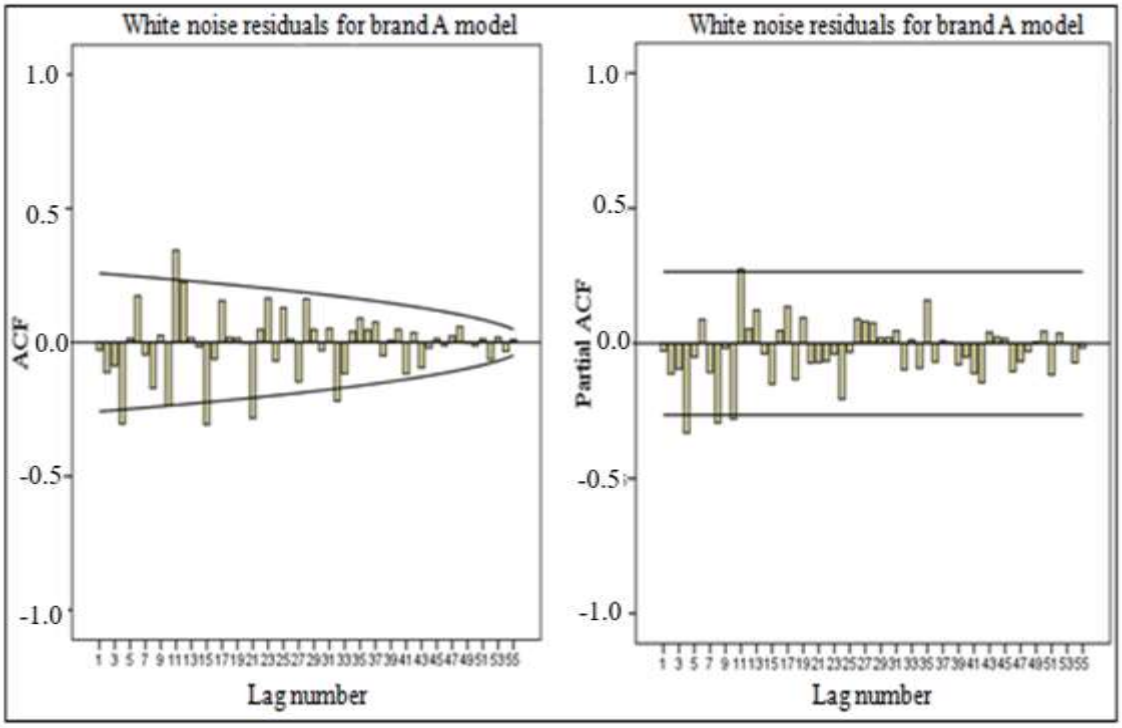

Figure 15- White noise ACF and PACF graph for A brand

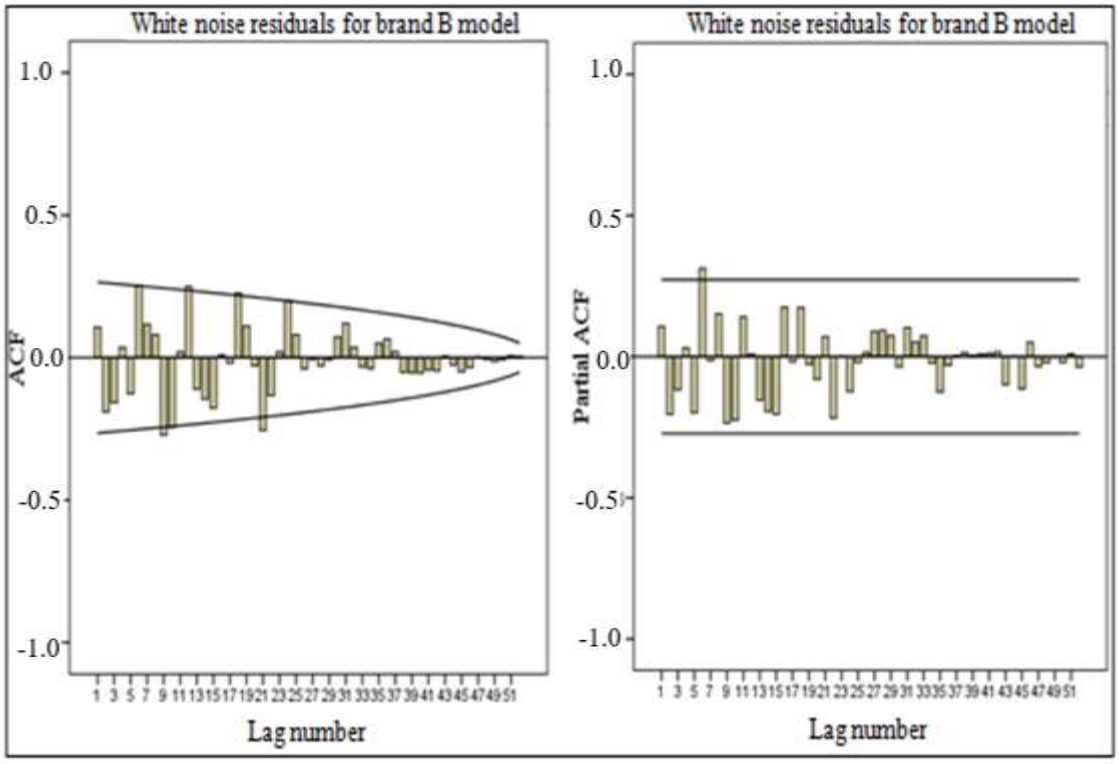

Figure 16- White noise ACF and PACF graph for B brand

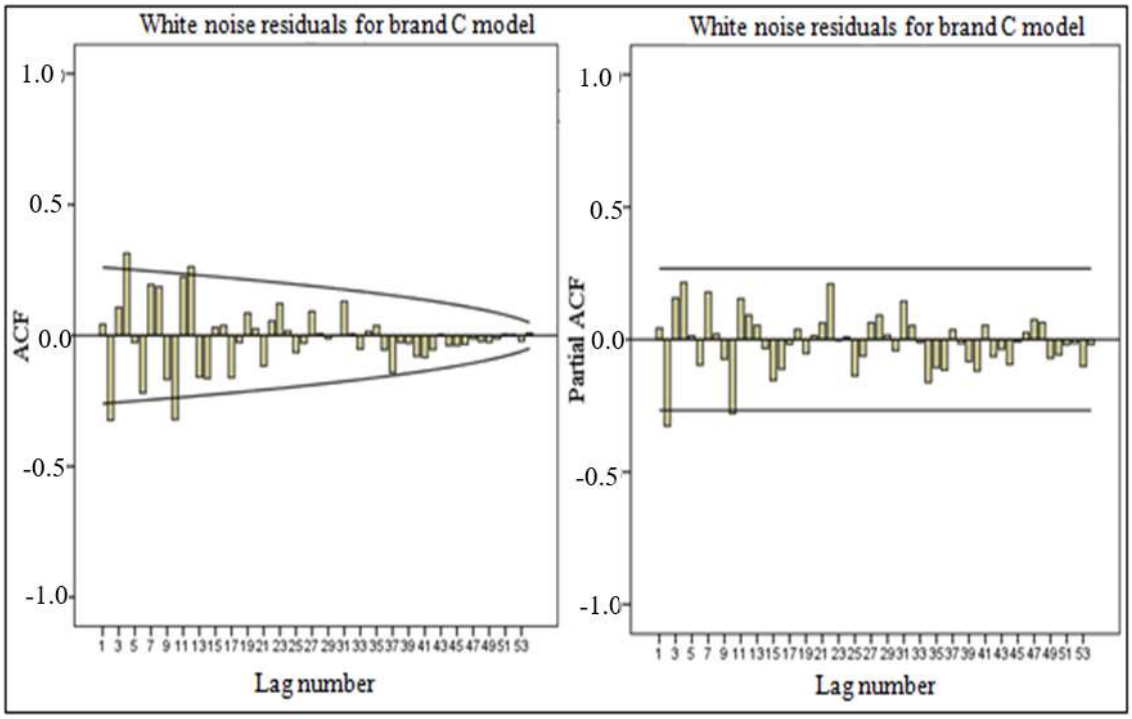

Figure 17- White noise ACF and PACF graph for C brand

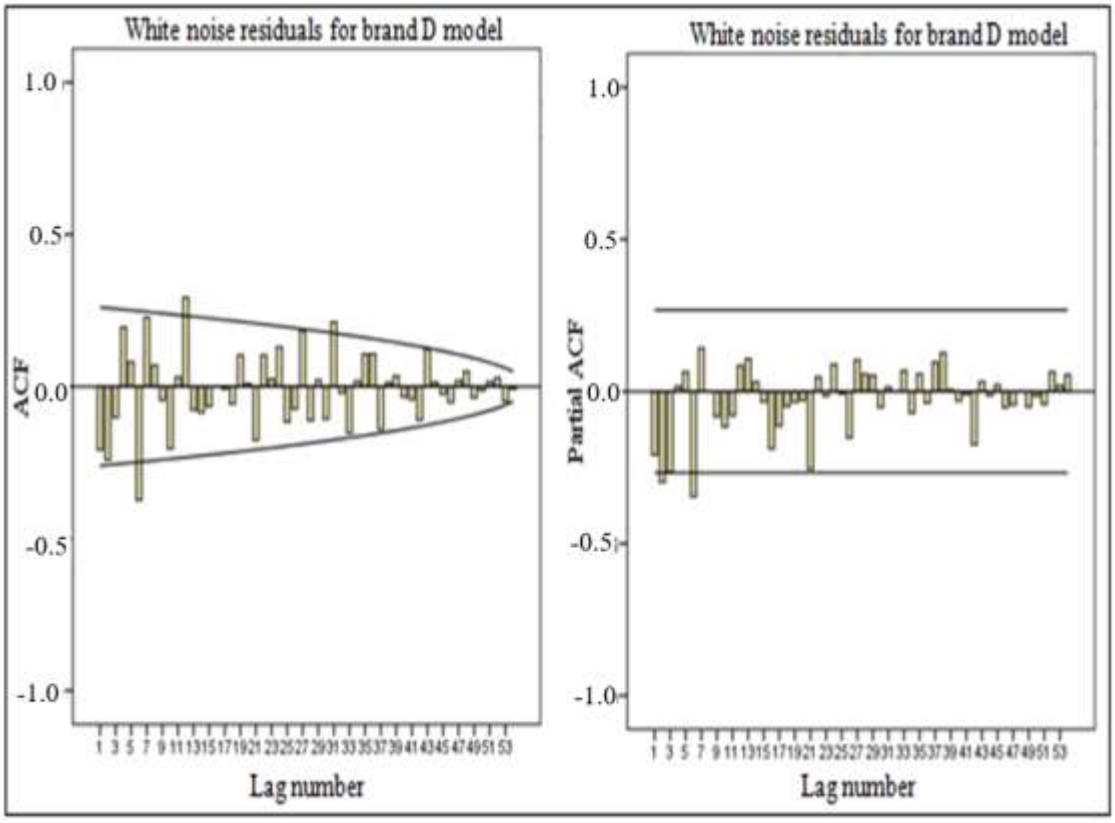

Figure 18- White noise ACF and PACF graph for D brand

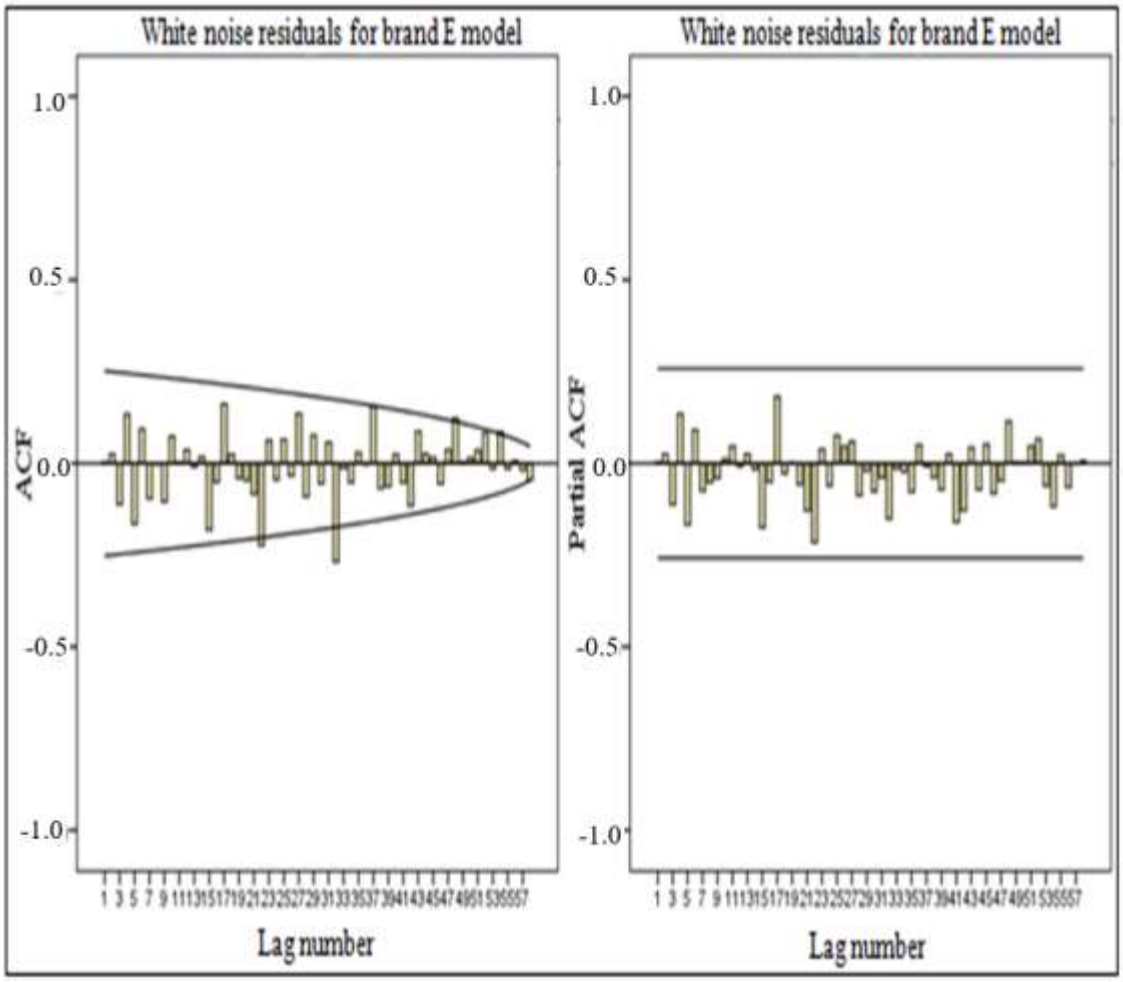

Figure 19- White noise ACF and PACF graph for E brand

After deciding on the most suitable model, the model is entered into SPSS program. Parameter values, error values and estimation values that we want them to be found during model inputs are marked and outputs are obtained in this way. As a result of the outputs obtained, the estimated values for the A, B, C, D and E tractor brands for 2010 are given in Table 8.

Table 8- Estimation values for tractors for 2010

Months	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Brand A	64	63	71	86	96	99	107	120	129	135	144	155
Brand B	65	46	61	65	64	41	57	59	62	38	54	56
Brand C	150	173	165	235	189	218	194	271	210	229	205	272
Brand D	961	1000	1125	1171	1198	1475	1481	1520	1716	1750	1786	2034
Brand E	209	185	172	165	161	159	158	158	157	157	157	157

3.2. Comparison of actual values and estimation values determined by model

Table 9 shows the average annual sales of five tractor brands for the years 2005-2009. When the table is analyzed, it is seen that the brand D (4831 units) with the highest sales averages between 2005 and 2009 and the brand with the lowest sales average is B (406). Annual average sales values were 445 units in A brand, 3589 units in brand C and 782 units in brand E. The brands A, B and E maintained their sales lines, while the C and D brands reached their maximum sales figures in 2006, and the C brand 2009 and the D brand suffered serious sales losses in 2009.

Table 9- Annual average sales volume of five tractor brands for 2005-2009 (TUİK 2010)

Year	*A*	*B*	*C*	*D*	*E*
2005	137	96	1200	1037	149
2006	119	101	1169	1149	170
2007	84	75	868	1064	183
2008	71	85	289	1056	155
2009	34	49	63	555	125
Total	445	406	3589	4861	782

Figure 20 shows the graph drawn by taking advantage of the monthly sales figures of 2005-2009 for five tractor brands on a monthly basis. In the graph, it is seen that the brands C and D, where A, B, and E brands maintain their stable structures, have a variable structure on a monthly basis.

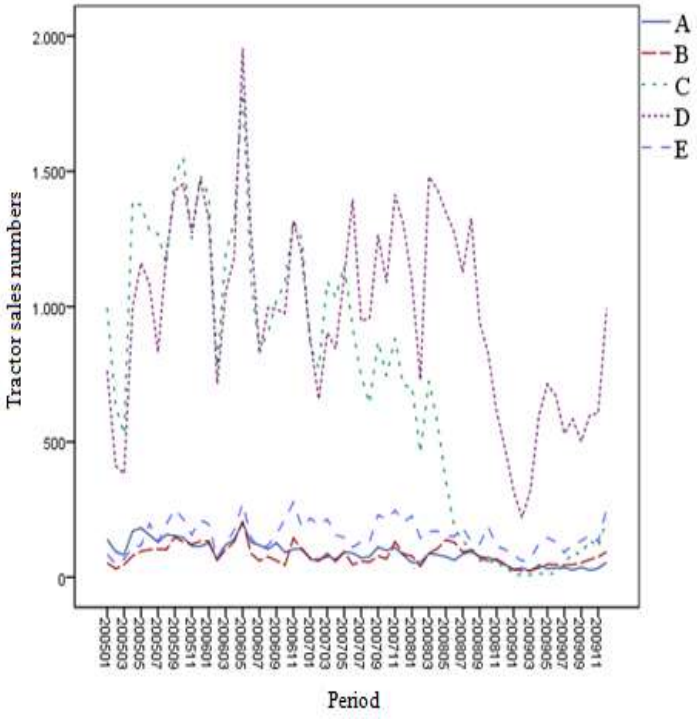

Figure 20- Sales figures distribution of five brands by months

The comparison of the actual sales data of 5 different tractor brands for 2005-2009 years and the estimation values for the same period as determined by the model established by the time series analysis method are given in Table 10. Table 10 shows the% deviation between the actual values and the estimated values. Percentage deviation values, minimum difference, and maximum difference values were found.

Table 10- Approximate deviation between actual values and estimated values

2005-2009	*A*	*B*	*C*	*D*	*E*
Average of actual values	89	81	718	972	156
Average of difference values	16	19	170	162	36
% Deviation	%18	%24	%24	%17	%23
Maximum difference	57	62	743	615	110
Minimum difference	0	0	4	1	1

A similar study was conducted for the total number of tractors belonging to the period of 1991-2009. The total number of tractors estimated by time series analysis was obtained and these estimation values were compared with the actual total tractor numbers. The result of the established model; Estimated values were found to be very close to the actual values,

and according to the difference between the average real and predicted values of the years 1991-2009, the difference between the real and predicted values was found to be 3%. This situation is shown in Table 11.

Table 11- The comparison of the actual values and estimated values of the total number of tractors in the period 1991-2009

Period (year)	*Actual total number of tractors (unit)*	*Estimated total number of tractors (unit)*	*Difference values (unit)*
1991	704373	669702	34671
1992	725933	686662	39271
1993	746283	704925	41358
1994	757505	765769	8264
1995	776863	787366	10503
1996	807303	805788	1515
1997	874995	815051	59944
1998	902513	830220	72293
1999	924471	865054	59417
2000	941835	970093	28258
2001	948416	1000804	52388
2002	970083	1013161	43078
2003	997620	987588	10032
2004	1009065	976386	32679
2005	1022365	995126	27239
2006	1037383	1027484	9899
2007	1056128	1041449	14679
2008	1070746	1046317	24429
2009	1073538	1049325	24213
Average	*913022*	*896751*	*16271*
Deviation (%)	*3*		

4. Conclusions

Based on our study, the tractor with the highest average sales volume is D brand, followed by the C brand. At the same time, a large amount of change is observed in the sales volumes of these brands. The high amount of change in C and D brands caused the maximum difference between the estimation and the real value to be high. However, the deviations of our estimation values for brands are acceptable. For the period 2005-2009, the% deviation values obtained between the actual values and the estimated values; it is calculated as 18% for brand A, 24% for brand B, 24% for brand C, 17% for brand D and 23% for brand E. In addition, time series analysis Box-Jenkins method was applied in a similar study and the suitability of the method and model was tried to be demonstrated. Estimation values for the same period have been obtained by using the total number of tractors belonging to the period of 1991-2009. In the comparison, it was observed that the estimation values for the period of 1991-2009 were quite close to the real values with a deviation of 3%. Time series analysis methods can be used as a guide for the decision makers or companies that want to break into many market sectors by providing information on market and sales forecasting. For public institutions or private enterprises, it is very important to make predictions and plan accordingly for the future. This tool can act as guide in certain areas, such as production planning, stock planning and sales planning. Based on this study, it is determined that the estimation method using Box-Jenkins method from time series analysis method can be used to make tractor sales forecasts. This study concludes that tractor companies can use time series method in their future planning to forecast their sales.

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Rangeland Condition and the Appropriate Rangeland Management Methods

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ABSTRACT
Improvement, development and proper exploitation of current rangelands conditions and selecting a range management method (balanced, natural and, artificial) to be implemented in the form of a range management plan (RMP). Data was collected from the rangelands of Haraz River watershed. Information including the total percentage of vegetation and each of the increasing species, rangeland condition and production were studied in 2016. To determine the best range management method, the analysis of variance was used. To compare the quantitative characteristics measured before and after the implementation, a two-sample independent t-test was used. Mann-Whitney U test was used to compare qualitative characteristics. Results showed that the vegetation percentage of palatable species composition did not increase, the balanced and natural methods were the best methods in good and fair rangeland condition. The reason for the lack of increase in the number of palatable species was the unsuccessful implementation of planned programs in RMPs. The artificial method was performed in the RMPs with poor vegetation types, the implementation of these plans had no significant effect on the rangeland condition of the study rangelands due to the high livestock population. Rangeland plans will be more effective when there is a balance between grazing capacity and livestock population.

Keywords: Rangeland condition, Rangeland, management methods, Rangeland trend

1. Introduction

In the middle of the last century, due to the socio-economic problems of beneficiaries, the exploitation, and management of rangelands have changed a lot. In the past, there were no problems in the rangeland management due to the conventional rules and the appropriate proportion of the number of beneficiaries and the number of livestock. However, nowadays, due to the management difficulties of rangelands, a large number of beneficiaries and high population of livestock and ranchers with poor economic status, the investment and rangeland development have been challenged (Kamrani 2004). Therefore, in the current situation, the most basic steps to prevent rangeland degradation and improve the rangeland condition are the provision and utilization of range management plans in the form of a compiled program.

In the last few decades, hundreds of rangeland management projects have been developed and implemented with the aim of developing basic utilization. These projects have played a positive role in improving rangeland conditions (Arzani et al 2011).

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Safari et al (2016) believe that by selecting the correct range management method and appropriate rangeland and improvement practices, the rangeland condition, livestock grazing management, watershed condition, and wildlife habitat can be improved.

Borhani et al (2014), through the technical and ecological evaluations of range management plans in Isfahan province, showed that the implementation of range management plans greatly influenced the increase of desirable species. This also improved the rangeland condition and reduced the uniformity of the rangelands, where RMPs were implemented. However, in some range management plans, the grazing capacity, time, and duration of range utilization are not well-respected. The reason is that these are not cost-effective (Arzani et al 2011). However, the capacity and capability of rangeland production are significantly increased in the projects where technical principles are well observed. Savory (1987), Walker (1993), and Teague & Dowhower (2002) argued that if the utilization of rangelands is designed and planned, the exploitation will be more sustainable.

There are three methods for range management, including Balanced, Natural, and Artificial methods, which can be chosen in the range management plan based on vegetation community condition. Balanced is used when the range is good and the land is covered with grass. However, the amount of plants needs to have high palatability, and the duration of growth and reproduction should be good. The objective of the balanced policy is to maintain the current desired condition of rangelands that are in good condition. Natural is used when the condition of the range is not so good and the plants have a little palatability, but there is hope for their reproduction and growth, and it is enough to rest them. In the natural policy, the objective is to improve vegetation condition by providing enough time for natural regeneration. This policy is used in the rangelands where the condition is fair and the vegetation cover, regeneration, and palatable species composition in the total vegetation cover are low. Artificial is used when none of the first and second modes are available, the erodible rangelands, the palatable plants are very low or absent, the range is weak, and there is no natural regeneration or the recovery requires a long time. Management in poor rangelands is seeking improvement and reclamation of vegetation using the artificial policy and implementing methods such as seeding, pit seeding and etc. (Arzani et al 2011).

Since rangeland beneficiaries, especially in private rangelands are thinking of exploiting more in the short-term, they are ignoring the range grazing capacity. Therefore, the range management principles such as the balance of livestock in these rangelands are less observed. Researchers such as Hardin (1998), Qandali (2001), and Antje (2004), have also this issue. Continuing this trend will result in reduced rangeland production, forage quality and livestock production (Houston & Woodward 1966, Launchbaugh 1967; Shoop & Mcllvain 1971). This problem can be solved by selecting the right grazing system and applying the appropriate rangeland management method under different rangeland conditions, as it both causes the proper distribution of livestock in the rangeland and improves the range trend. Among these methods, the artificial policy could be conducted only in case of government funding, since it is associated with the implementation of improved practices and cost spending.

Since the government intervention in improving the rangeland condition is common in Iran, among these methods, the artificial method, which is accompanied with costly reclamation and improvement practices, could be implemented in the low-area rangelands in case of government financial assistance. Therefore, not much attention is paid to this method by the rangeland beneficiaries. (Arzani et al 2011). It is clear that the range management costs vary depending on the range condition. Regardless of this fact, sometimes the experts make a mistake in preparing the range management plans and selecting the right range management method. Due to the little data published on this issue, the present study was carried out to investigate the role of rangeland condition in selecting the best rangeland management method in the form of a range management plan in a vegetation community. It should be noted that this is the first study in the rangelands of the central Alborz region with a fixed and mobile rural livestock and a dominant combination of sheep and goats (85% sheep).

2. Material and Methods

2.1. Study site

The research was conducted in the Gazanak rangelands with an area of 18416 hectares in the south of Amol city and in the Larijan district, north of Iran. It is located between 59˚ 45' 90" and 61˚ 54' 50" eastern longitudes and 39˚ 63' 900" and 39˚ 25' 800" northern latitudes, including nine range allotments with rangeland management plans. These plans had been started in the 1990s. The average rainfall of the region is 602.3 mm annually, with the highest and lowest rainfall of 89.9 mm in March, and 12.6 mm in August. The species are mostly and belong to the *Poaceae* family. The dominant species are *Festuca ovina, Bromus tomentellus,* and *Onobrychis cornuta*.

2.2. Measurements of range management plans

Out of the range management plans implemented in the 1990s, three plans of each range management type and a total of nine plans were randomly selected. The characteristics of each plan included the range management method for livestock grazing (the rangeland type, number of livestock and proposed grazing system), vegetation percentage, production, and rangeland condition and trend, recorded in Excel software (Table 1). The required number of plots was calculated based on Eq.$N = \frac{t^2 S^2}{P^2 \bar{x}^2}(1 + 2/n)$ (Mesdaghi 2007). Therefore, 80 plots of 1 square meter (totally 240 plots for all three methods) were applied in each of the areas that Rangeland management methods were implemented. Systematic random sampling was used to establish the plots along four 50-meter transects. The vegetation characteristics were then re-measured as the percentage of total canopy cover, vegetation percentage, and composition of important rangeland species (desirable and undesirable to the grazing livestock) in 2016. Clipping and weighing method was used to measure rangeland production. In order to determine the rangeland condition in each vegetation community, the 4-factor method suggested by Parker & Harris (1951) was used. To compare quantitative characteristics, the two-sample independent t-test was used at significant levels of 1% and 5%. The Mann-Whitney U test was used in order to compare qualitative characteristics.

Table 1- Comparison of the range condition and range trend in 1990 and 2016

Range allotment	*Rangeland type*	*Proposed grazing system in 1991*	*The start year of RMP*	*Rangeland condition in 1991*	*Rangeland trend in 1991*	*Rangeland condition in 2016*	*Rangeland trend in 2016*	*Animal Unit (AU)*
Rinekoh	Br.to- Fe.ov-As.sp	Rotation	1997	good	positive	good	positive	3579
Raiskoh	Fe.ov-Br.to-On.co	Delayed rotation	1999	fair	constant	fair	constant	2897
Malarkoh	Fe.ov-Br.to-On.co	Rotation	2005	good	positive	good	positive	1120
Gazanak	Fe.ov-Br.to-On.co	Delayed rotation	1993	fair	constant	poor	constant	1295
Asklat	Fe.ov-Br.to	Rotation	2005	good	positive	good	positive	1839
Anji	Fe.ov-As.sp-Ar.au	Rest rotation	1999	fair	constant	fair	constant	2090
Amlaira	Fe.ov-Br.to-On.co	Delayed rotation	1997	fair	constant	fair	constant	237
Abgarm	Fe.ov-Br.to-On.co	Rest rotation	1997	good	positive	good	positive	909
Ghazimaz raeask	Fe.ov-Br.to-On.co	Rotation	2009	good	positive	fair	constant	2035

3. Results and Discussion

3.1. Measurements of range management plans

Comparison of differences in the mean values of canopy cover and production in various range management policies showed a difference between the various management policies for these variables (Figures 1 and 2). In other words, the implementation of Rangeland management methods is effective on the percentage of canopy cover and rangeland production, and consequently, on the rangeland condition. The rangelands selected for the implementation of balanced rangeland management method had a good rangeland condition, the highest mean production (390.06 kg ha^{-1} Dry Forage), and the highest mean canopy cover percentage (71.64%). However, the lowest mean production (345. 67 kg ha^{-1} Dry Forage) and the lowest mean percentage of canopy cover (50.16%) were obtained for the poor condition rangelands, where the artificial method was implemented.

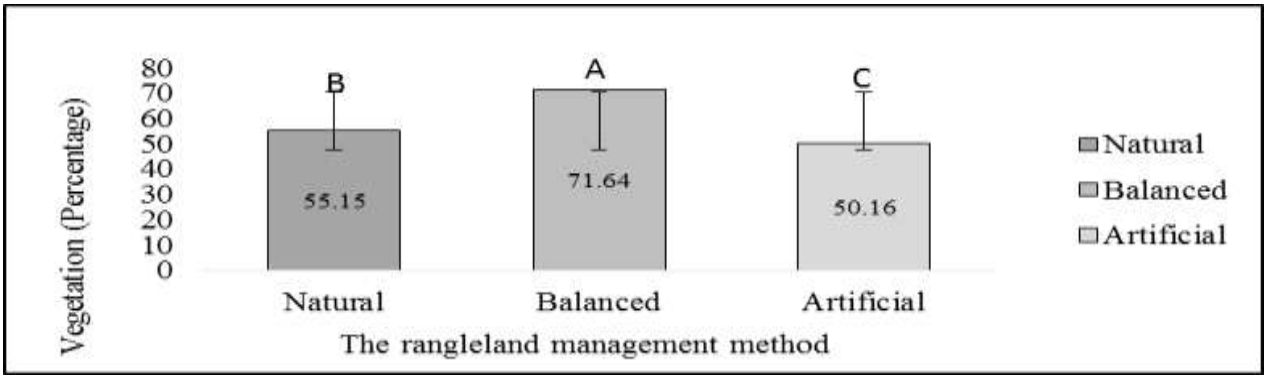

Figure 1- Vegetation percentage of each rangeland management method (2016)

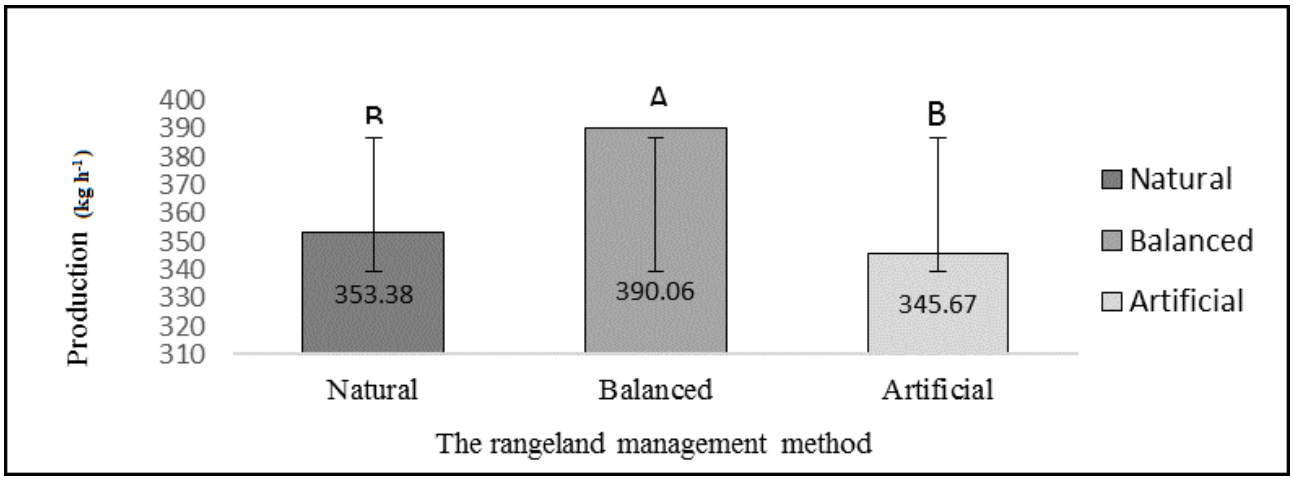

Figure 2 - Production of each rangeland management method at the time of the study (2016)

3.2. The study of changes in rangeland condition and trend

The study of vegetation percentage analysis showed that the balanced policy with an average of 71.64% was the best rangeland management method for the good rangeland condition in the study area. Moreover, the results of production proved that the artificial and natural policies with an average production of 345.67 and 353.38 kg ha^{-1} Dry Forage had no significant difference, and the balanced policy was determined as the best. The rangelands selected for the implementation of balanced range management- that were in more than a good condition- had the highest mean production (384.3 and 448.2 kg ha^{-1} Dry Forage) and that of the artificial range management had the lowest average production (257.6 and 317.3 kg ha^{1} Dry Forage). In addition, the assessment of the significance of condition changes (very good, good, fair, poor, and very poor) and condition trend (positive, negative and constant) was conducted in 2016 using the non-parametric Mann-Whitney U test (Zare Chahuki, 2012). These studies showed that changes in the rangeland condition and trend between the two assessment periods were not significant in any of the Rangeland management methods.

The vegetation composition in terms of percentage of palatable and increasing species was investigated and compared. This showed that the palatable species like alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.), and sheep fescue L. (*Festuca ovina*) did not increase compared to the year of preparation of range management plan (the 70s). However, the increasing species, such as bulbous meadow grass (*Poa bulbosa*) and common mullein (*Verbascum thapsus*) were increased.

The study of changes in plant composition and production of desirable species in the 1990s and 2016 showed that a significant difference was found between these values as well as among the range management policies. In other words, implementing the range management plans showed a positive effect on the production of rangeland species. Arzani et al (2011) conducted a study on the effects of rangeland management plans on the condition and capacity of rangelands in

both arid and semi-arid regions. According to the final report of the study, the effectiveness of range management plans could be increased by economizing rangelands' area, prioritizing the private rangelands when planning RMPs, proper selection of measuring methods, determining the capacity, controlling livestock, and paying attention to the socio-economic issues. The changes in rangeland condition and trend were insignificant in all the rangeland management policies in 2016. Furthermore, no positive changes were observed in the degree of rangeland condition and/ or trend, as a result of the implementation of the rangeland management plan. This suggests that the mere planning is not enough, but monitoring, controlling the number of livestock, and executing the program are also very important. In this regard, Arzani (1994) and Valentine (2001) believe that determining a regional production index and ultimately long-term grazing capacity, as well as the correct selection of livestock number, are the most important options to improve the vegetation, livestock production, and economic returns. They also concluded that the sustainable utilization of rangelands requires a management program, correct estimation of grazing capacity, and a balance between the number of livestock and rangeland production.

It should be noted that the results obtained from this research are inconsistent with the results of Mazaheri et al (2009) conducted in the Khorasan Razavi Province, Eftekhari et al (2016) in Markazi Province, and Ariapoor et al (2016) in the Hamedan Province. All the studies showed that the implementation of rangeland management plans had an effect on increasing the vegetation cover, production, and improvement of vegetation composition in favor of palatable species. The main reasons for the differences between the findings of this research and the results of the above-mentioned researchers are the lack of adequate supervision, lack of credit and facilities, and finally the lack of enough knowledge of rangeland beneficiaries about the benefits of implementing range management plans. A study was conducted on the changes in the percentage of vegetation of palatable (Class I) and increasing species after implementation of rangeland management plans. It showed that species such as *Medicago sativa* (alfalfa), *Trifolium repens* (clover), and *Festuca ovina* (grass) had no significant change as a result of the implementation of natural and balanced rangeland management plans. However, the rangeland beneficiaries tried to increase the percentage of palatable species using rangeland improvement methods such as water resources improvement and development (installation of watering place, spring repair and water transfer) as well as seeding projects with species of *Medicago sativa*, *Trifolium repens*, and *Poa bulbosa*. The implementation of these practices is not only costly but also requires a lot of time to achieve the results. While the rangeland beneficiaries with the guidance of natural resources experts could achieve this goal appropriately by applying the grazing systems and appropriate Rangeland management methods at the least cost. Thus, it is important to choose the correct management method in each vegetation community. In practice, the vegetation percentage of *Poa bulbosa* increased by 49.43%, but alfalfa (59.6%) and clover (58%) were significantly reduced. Undoubtedly, the reason is the proper establishment of *Poa bulbosa* seeds and livestock preference in the rangelands of the region to graze on alfalfa and clover. In contrast, as a result of the implementation of natural and balanced rangeland management methods, the two increasing species of the Alborz summer rangelands, *Poa bulbosa*, and *Verbascum thapsus*, had no significant change; however, the implementation of artificial policy in 2016 resulted in a significant increase for the two species, as compared to the 1990s. Therefore, the percentage of vegetation was significantly increased in *Festuca ovina* (73.25%) and *V. thapsus* (45.76%). The reason for this is related to the excessive livestock grazing, the lack of proper implementation of livestock grazing management, and the lack of complete implementation of reclamation practices due to the lack of funds and facilities, which have weakened the rangelands. In addition, in the natural rangeland management method, *Festuca* and Alfalfa increased by 6.8% and 5%, respectively. The vegetation percentage of white clover was reduced by 20%, with no significant change. These insignificant changes in the invasive species were associated with a reduction in the vegetation percentage of *Festuca* and *Verbascum thapsus*. In the balanced rangeland management method, the canopy of *Festuca* and Alfalfa increased by 8.74% and decreased by 8.13%, respectively, and the vegetation percentage of white clover reduced by 15.21% with no significant change. No significant changes were observed in the invasive species studied except for a slight reduction in *Festuca* species and an increase in *Verbascum thapsus* (Table 2). These changes occur due to increased grazing pressure. However, considering the good and fair rangeland condition of the study area, the selection of artificial rangeland management method imposes many costs, such as the supply and purchase of inputs, building materials, and the labor and expertise cost in implementing improvement and reclamation projects (seeding, construction of water storage, water reservoir and so on). In other words, the artificial rangeland management method only affected the total vegetation percentage without increasing the percentage of desirable species. It is in conflict with the main purpose of Rangeland management methods, which is to increase the percentage of palatable and desirable species for livestock. In contrast, the balanced range management method as the first option and then the natural range management method regardless of the fact that there are far fewer administrative costs, have better performance and can be easily performed as compared to the artificial policy. Therefore, the rationale is that the cost-effective, simple and quick policies are implemented rather than costly and complex management methods. The results of this study are consistent with the findings of Danckworts & Madam (1991), Holecheck (2002), Hoshino et al (2009), and Moradi and Mofidi (2012). They

showed that the increased grazing pressure caused the reduction of palatable species and an increase in toxic and unpalatable species.

Table 2- The average percentage of palatable and increasing species in 1990 and 2016

The average percentage of vegetation									
Species	*Natural policy*			*Balanced policy*			*Artificial policy*		
	1990	*2016*	*percentage*	*1990*	*2016*	*percentage*	*1990*	*2016*	*percentage*
Medicago sativa	6	6.3	5	8.6	7.9	-8.13	2.5	1.01	-59.6
Trifolium repens	4	3.2	-20	4.6	3.9	-15.21	1.5	0.63	-58
Festuca ovina	20	21.36	6.8	20.69	22.5	8.74	8.03	12	49.43
Poa bulbosa	2.6	2	-23.07	0.5	0.45	-10	4	6.93**	73.25
Verbascum thapsus	1.2	10.01	-15.83	0.58	0.69	18.9	2.6	3.79**	45.76

**, The Significant difference at the 1% level

Generally, in the rangelands, where the grazing capacity is taken into consideration and rangeland management plans are carefully implemented with the participation of beneficiaries, the quantity and quality of the plant composition will undoubtedly increase. The effectiveness of rangeland management policies on vegetation factors in the rangelands where the balanced and natural methods are applied indicates that the positive or negative changes in vegetation are not sufficient to change the degree of rangeland condition, and these rangelands mostly have a constant trend. Therefore, it is necessary to increase the effectiveness of the rangeland management plans by more supervision, increasing the income of beneficiaries by multipurpose utilization, the balance between livestock and long-term grazing capacity, and applying grazing systems. The balanced and natural rangeland management policies have a positive and significant effect on the total vegetation in the rangelands with a good and fair condition. However, it did not lead to an increased degree of rangeland condition or a significant increase in vegetation percentage and an increased percentage of palatable species composition. It should be noted that in the fair rangeland condition, the incorrect implementation of the artificial method has had no positive effect on the rangeland condition and trend. It means that despite the increased vegetation percentage of increasing species, the vegetation percentage and production of these rangelands decreased compare to the time of preparation the RMP. Failure to correctly execute programs and the lack of attention to the livestock stocking rate and its related variables as well as collective rangelands and failure to observe the grazing season by beneficiaries are the main reasons for the weakening of these rangelands. It seems that the effectiveness of rangeland management plans will be more when the plans are implemented on rangelands where there is a balanced livestock grazing (Tawafi & Arzani 2012; Gillen & Sims 2006). Therefore, the most important management action in the rangelands of the region is the choice of balanced rangeland management method, since it is easier, more cost-effective, and more feasible. Among nine range allotments studied in this research, the rangeland management method has not been selected correctly in five range allotments, so that in two range allotments, the natural policy was applied instead of the balanced policy. As well, in three range allotments whose rangeland condition was fair, regardless of climate conditions and topography and soil characteristics, the artificial policy was applied along with implementing the projects such as seeding and pit seeding, instead of the natural method within the proper grazing systems (Table 3). Due to the continuous presence of livestock and unsuccessful implementation of grazing management programs, the rangeland production continued to decline (Table 3). Overall, the selection of artificial rangeland method not only has contributed to increasing the amount of vegetation but also has imposed the costs of implementing reclamation and improvement practices on beneficiaries.

Table 3- Comparison of the range condition and range management method proposed during the 1970s and 2016

No	*Ranch Units*	*The rangeland condition in 1991*	*The rangeland management method*	*The rangeland condition in 2016*	*The proposed rangeland management method*
1	Rinekoh	good	Natural*	good	Balanced
2	Raiskoh	fair	Natural	fair	Natural
3	Malarkoh	good	Balanced	good	Balanced
4	Gazanak	fair	Artificial*	poor	Artificial
5	Asklat	good	Balanced	good	Balanced
6	Anji	fair	Artificial*	fair	Natural
7	Amlaira	fair	Artificial*	fair	Natural
8	Abgarm	good	Natural*	good	Balanced
9	Ghazimazraeask	good	Balanced	fair	Natural

4. Conclusions

Our results clearly showed that it is important to choose a suitable, practical, and not expensive range management method to utilize the rangelands and promote its condition. Rangeland condition is a good criterion to select a suitable policy. Where the rangeland condition is good or fair, the artificial policy is not recommended for range improvement. Considering that the implementation period of 85% of range management plans in the summer rangelands of the study area is finished (over) and needs to be revised, therefore, while observing ecological principles, beneficiaries' economic power and rangeland beneficiaries' views, the revision of range management plans should be put on the agenda by the custodians of natural resources. Accordingly, it is necessary to economize range management and, consequently, improve the livelihoods of beneficiaries through focusing on other rangeland uses including by-products, fattening, apiculture, tourism, and aquaculture in range management plans. Finally, it seems that another similar study in winter rangelands can provide interesting results regarding the effects of range management plans on the rangeland condition.

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