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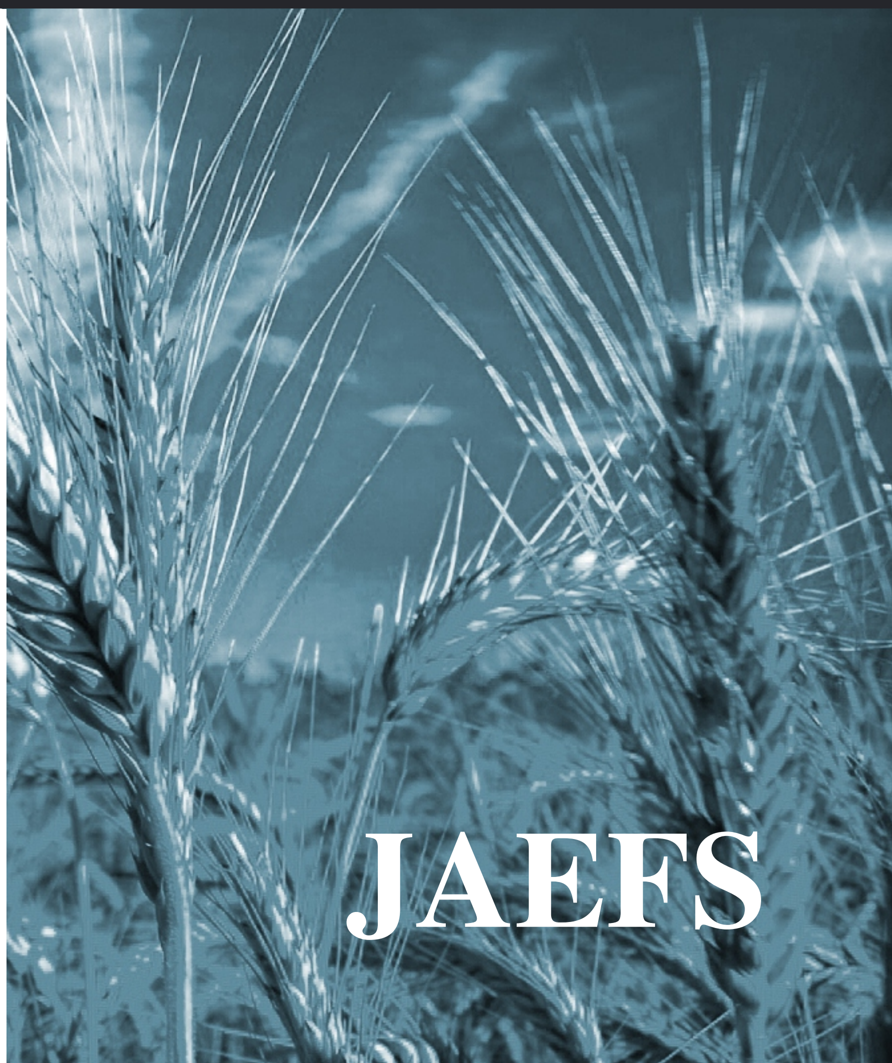

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Relationship between photosynthesis and fruit quality of ‘Clemenules clementine’ mandarin variety budded on various rootstocks

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Abstract

‘Clemenules’ (Nules, Clementina de Nules) has been a very popular variety in fresh mandarin markets especially in the Mediterranean region countries. It is commercially grown on sour orange rootstocks in the calcareous soils of Turkey. However, production of ‘Clemenules’ needs a substituted rootstock in addition to sour orange due to hypersensitive of sour orange to the “Citrus Tristeza Virus”. ‘Clemenules’ mandarin grafted onto ten rootstocks was evaluated in order to determine the influences of rootstocks on fruit yield, quality and photosynthetic variables of the scion as well as their relationship. Rootstocks significantly affected ($p<0.05$) fruit yield and using Volkameriana significantly increased fruit yield of ‘Clemenules’ (44.71 kg tree^{-1}). Similarly, sour orange and Volkameriana rootstocks positively affected fruit weight and height ($p<0.05$). Total acids (%) and ripening index varied within rootstocks and FA-517 resulted the highest total acids in fruits juice samples of ‘Clemenules’ whereas the lowest ripening index was determined in fruits grafted on Flhorag1. Leaf chlorophyll concentration (Chl) and leaf chlorophyll fluorescence in the light adapted stage (Fv'/Fm') of the scion differed based on rootstocks. In addition to fruit yield and characteristics, rootstocks also significantly affected variables related to photosynthesis. Cleopatra mandarin, sour orange and Volkameriana increased the photosynthetic rate (P_N), while transpiration rate (E), and stomatal conductance (g_S) of the scion were higher on Volkameriana rootstocks. FA-5 maintained the highest water use efficiency (WUE) in comparison to other rootstocks evaluated. The present research has clearly shown that rootstocks were able to influence the quality of fruits and the physiological activity. Regarding fruit yield and photosynthetic performance of ‘Clemenules’ mandarin variety, Volkameriana and sour orange performed well. However, considering the calcareous soils of the Mediterranean Region, FA-5 citrandarin proved to be potential rootstock for enhanced photosynthetic rate and WUE.

Keywords: Citrus, Fruit quality traits, Chlorophyll concentration, Net photosynthetic rate

Introduction

Mandarins are called ‘easy-peelers’ because of their sweet flavor and aroma, loose skins, relatively small fruit size among the edible citrus and are easy to peel and separate into segments (Demirkeser et al., 2009). Currently, mandarins remain the most consumed and demanded citrus species due to some important advantages, such as small fruit, thin skins and easy peeling in all over the world. Turkey has exceptionally reasonable environmental conditions and citrus-producing prospective, with 4.769.726 tons of citrus fruit produced in 2017. At present 65 percent of the export of fresh fruits in Turkey is citrus and the export of citrus fruit, particularly for mandarins, has increased considerably in recent years. Mandarins remain as one of the most the most popular citrus fruits in Turkey, accounting for approximately 30% of the total production (FAO, 2020).

The quality of fruit was always a major interest breeders, producers and consumers which can be manipulated by the us-

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age of rootstocks (Castle, 1995). In addition, rootstocks positively affect scion growth, fruit internal/external quality and fruit yield (Castle et al., 2009; Forner-Giner et al., 2003a), along with photosynthesis (González-Mas et al., 2009). Amongst other physiological progressions, photosynthesis (P_N) is one of the elementary factors of plant productivity and the ability to sustain the carbon assimilation rate under environmental stress (Lawlor, 1995).

Sour orange is commonly preferred as a rootstock for many citrus varieties in Turkey. While sour orange offers many excellent horticultural advantages, its susceptibility to Citrus Tristeza Virus (CTV). In many areas, particularly the western Mediterranean basin, CTV has significantly reduced the use of this rootstock. Sour orange is a good rootstock in areas not subjected to CTV. On the other hand, the CTV-induced problems have destroyed or degraded more than 80 million trees grafted into sour orange rootstock. Citrus breeders are seeking new rootstock genotypes until 1900's. Some of the important targets of citrus rootstock breeding are tolerance/resistance to CTV, alkalinity, cold weather, and positive effect on high fruit yield and quality. So far, Carrizo citrange, Troyer citrange, Swingle citrumelo, C-35 citrange, C-32 citrange obtained by hybridization method and all of them using in citrus production areas successfully (Castle, 1995; Castle et al., 2009; Cimen and Yesiloglu, 2016). Furthermore, two new rootstocks published in Spain have been recorded in Forner et al. (2003b). The two interspecific hybrids that are CTV and have been documented as more tolerant to lime-induced iron (Fe) chlorosis than Carrrizo citrange. Gonzalez-Mas et al. (2009) also carried out a field rootstock experiment in the calcareous soil in order to explore the effects of rootstock on the leaf photosynthesis of 'Navelina' navel and recorded the best performances of the trees grafted on FA-5 under calcareous soils.

Although sour orange (*Citrus aurantium* L.) is still a popular rootstock in the Mediterranean region of Turkey, producers have begun to prefer using citranges (Carrizo and C-35 citranges) in most recently established mandarin orchards due to their positive effects on fruit quality. Sour orange and citranges have generally been satisfactory and, thus, there are limited rootstock studies involving mandarin varieties. In Mediterranean basin, producers still try to find a good rootstock not only tolerant to alkalinity, but also tolerant to CTV.

Regarding these matters, the present study was carried out to evaluate fruit quality and photosynthetic performance of 'Clemenules' mandarin variety budded onto ten important rootstocks in citriculture including commonly used sour orange under calcareous soil condition of the Mediterranean region.

Materials and Methods

Plant Material

Nine years old trees of 'Clemenules cementine' (*Citrus clementina* hort. ex Tanaka) mandarin variety grafted on various rootstocks as presented in Table 1 were used as plant material. Samples of fruits have been harvested from trees located on the citrus rootstock experimental orchards of Cukurova University, Faculty of Agriculture, Department of Horticulture (Latitude, 37°1'27.65" N; Longitude, 35°22'29.30" E; Altitude 49 m) at optimum maturity stage in November and randomly selected from trees. The soil pH ranged from 7.6 to 7.9 at a depth of 0-90 cm in the rootstock experiment orchard which represent slightly alkaline soil conditions of the Mediterranean Region of Turkey with a clay-loam character. The trees were irrigated weekly from May to October using drip irrigation.

Table 1. Genotypes evaluated as rootstocks to 'Clemenules' mandarin variety and scientific names

Genotype	Latin name	Resource*
C-35 citrange	*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.	TGK1131
Carrizo citrange	*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.	TGK0627
FA-5	*C. reshni* x *P. trifoliate* 'Rubidoux'	IVIA
FA-517	*Citrus nobilis* Lour. x *P. trifoliata*	IVIA
FAO-SRA	[*C. sunki* (Hayata) hort.ex Tanaka x *Poncirus trifoliata* (L.) Raf.]	SRA
Flhorag1	*Poncirus trifoliata* L. Raf. + *Citrus deliciosa* Ten.	SRA
Cleopatra mandarin	*Citrus reshni* Tan.	TGK0947
Swingle citrumelo (Citrumelo 4475)	*Citrus paradisi* Macf. var. Duncan x *Poncirus trifoliata* (L.) Raf.	TGK0702
Sour orange	*Citrus aurantium* L.	TGK1065
Volkameriana	*Citrus volkameriana* V. Ten. Pasq	TGK0623

*TGK, Turkish citrus germplasm. SRA, French citrus research center; IVIA, Spain citrus research center.

Fruit Characteristics and Yield

Fruit yield of each tree evaluated as replicate in this study was weighted during the harvesting period. The fruit weight (g), height (mm), diameter (mm), fruit shape index (fruit height/diameter ratio), rind thickness (mm), juice content (%), total soluble solids (%), titratable acidity (%), and ripening index (RI) were determined. Mature fruits of 'Clemenules' variety budded on ten rootstocks randomly selected (25 fruits for each replicate) from five trees. Fruit samples were immediately transferred to citrus physiology laboratory for quality analysis. Fruits were randomly selected and weighed to determine the average fruit size. The fruit was sized at the equatorial diameter and height with a digital caliper (Mitutoyo CD-15CPX). The fruits were halved and the thickness of the rind was measured with a digital caliper. 25 fruits were weighed then juiced with a regular juicer. As a % of the total fruit weight, the juice was expressed. A portable refractometer was used to detect the overall soluble solid (TSS) content. A titration with 0.1 N sodium hydroxide (NaOH) was used to evaluate the overall acidity (TA) of the juice. The relationship between TSS and TA was determined as ripening index (Lado et al., 2014).

Leaf Chlorophyll Concentration and Photosystem II Efficiency

For the estimation of leaf Chl concentrations by SPAD-502 chlorophyll meter, fully expanded young leaves of 'Clemenules' were used (Minolta Inc., Osaka, Japan). Because SPAD reading and chlorophyll levels in citrus leaves are strongly linked, SPAD reading was used to estimate the concentration of Chl leaves (Jifon et al., 2005). Also, PSII maximum efficiency (*Fv'/Fm'*) readings in a light-adapted leaf phase were measured at the same leaves by using a portable fluorimeter (FluorPen FP100, Photon System Instruments Ltd, Drasov, Czech Republic).

Gas Exchange Measurements

A portable photosynthesis system detected the leaf gas exchange parameters of fully developed 4th to 5th leaves from the shooting apex (model LCA-4, ADC Bioscientific Ltd., Hoddesdon, UK) per each rootstock (Cimen et al., 2014). Portable photosynthesis system measured stomatal conductance, g_S (mmol $m^{-2}s^{-1}$); transpiration rate, E (mmol $m^{-2}s^{-1}$); and net photosynthetic rate, P_N [µmol (CO_2) $m^{-2}s^{-1}$] in each single measurement. The instantaneous photosynthetic water use efficiency was predicted as 'WUE = P_N/E' according to Ribeiro et al. (2009). During the gas exchange measurements, leaf temperature varied from 24 and 26°C and the relative humidity was 55%, where PFD was detected as 750-850 µmol m^{-2} s^{-1}.

Statistical Analysis

The experiment was organized as ten rootstocks and five replicates for each rootstocks in a 'Randomized Block Design'. The data were tested by an analysis of variance (ANOVA). The means and calculated standard deviations were stated. Least significant difference (LSD) test was used for mean comparison within rootstocks when the F test was significant at $p < 0.05$. In addition, the 'correlation coefficients' between all measured parameters were calculated. Data subjected to ANOVA by the SAS v9 statistical analyses software and SigmaPlot® v11 (Systat Software, San Jose, CA, USA) was used for data presentation.

Results and Discussion

Fruit yield have been reported as an important factor in citriculture which is directly affected by rootstock as well as many other deciduous fruit trees (Castle, 1995). In the present study we evaluated the influences of various rootstocks on fruit yield and quality traits of 'Clemenules' mandarin variety. Fruit yield significantly ($p < 0.05$) varied among the investigated rootstocks based on one-year results (Fig 1). The highest fruit yield per tree was determined from the trees grafted on Volkameriana, followed by sour orange. On the other hand, similar fruit yield per trees were recorded from 'Clemenules' variety grafted on C-35, Carrizo, FA-5, FA-517, Flhorag1 and Cleopatra mandarin according to LSD test at α= 0.05. Using FAO-SRA and Swingle citrumelo as rootstocks to 'Clemenules' resulted the lowest fruit yield per tree. On the other hand, long-term studies are needed to determine the effect of rootstock on fruit yield and at least 2-3 years of data are required for an exact statement.

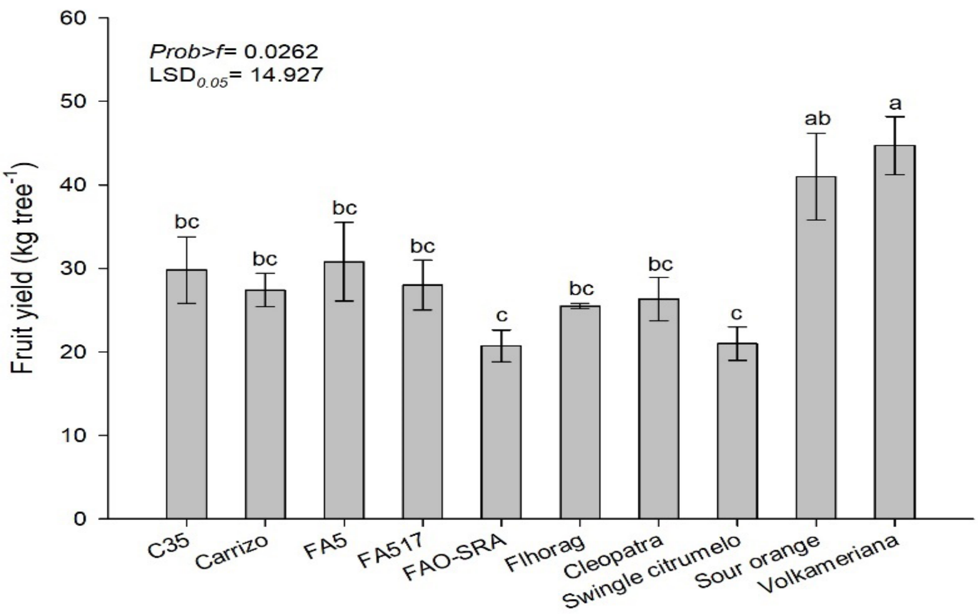

Figure 1. Fruit yield (kg $tree^{-1}$) of 'Clemenules' mandarin variety on various rootstocks

Rootstocks significantly affected variables related to fruit size such as fruit weight ($p < 0.05$) and fruit height ($p < 0.01$) but not significantly affected rind thickness and total soluble solids based on one-year results (Table 2). For producers and consumers, the fruit size is a valuable trait. Medium to large fruits lead to full attention on the fresh market for consumers (Hussain et al., 2013). In our study, except Cleopatra mandarin and Flhorag1, rootstocks produced medium to large fruit size according to recorded fruit weight values. The highest fruit weight was 150.14 g in scion on sour orange followed by Volkameriana (148.60 g) whereas the lowest fruit weight was as 111.48 g from fruit samples on Flhorag1 allotetraploid somatic hybrid. Similar to fruit weight the highest fruit height was determined from fruits on sour orange whereas it was the lowest in for that of Flhorag1. Fruit diameter of 'Clemenules' grafted on various rootstocks varied from 62.88 to 69.11 mm. Although there was no significant rootstock effect on fruit diameter, fruit samples collected from trees on sour orange and Volkameriana had slightly higher fruit diameters in comparison to fruits on other rootstocks. Rootstock had no significant effect on rind thickness which ranged from 3.09 to 4.21 mm.

Table 2. Effects of various rootstocks on fruit weight (g), fruit height (mm), fruit diameter (mm), fruit index and rind thickness (mm) of 'Clemenules' mandarin variety

Rootstock	Fruit weight (g)	Fruit height (mm)	Fruit diameter (mm)	Fruit index	Rind thickness (mm)
C-35 citrange	128.34^{bcd}	59.00^{abc}	65.62	1.11^{c}	3.39
Carrizo citrange	136.11^{abc}	60.05^{ab}	66.36	1.11^{c}	4.21
Cleopatra mandarin	119.00^{cd}	59.70^{abc}	66.26	1.11^{c}	4.04
FA-5	132.01^{a-d}	57.23^{abc}	65.87	1.15^{bc}	3.85
FA-517	124.42^{cd}	56.82^{a-d}	64.97	1.14^{bc}	3.23
FAO-SRA	135.65^{abc}	55.68^{bcd}	66.49	1.20^{a}	3.09
Flhorag1	111.48^{d}	52.37^{d}	62.88	1.20^{a}	3.57
Swingle citrumelo	124.48^{cd}	55.35^{cd}	65.43	1.18^{ab}	3.66
Sour orange	150.14^{a}	60.84^{a}	69.00	1.14^{bc}	3.16
Volkameriana	148.60^{ab}	60.46^{ab}	69.11	1.14^{bc}	3.41
Prob>F	*0.0352*	*0.0187*	*0.1295*	*0.0445*	*0.2296*
$LSD_{0.05}$	*19.773*	*4.284*	-	*0.062*	-

Based on one-year results, rootstocks also did not affect total soluble solids (TSS) of 'Clemenules', however fruit samples collected from those grafted on Volkameriana (11.15%) had slightly lower TSS values than those grafted on other rootstocks under evaluation. In contrast, significant rootstock effect ($p < 0.01$) on total acidity of 'Clemenules' variety was obtained according to a one-way ANOVA (Table 3). Total acid values ranged from 1.14% to 1.32%. The lowest total acids were determined in fruit samples grafted on Cleopatra mandarin and Volkameriana, whereas TA values were higher in fruit samples collected from the rest of the rootstocks. The fruit ripening index is a widely used indicator to determine the citrus fruit maturity level (Lado et al., 2014). Significantly high values of RI were confirmed in fruits of 'Clemenules' grafted on Cleopatra mandarin (16.07), Swingle citrumelo (15.65), and sour orange (15.67), as presented in Table 3. Rootstock had no effect on juice content of 'Clemenules' and fruit juice content ranged from 31.37% to 41.68%.

Fruit internal and external properties are the most important quality parameters and are affected by many factors, such as genetic variability, climate and environment, and rootstock. Several researches revealed that rootstocks significantly affect fruit dimensions and shape (Castle, 1995; Georgiou, 2002; Hussain et al., 2013; Legua et al., 2011). On the contrary, Georgiou (2002) reported that fruit diameter of 'Clementine' mandarin on sour orange, Carrizo citrange and Swingle citrumelo was not significantly affected which is parallel to the results of the present study. In addition, Bassal (2009) reported that the fruits of Marisol mandarin from trees budded on sour orange had higher total acidity (%) than those of Cleopatra mandarin, which is in agreement with the present study.

As a result of the positive linear relationship (r^2 >0.8) between SPAD readings and Chl leaf concentrations reported by Jifon et al (2005), SPAD measurements were used to predict cholorophyll (Chl) levels of leaves in the present study. According to the estimation of Chl concentration by SPAD readings, rootstocks had a significant impact on the leaf Chl content of 'Clemenules' (Table 4). Regarding slightly alkaline soils of the Mediterranean region of Turkey, where the experiment orchard was located at, a remarkable decrease of leaf Chl content in 'Clemenules' grafted on Swingle citrumelo and C-35 citrange. Previously, these rootstocks reported as susceptible to lime-induced iron (Fe) chlorosis by several authors (Castle et al., 2009; Cimen et al., 2014; Pestana et al., 2005). In contrast, the highest leaf Chl content was estimated in the leaves on Volkameriana (58.47), followed by Cleopatra mandarin (58.30). Chl concentrations in the leaves of 'Clemenules' mandarin on Carrizo citrange and FA-5 growing in slightly calcareous soil were not significantly separated and determined in the same sub-group according to the post-hoc test conducted (Table 4). The present study revealed that the Cleopatra mandarin and Volkameriana performed tolerance to slightly alkaline soils likely to sour orange regarding the results obtained for one year. Fe is especially important in the synthesis and stabilization of chlorophyll (Pestana et al., 2011). A number of authors have classified Fe tolerance to citrus rootstocks based on shoot and leaf chlorosis parameters. Sour oranges kept significantly higher Fe concentration in their leaves than trifoliate orange and hybrids (Byrne et al. 1995; Castle et al. 2009).

Similar to leaf Chl concentration, the rootstocks significantly affected PSII efficiency (*Fv'/Fm'*) of 'Clemenules' ($p<0.05$). Cleopatra mandarin and Volkameriana significantly increased PSII efficiency of the scion (Table 4). The declines in chlorophyll fluorescence variables in the case of using trifoliate orange hybrids have been previously reported by Gonzáles-Mas et al. (2009) and Cimen et al (2015). In addition, Pestana et al. (2011) indicated that chlorophyll fluorescence of Newhall navel grafted on Troyer rootstock decreased under iron deficiency. In the present study, PSII activity, in the light adapted period of the leaves, was slightly lower in Swingle citrumelo, in comparison to the rootstocks evaluated.

The precise measurements of leaf gas exchange were conducted with a portable photosynthesis system and the results revealed that the photosynthetic activity of the scion did significantly vary depending on the rootstocks in use. Variables related to photosynthesis of 'Clemenules' mandarin variety grafted on various rootstocks were presented in Table 4. The results of a one-way ANOVA indicated a significant effect ($p < 0.01$) on the net photosynthetic rate (P_N) of 'Clemenules' leaves. P_N of the scion ranged from 1.88 to 5.77 μmol-m^{-2}s^{-1}. 'Clemenules' grafted on Cleopatra mandarin, sour orange and Volkameriana had the highest P_N values with 5.66, 5.44 and 5.77 μmol-m^{-2}s^{-1}, respectively. On the contrary, the lowest P_N was determined in the leaves of 'Clemenules' grafted on Swingle citrumelo (1.88 μmol-m^{-2}s^{-1}). The inhibition of P_N of the leaves on Swingle was found to be corresponding with relatively lower Chl concentration and PSII efficiency (*Fv'/Fm'*) of the leaves on the same rootstock. A one-way ANOVA indicated that rootstock had also significant effect ($p < 0.01$) on leaf transpiration rate (E) of 'Clemenules'. Leaves on sour orange and Volkameriana had the highest E similar to P_N values of these rootstocks. Besides, significant rootstock effect ($p < 0.05$) on leaf stomatal conductance (g_S) was determined. The highest g_S was 101.10 mmol m^{-2}s^{-1} in the leaves of shoots grafted on to Volkameriana rootstock followed by sour orange (92.28 mmol m^{-2}s^{-1}). The lowest g_S was recorded in the leaves of shoots on C-35 citrange (58.33 mmol-m^{-2}s^{-1}). Leaf water use efficiency (WUE) significantly varied between leaves on different rootstocks ($p < 0.01$). The highest WUE was determined on the leaves grafted on FA-5 whereas the lowest WUE values were determined from the leaves on C-35 citrange and sour orange (Table 4). Similar to our findings, FA-5 previously reported to perform better than citranges in terms of photosynthetic activities under high pH conditions (González-Mas et al., 2009)

Physiological parameters like measurements of CO_2-gas exchange can confirm the performance of the plants under high pH conditions (Bavaresco et al., 2006; Chouliaras et al., 2005; Larbi et al., 2006; Molassiotis et al., 2006; Morales et al., 2000; Nenova, 2008). In addition, the lime-induced iron deficiency has been reported to reduce Chl and P_N levels in citrus leaves (Byrne et al., 1995; Hamzé et al., 1986). On the other side, leaf water potential straightforwardly directs leaf transpiration, gas trade, and stomatal conduction in trees developed in alkaline soils (Brodribb and Holbrook, 2003; Eichert et al., 2010; Meinzer, 2002; Sperry, 2000).

Table 3. Effects of various rootstocks on seed number per fruit, total soluble solids (%), total acids (%), TSS/TA, and juice content (%) of 'Clemenules' mandarin variety

Rootstock	Seed number	Total Souble Solids (%)	Total acids (%)	TSS / TA	Juice content (%)
C-35 citrange	10.37^{abc}	12.00	0.90^{ab}	13.44^{ab}	36.48
Carrizo citrange	7.90^{cd}	11.72	0.84^{abc}	14.17^{ab}	39.81
Cleopatra mandarin	13.29^{a}	11.90	0.74^{c}	16.07^{a}	31.37
FA-5	10.15^{abc}	12.20	0.82^{bc}	15.00^{ab}	38.00
FA-517	10.99^{ab}	11.86	0.96^{a}	12.86^{b}	39.07
FAO-SRA	8.59^{bcd}	12.08	0.81^{bc}	15.11^{ab}	36.09
Flhorag1	11.07^{ab}	11.65	0.93^{ab}	12.72^{b}	36.44
Swingle citrumelo	9.26^{bc}	12.18	0.79^{bc}	15.65^{a}	38.91
Sour orange	8.83^{bc}	12.34	0.79^{bc}	15.67^{a}	41.68
Volkameriana	5.28^{d}	11.15	0.70^{c}	16.08^{a}	38.63
Prob>F	*0.0080*	*0.2796*	*0.0245*	*0.0401*	*0.5632*
$LSD_{0.05}$	*2.798*	-	*0.125*	*2.337*	-

Table 4. Leaf chlorophyll concentration and variables related to photosynthesis of 'Clemenules' mandarin variety grafted on various rootstocks

Rootstock	SPAD	PSII (*Fv'/Fm'*)	P_N	E	g_S	WUE
C-35 citrange	51.07±1.42^{ef}	0.634±0.027^{c}	2.51±0.15^{d}	0.57±0.03^{e}	58.33±2.19^{e}	4.40±0.19^{d}
Carrizo citrange	55.33±0.41^{abc}	0.691±0.004^{ab}	4.11±0.12^{c}	0.77±0.02^{c}	75.67±1.20^{d}	5.34±0.19^{ab}
Cleopatra mandarin	58.30±0.89^{ab}	0.710±0.002^{a}	5.66±0.20^{a}	1.05±0.04^{b}	86.32±3.11^{bc}	5.39±0.37^{ab}
FA-5	55.93±1.44^{abc}	0.697±0.007^{ab}	4.77±0.12^{b}	0.83±0.03^{c}	77.17±1.01^{cd}	5.75±0.16^{a}
FA-517	54.97±1.18^{bcd}	0.679±0.036^{abc}	4.04±0.14^{c}	0.71±0.01^{cd}	65.33±5.17^{e}	5.69±0.19^{ab}
FAO-SRA	51.90±0.96^{def}	0.667±0.020^{abc}	2.99±0.13^{d}	0.57±0.02^{e}	62.00±1.73^{e}	5.25±0.16^{ab}
Flhorag1	54.10±1.59^{cde}	0.659±0.003^{bc}	3.03±0.05^{d}	0.59±0.01^{de}	63.67±1.20^{e}	5.14±0.03^{bc}
Swingle citrumelo	49.30±0.36^{f}	0.548±0.023^{d}	1.88±0.13^{e}	0.63±0.05^{de}	63.13±0.47^{e}	2.98±0.28^{e}
Sour orange	56.30±0.57^{ab}	0.687±0.001^{ab}	5.44±0.13^{a}	1.23±0.05^{a}	92.28±4.10^{ab}	4.42±0.16^{d}
Volkameriana	58.47±1.66^{a}	0.716±0.002^{a}	5.77±0.50^{a}	1.26±0.09^{a}	101.10±2.07^{a}	4.58±0.08^{cd}
Prob>F	*0.0002*	*0.0001*	*0.0001*	*0.0054*	*0.0024*	*0.0098*
$LSD_{0.05}$	*3.375*	*0.051*	*0.599*	*0.126*	*9.757*	*0.590*

Table 5. Correlation coefficients analysis between investigated parameters. ** – $p<0.01$, * – $p<0.05$, ns – not significant

Variable	SPAD	PSII	P_N	E	g_S	WUE	Yield	FW	TSS	TA	TSS/TA	Juice (%)
SPAD	1.00	0.75**	0.78**	0.66**	0.68**	0.41*	0.40*	0.15^{ns}	-0.26^{ns}	-0.30^{ns}	0.20^{ns}	-0.14^{ns}
PSII		1.00	0.75**	0.50*	0.49*	0.68**	0.22^{ns}	0.15^{ns}	-0.30^{ns}	-0.24^{ns}	0.13^{ns}	-0.10^{ns}
P_N			1.00	0.88**	0.83**	0.44*	0.47*	0.41*	-0.28^{ns}	-0.40*	0.28^{ns}	-0.09^{ns}
E				1.00	0.89**	-0.02^{ns}	0.60**	0.30^{ns}	-0.32^{ns}	-0.39*	0.27^{ns}	0.01^{ns}
g_S					1.00	0.05^{ns}	0.44*	0.31^{ns}	-0.38^{ns}	-0.40*	0.26^{ns}	-0.06^{ns}
WUE						1.00	-0.10^{ns}	-0.04^{ns}	0.02^{ns}	-0.09^{ns}	0.08^{ns}	-0.21^{ns}
Yield							1.00	0.04^{ns}	0.04^{ns}	0.10^{ns}	0.02^{ns}	-0.11^{ns}
FW								1.00	0.22^{ns}	-0.43*	0.43*	0.28^{ns}
TSS									1.00	-0.23^{ns}	0.49*	-0.13^{ns}
TA										1.00	-0.94**	0.04^{ns}
TSS/TA											1.00	-0.11^{ns}
Juice (%)												1.00
Mean	*54.57*	*0.67*	*4.02*	*0.82*	*74.50*	*4.89*	*30.22*	*131.15*	*11.95*	*0.84*	*14.57*	*37.88*
StD	*3.35*	*0.05*	*1.37*	*0.26*	*14.88*	*0.85*	*13.21*	*18.12*	*0.61*	*0.12*	*2.08*	*5.79*
n	*30*	*30*	*30*	*30*	*30*	*30*	*47*	*46*	*46*	*46*	*46*	*46*

Correlation coefficients revealed significant relationship between variables related to both fruit quality and photosynthesis (Table 5). Higher coefficient between leaf chlorophyll concentration and P_N showed that photosynthesis increased with the increasing amount chlorophyll concentration in the leaves regarding rootstocks. Besides, increasing P_N in the leaves resulted as a significant increase in E. Thus, a strong relationship between leaf chlorophyll concentration and photosynthetic variables was observed. Regarding the increase in E in the leaves of 'Clemenules' tress increased the fruit yield of the variety, in the present study. In addition to the relationship between these variables, a significant increase was determined in fruit weight according to pairwise correlations. Therefore, regression analyses were performed between these correlations and strong relationships were determined concerning high r^2 values. The regression analysis confirmed that P_N rate was increased by the high Chl content in leaves and similarly high P_N increased fruit weight of 'Clemenules' mandarin variety (Figure 2).

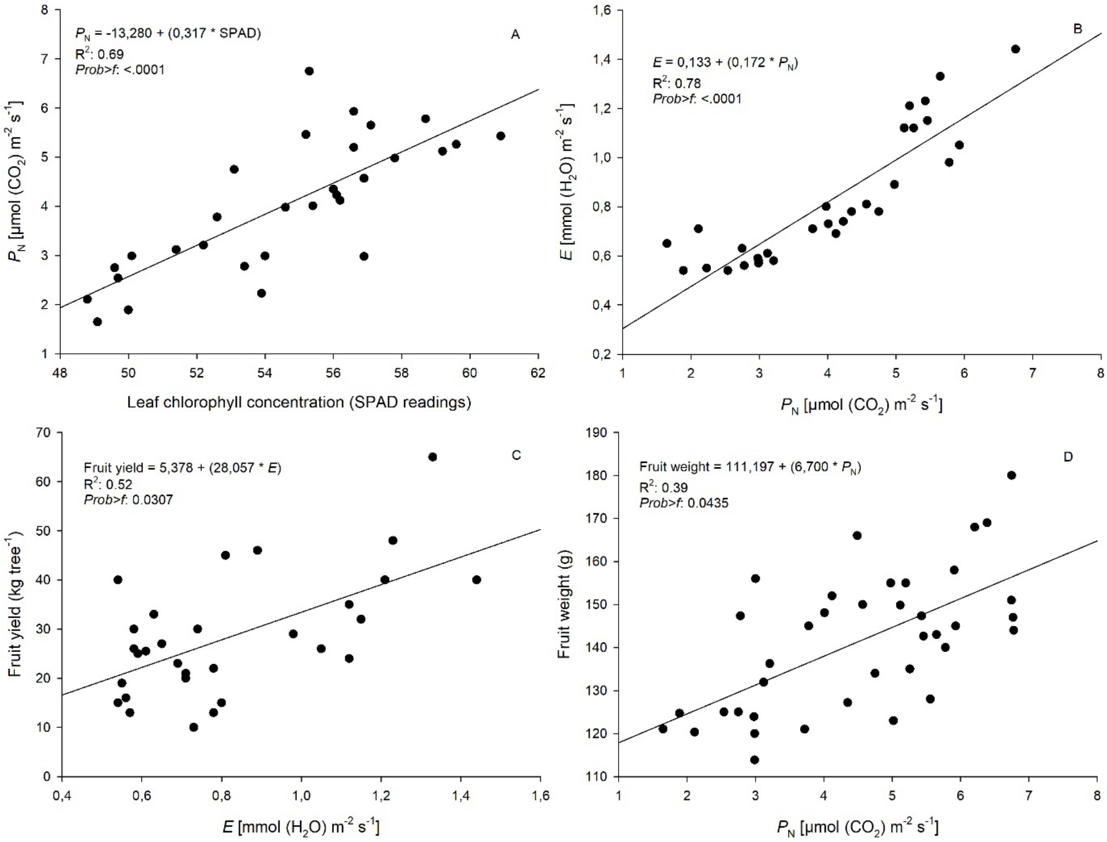

Figure 2. Regressions between A= SPAD readings vs P_N, B= P_N vs E, C= E vs fruit yield, D= P_N vs fruit weight

Conclusion

The present study investigated the effects of rootstock on some fruit quality characteristics and photosynthesis of 'Clemenules' mandarin which is preferred by both Turkey's mandarin growers and consumers. Especially the fruit yield and the fruit weight were remarkably affected by the usage of different rootstocks as well as total acids and ripening index. Sour orange and Volkameriana significantly increased fruit yield due to their high photosynthetic performances. On the other hand, the rootstocks were insignificant on fruit diameter, rind thickness, total soluble solids, and juice content of 'Clemenules'. Since the Mediterranean region of Turkey has calcareous soils, concentration of leaf Chl in 'Clemenules' budded on Swingle citrumelo significantly reduced. Generally, trees budded on Carrizo citrange, FA-5, and FA-517 performed much better than those of C-35 and Swingle citrumelo in terms of positive effects on photosynthetic performance and fruit quality traits. Thus, using these rootstocks for 'Clemenules' should be beneficial especially in terms of their capability to allow high density planting. However, it is worth to mention that long-term studies are needed to determine the exact effects of rootstocks on scion. Therefore, the effects of these rootstock evaluated in the present study will be investigated in terms of fruit yield and quality in the following years.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Response of some physiological components of cotton to surface and subsurface drip irrigation using different irrigation water levels

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Abstract

This study was carried out to determine the leaf water potential (LWP), stomatal conductance (SC) and leaf area index (LAI) of cotton crop using subsurface drip irrigation (SSDI) and surface drip irrigation (SDI) and different irrigation water levels based on the FAO-56 Penman-Monteith (PM) during the 2016 and 2017 growing seasons. The critical LWPs in vegetative period, flowering stage and boll formation stage in SDI for irigation time were -24, -23 and -24 bar, respectively. Considering the same putting in order for the crop development stages in SSDI-40 cm, those were -23, -23 and -24 bar, respectively. The values of LWP in SSDI-30 cm were the same levels in SSD-40 cm. LWP in the boll formation stage were, in general, lower (bigger in minus numerical number) compared to the first two development stages of the crop. The critical SCs in vegetative period, flowering stage and boll formation stage in SDI were 312.8, 201.8 and 198.9 mmol m^{-2} s^{-1}, respectively. The values of SC in the same putting in order for the crop development stages in SSDI-30 cm and SSDI-40 cm were 368.8, 182.6 and 221.8 mmol m^{-2} s-1; and 371.7, 185.9 and 186.8 mmol m^{-2} s^{-1}, respectively. SC decreased from the vegetative period through generative period of the crop. The SCs increased together with increasing amount of irrigation water and it decreased with increasing water stress conditions. The LAIs were 2.99, 3.11 and 3.45 in SDI, SSDI-30 cm and SSDI-40 cm, respectively. The values of LAI increased from the surface drip irrigation and lower irrigation water level applied through subsurface drip irrigation and highest level of amount of irrigation water. Although some plant physiological indicators such as LWP and SC might be used for irrigation scheduling and irrigation time, these indicators are highly affected by soil water status, temperature, light, air humidity and calibration of the devices used.

Keywords: Cotton, Leaf water potential, Stomatal conductance, Leaf area index, Drip irrigation

Introductıon

Cotton is the major industrial crop produced in Turkey and it is crucial to the wider economy since it provides the fiber for textiles. Cotton is primarily grown in the Southeast Anatolia Region which is in the study area of Turkey. Cotton requires a large amount of water (about 1000 mm) using surface irrigation methods since climatological and farmer conditions (Kanber et al., 1991; Cetin and Bilgel, 2002).

In the last decade, use of drip irrigation for cotton increased enormously by means of subsidizing of Turkish Government and awareness of farmers considering water saving. However, use of modern technology, surface and subsurface drip irrigation, need more attention and high experience to accurate and to precise irrigation water. Thus use of irrigation water considering water saving and/or higher irrigation water productivity will be essential if farmers are to minimize risks associated with deficit water while also minimizing the negative outcomes of overirrigating (Chastain et al., 2016).

On the other hand, some data and parameters pertaining to the soil or plant water status for crop irrigation scheduling must be known for an accurate irrigation. However, this is not always reliable, as different physiological behaviours of

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plants might correspond to the same soil water content. In addition, it is sometimes difficult to know where to measure soil water content because of variation in the soil water content in soil volume surrounding the roots (Patane, 2011). Thus, some physiological criteria such as leaf water potential (LWP) and stomal conductance (SC) might be usefull for irrigation scheduling and/or to show the water stress conditions for plants.

LWP could be defined as a measurement of the negative hydrostatic pressure that occurs in the xylem tissue of a plant. LWP can have been, thus, estimated as the negative pressure value required to obtain liquid on the surface of the xylem exposed to atmospheric pressure (Scholander et al., 1965; Campbell, 1985; Busso, 2008). LWP can be used as an effective irrigation measure and/or scheduling to maximize water productivity for cotton irrigation with considerably differences in water availability (Chastain et al., 2016). However, some environmental factors can limit the use of indirect measurements of water status in the plant. This can potentially be accounted for by a calibration pertaining to the specific region using a direct measure of plant water status (Jones, 2004). Concerning measurement of water potential in the midday is highly indicative for the physiological state of the plant (Turner et al., 1986; Pettigrew, 2004; Ennahli and Earl, 2005; Chastain et al., 2016) and a strong indicative of water-induced variation in productivity (Grimes and Yamada, 1982).

Stomatal conductance (SC) is a measurement of the degree of stomatal opening and can be used as an indicator of plant water status. The SC can show the stress conditions of plants through use of a porometer device. Some evidence indicates that the SC and photosynthetic rate of leaves are correlated across diverse environments. The correlation between SC and photosynthetic rate has led to the postulation of a "messenger" from the mesophyll that directs stomatal behavior (Radin et al., 1988).

On the other hand, leaf area index (LAI) directly specify canopy structure, and can be used to estimate primary productivity and growth of crops. LAI is commonly used in ecosystem and crop. Thus many ecosystem and crop models require LAI as an input variable.

The main purpose of this study is to evaluate some crop physiological stress indicators such as leaf water potential and stomatal conductance and leaf area index associated with yield and different irrigation water levels using surface and subsurface drip irrigation for cotton.

Materials and Methods

Study area

This study was carried out at the Research and Experimental Station, Faculty of Agriculture, Dicle University (Diyarbakir, Turkey) during the 2016 and 2017 cotton growing seasons. The experimental site is located at 37^o 54' N, 40^o 14' E, at an elevation of 660 m above sea level. The soil texture is heavy texture, clay content is about 65%. The climate in the study area fall into terrestrial climatological properties. The average annual rainfall of 490 mm is concentrated in winter season and there is no almost precipitation during the cotton irrigation season. The bulk density of soil ranged from 1.19 to 1.27 g cm^{-3} in the soil profile. The infiltration rate was 8 mm h^{-1}. There were no any risk in terms of water table, salinity and irrigation water used.

Experimental design and treatments

The field trials were performed using a split plots in randomize blocks with three replications (Yurtsever, 2011). Main plots are surface and subsurface drip irrigation systems, and sub-plots are different amount of water based on FAO-56 Penman-Monteith method (PM) and Kc approach (Allen et al., 1998). The experimental treatments are given in Table 1.

The irrigation amount of water was applied according to the estimated crop evapotranspiration (ETc) based on FAO-56 Penman-Monteith method and using actual climatological data pertaining to experimental site (Allen et al., 1998). For this, the reference evapotranspiration (ETo) was daily calculated using the meteorological data pertaining to the study site according to the FAO-56 Penman-Monteith method equation. Then, amount of irrigation water was computed using Kc coefficients in the crop development stages of cotton. Irrigation cycle was at each 5 days. The last irrigation was ended at the approximately 10% of boll opening (Bilgel, 1994).

The equations (1 and 2) given below were used to calculate amount of irrigation water applied (Allen et al., 1998).

$$ETc = Kc\ ETo \tag{1}$$

$$I = A\ .\ ETc\ .\ K.\ Pc \tag{2}$$

Where ETc is estimated crop evapotranspiration (mm d^{-1}), Kc crop coefficient (dimensionless) and ETo reference crop evapotranspiration for grass (mm d^{-1}). I is amount of irrigation water applied to the experimental plot (Liters), A is plot area (m^2), K is different rates of ETc, Pc is canopy cover (%).

The plot size is 4.20 m x 8.00 m (33.60 m^2). One lateral has irrigated two cotton rows, thus, the lateral spacing is 1.40 m. The sowing date was at the beginning of May and the harvest date was at the beginning of October for two experimental years.

Measurement of LWP and SC

The physiological indicators, LWP and SC were measured in five cotton plants for each treatments and plots under different irrigation treatments and irrigation systems (SDI and SSDI) for three critical stages of cotton, vegetative period (I), flowering stage (II) and boll formation (III) before irrigation (Kara and Gunduz, 1998). LWP and SC were measured by a pressure chamber as bar and a diffusion porometer as mmol H_2O m^{-2} s^{-1}, respectively. To measure SC was used a portable porometer. The instruments were calibrated according to the manufacturer's instructions before each measurement cycle.

The measurements were carried out at noon (13.00–14.00) on the lower surface of the last fully expanded leaf on five samples per plot (Martinez et al., 2013; Koksal et al., 2010).

Leaf area index (LAI)

LAI is the ratio of all leaves' area on the plant to the certain and cropped ground area. For this, all leaves on the five plants in each plot were collected and they were scanned and computed all area of the leaves using a computer programme (software). Then, this area of the leaves were divided to the total area of certain cropped in the field.

Table 1. Experimental treatments according to the split plots design

Main plots (Drip irrigation systems)	Sub-plots (Irrigation water)
I_1 : Surface drip irrigation **I_2:** Subsurface drip irrigation (depth of 30 cm) **I_3:** Subsurface drip irrigation (depth of 40 cm)	**K_1**: I=1.25 × ETc (crop evapotranspiration based on FAO-56 Penman-Monteith **K_2**: I=1.0 × ETc **K_3**: I=0.75 × ETc

Results and Discussion

Leaf water potential (LWP)

The values of LWP ranged from -20.7 to -26.3 in 2016 and from -16.6 to -26.3 depending on crop stages, irrigation water levels, surface and subsurface drip irrigation systems and experimental years. However, the average values for 2 experimental years are given in Figure 1a. In addition, Figure 1a shows the values of LWP in both different drip irrigation systems (SDI and SSDI) and different amount of irrigation water according to the different development stages of cotton. Thus, LWPs in the first two stages of the crop, vegetative development and flowering, were higher compared (smaller in minus numerical number) to those in boll formation stage. In general, the values of LWP in the boll formation stage, generative stage of crop, were, thus, lower (bigger in minus numerical number) compared to the first two development stages of the crop. This result clarified that boll formation stage was more consumptive water use because of generative stage of the crop (Kara and Gündüz, 1998). On the other hand, considering the different development stages of crop , the range and/or treshold of LWPs for flowering stage were same for all drip irrigation systems (Figure 1a).

According to the variance analysis, the treatments in the main plots, surface and subsurface drip irrigation, significantly affected ($P \leq 0.01$) LWPs in the vegetative stage of crop. It is understood from these results that the plants in the subsurface drip irrigation at 40 cm depth has got less water stress compared to the surface and the other subsurface drip (30 cm) irrigation. There were significantly effects ($P \leq 0.05$) of different amount of irrigation water on LWPs in the boll formation of crop. LWPs increased (smaller in minus numerical number) as long as increased amount of irrigation water applied (Figure 1b). Different amount of irrigation water applied in the boll formation of crop which has got more stress affected the LWP. For instance, the treatment of I=ETc x 1.25 (K_1, more irrigation water) showed lower LWP (bigger minus numerical number) however the treatment of I=ETc x 0.75 (K_3, less irrigation water) showed higher LWP (smaller minus numerical number) in both SDI and SSDI depending on amount of irrigation water applied (Figure 1b and Figure 2). Thus, LWP dependent on amount of irrigation water rather than different drip irrigation systems. Kaufman (1981) stated that the critical treshold value of cotton on LWP for irrigation is between -17 and -18 bar. Maya (2017) reported that the values of LWP of cotton for full irrigation, deficit irrigation and severe stress conditions were about -15.5, (-16)-(-18) and (-22)-(-23) bars, respectively. Yazdıç and Değirmenci (2018) measured the LWP to be (-23.4)-(-26.9) for cotton under the Mediterranean conditions. Although there have been some similar results taking into acoount the previous studies, the values of LWP at the crtical development stages of cotton were more or less different. The values of LWP in our study were lower (bigger minus numerical number) compared to these values. The reasons of these differences might be probably the lower available water in the soil since very heavy soil texture (clay content of soil is about 65%) and compaction, in another word, water retention energy by clay particules is very high thus water moving from soil into the plants are not easy. Thus, the values of LWP could be different considering environmental, soil and agronomic conditions. These could be considered such as soil water availability, soil temperature, absolute humidity and wind spead (Kaufman and Hall, 1974; Hake and Grimes, 2010). Considering some extreme climatic and soil conditions such as maximum temperatures (up to 45 ^{o}C), some extra advection to the study area, soil texture (very high clay content) and soil structure in the study region, obtaing different values of LWPs is expected results.

According to the previous studies, LWP is an using way in terms of internal dynamic for the plants (Jones et al., 1991), and LWP shows inverse correlation with relative water content of leaves, stoma dimension and numbers, and agronomic applications (Saleem et al., 2016). In addition, relative water content in the leaves and photosynthesis rate decreased as long as decreasing (increasing negative number) LWP (Lawlor and Cornic, 2002). Stomal and non-stomal irregularity under the deficit irrigation conditions for cotton caused in decrease of photosynthesis rate (Leidi et al., 1999).

On the other hand, LWP is a physiological criteria frequently used in irrigation scheduling. LWP shows energy status of water in the leaves, in another word it is described as a collimator power of water moving. LWP might be varied according to the transpirational flow and water content in the soil, thus it is an important criteria for the assessment of plant water relationships (Camacho et al., 1974).

Stomatal conductance (SC)

The SC could not measure in only vegetative period in 2016 since the porometer was out of order. In addition, although there has been a special calibration for porometer before reading and this was made, some readings (base or treshold values) might show deviation from readings from each stage of crop. Because, rapid stomatal closure in the porometer cuvette is another problem that can limit porometer accuracy in certain cases. There is substantial variation in sensitivity to leaf surface humidity among plants (McDermitt, 1990).

In the study, the SC were ranged 82.6-312.5 mmol $m^{-2} s^{-1}$ and 143.4-437.3 mmol $m^{-2} s^{-1}$ for all treatments in 2016 and

2017, respectively. However, the average values of SC pertaining to the experimental treatments and years are shown in Figure 3a and 3b.). According to the results of variance analysis, there were significantly differences (P≤0.01) between SDI and SSDI, and different amount of irrigation water applied in flowering stage in 2016. The maximum SC reached in the treatment that the maximum amount of irrigation water applied in K_1: I=1.25x ETc (Figure 3b). As expected, the SC increased together with increasing amount of irrigation water and decreased with increasing water stress conditions (decreasing amount of irrigation water) (Figure 4.). That the values of SC have been showed difference might be attributed to the development crop stage, different application of irrigation water, climatic conditions and variations in the porometer device.

On the other hand, SC increased for the crops on the plots in which grown under the subsurface drip irrigation at 40 cm compared to the other treatments during the vegetative development stage (Figure 3a). Considering the stage of boll formation, there were significantly difference (P≤0.01) between SC under the applications of different amount of irrigation water. In this stage, there was no any effect on SC under surface and subsurface drip irrigation. For this, the lowest SC was obtained under the lowest level of irrigation water (Figure 3b and 4). However, there were no significantly difference on SC during the boll formation. This might be attributed that the irrigation was ended different period considering the different treatments and the plants consumed more water.

According to the results in 2017, there were significantly differences (P≤0.05) between SCs on the SDI and SSDI during the flowering stage, however there were significantly differences (P≤0.01) between SCs on different amount of irrigation water during the boll formation. The SCs increased as long as amount of irrigation water increased (Figure 4).

Considering the treatment in which subsurface drip irrigation at 40 cm and irrigation water application based on crop evapotranspiration using FAO-PM (I=1.0xETc), the values of SC were 369, 183 and 222 mmol m^{-2} s^{-1} for the stages of vegetative development, flowering and boll formation stage, respectively. The values of SC during the vegetative stage were higher compared to those in the the other stages because this stage is rapidly development stage. In another word, during the vegetative growth stage, roots develop rapidly (Hake and Grimes, 2010). The values of SCs decreased in the next stages of crop (Figure 4). One of the factors indirectly affecting on SC might be soil compaction because of soil texture (very high clay content up to 65%) and clay type in the experimental site. For that reason, this condition affects soil water availability for crops.

Similarly, Ephrath et al. (1990) reported that SC decreased with increasing water stress conditions, the correlation between radiation and SC decreased as long as the plants exposed to water stress and there was a asymptotic relationship between photosynthesis rate and SC. All these findings show similar results from this study. Meidner and Mansfield (1968) stated that SC is also depending on CO_2 concentration in the surrounding environment and difference on leaf-air vapor pressure. Cell growth in cotton is, thus, more sensitive than stoma closure during the limited water conditions. For this, sensitivity to cell gowth and increase on plants height and leaf area are more responsive than the results related to stoma closure on transpiration and photosynthesis (Turner et al., 1986; Puech-Suanez et al., 1989).

On the other hand, photosynthesis and stomal conductance become discrete under the higher temperatures. Because, the higher temperature decrease absisic acid level, thus transpiration become maximum, thus this cause cool of leaves. Biological water use efficiency and water saving become maximum (Radin, 1992). Connecting this, in this study region has very hot climate regime during the growing season up to 45 ^{o}C. Thus, the effects of higher temperatures on stomal conductance are effective as reported by Radin (1992). In addition, the main effects of deficit water on cotton production occur on decreasing of stoma closure and C fixation in the leaves and leaf growth (Patterson et al., 1978; Inamullah and Isoda, 2005). The plants adapts differently to water stress. Cotton adapts to water stress by maintaining higher transpiration compared to the other crops such as soybean. The higher transpiration in cotton was due to a higher SC, which was supported by a higher flow rate of stem sap, larger stomatal area, and probably the diaheliotropism (Inamullah and Isoda, 2005).

Leaf area index (LAI)

LAI was only computed in 2017 and it was measured at the generative stage of crop (boll formation). Accoroding to the results, the LAIs ranged from 1.60 through 4.09 depending on the treatments and experimental years. However, the average values of LAI are given in Figure 5. The lowest LAI was obtained from the treatment in which the lowest amount of irrigation water was applied and the maximum amount of irrigation water resulted in the maximum LAI. The values of LAI increased from the surface drip irrigation and lower amount of irrigation water applied through subsurface drip irrigation and highest level of amount of irrigation water (Figure 5).

According to the results of variance analysis, increasing amount of irrigation water applied significantly ((P≤0.05) increased the values of LAI. Although there were no significantly difference on the values of LAI between surface and subsurface drip irrigation systems, the subsurface drip irrigation system at 40 cm resulted in the maximum LAI (Figure 5).

Considering the average seed cotton yields, there was a linear relationships between seed cotton yield and LAI (for SDI: y = 1093+873.7X, R^2 = 0.97**, P ≤ 0.01; for SSDI-30 cm: y = -8664+4113.5X, R^2 = 0.70*, P ≤ 0.01; and for SSDI-40 cm: y = 905.9+1045.4X, R^2 = 0.99**, P ≤ 0.01), and the regression curves with regard to each drip irrigation system are shown in Figure 6.

Ashley et al. (1964) reported that LAI reachs to 1.0 at 6 or 8 weeks after emergency of the plants. Considering the relationship between boll formation and LAI, LAI rises up to 5.0 during the boll formation. In addition the evaporation from the soil surface decreases as long as LAI and canopy cover increase (Luo et al., 2011). In the previous studies, the values of LAI ranged from 3.62 through 3.71 depending on different cotton varieties (Ekinci et al., 2008), the maximum LAI was obtained to be 4.0-5.8 at the treatment in the maximum crop evapotranpiration (Kanber et al., 1991) and LAI was 3.37 for cotton grown at the Lower Seyhan Plain of Turkey (Baydar, 2010). Ödemiş et al. (2018) determined LAI to be 3.59 for full irrigation conditions. In addition, Ertek and Kanber (2001) reported that the values of LAI ranged from 3.24 through 4.40. LAI were found between 3.10 and 5.54 depending on amount of irrigation water applied and cottton variety and LAI increased as long as increasing irrigation water (Keten, 2016). The valus of LAI in this study are, in general, similiar to the results of previous studies. However, LAI might be different as it is in this study depending on cotton varieties, irrigation systems and/or methods and amount of irrigation water and climatological conditions. On the other hand, ageing of leaves accelerate under the deficit irrigation, LAI continue to increase during the crop development stages under the full irrigation conditions but it decrease under the deficit irrigation (Saleem et al., 2016; Noreen et al., 2013). In addition, LAI is one of the criteria on measurement of photosynthesis capacity. Thus LAI might be used to determine the variation rate of CO_2 in the plants regardless the shape and dimension of (Pegelow et al., 1977).

On the other hand, there were significantly relationships (P≤0.01) between LAI and amount of irrigation water using regression analysis in both surface and subsurface drip irrigation systems (Figure 7). For this, seed cotton yield increased with increasing of LAI. As a result irrigation water applied increased directly cotton yield and LAI (Chen et al., 2017).

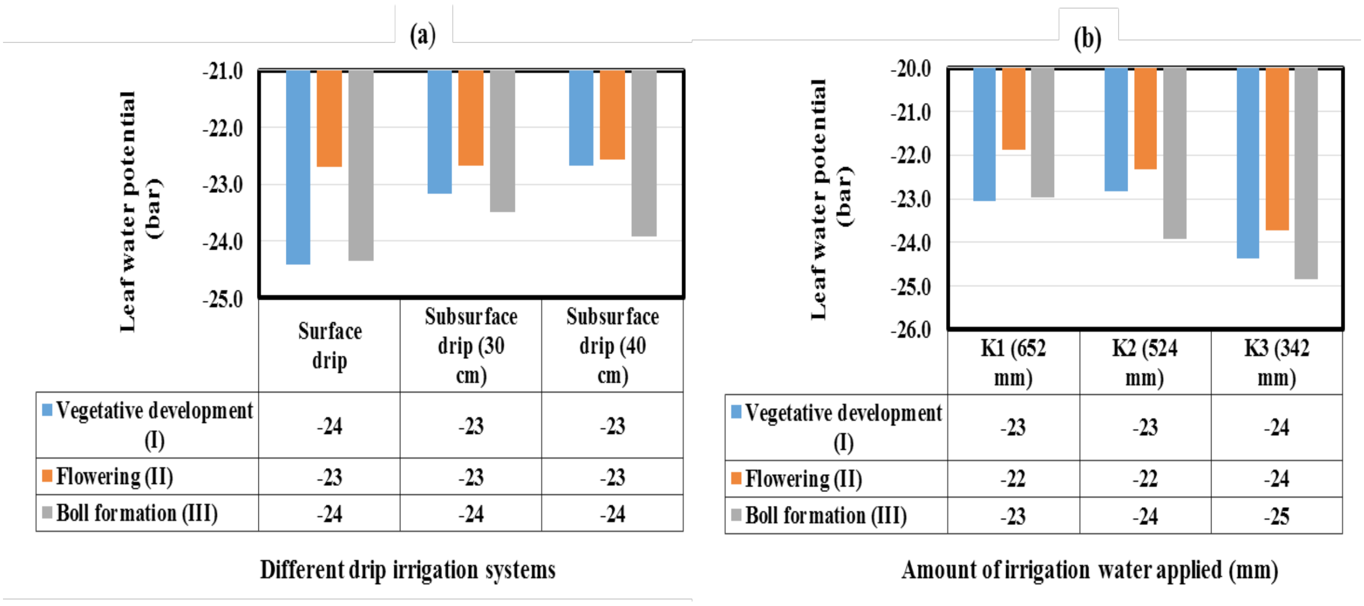

Figure 1. The effects of different drip irrigation systems (a) and different amount of irrigation water (b) on leaf water potential according to the crop development stages for the average of two experimental year.

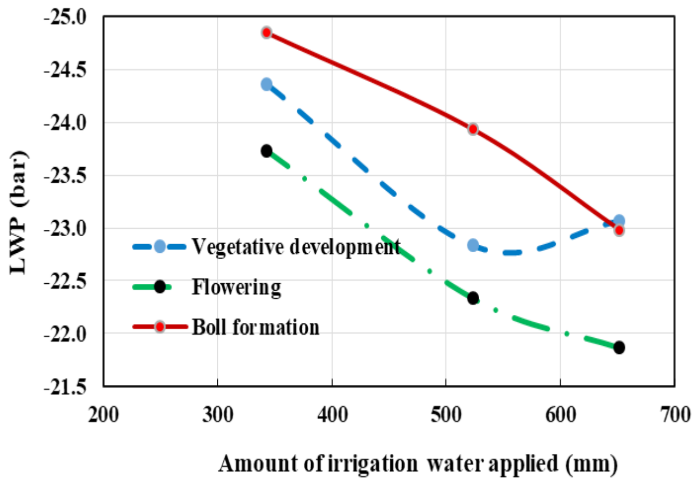

Figure 2. Relationship between amount of irrigation water and LWP

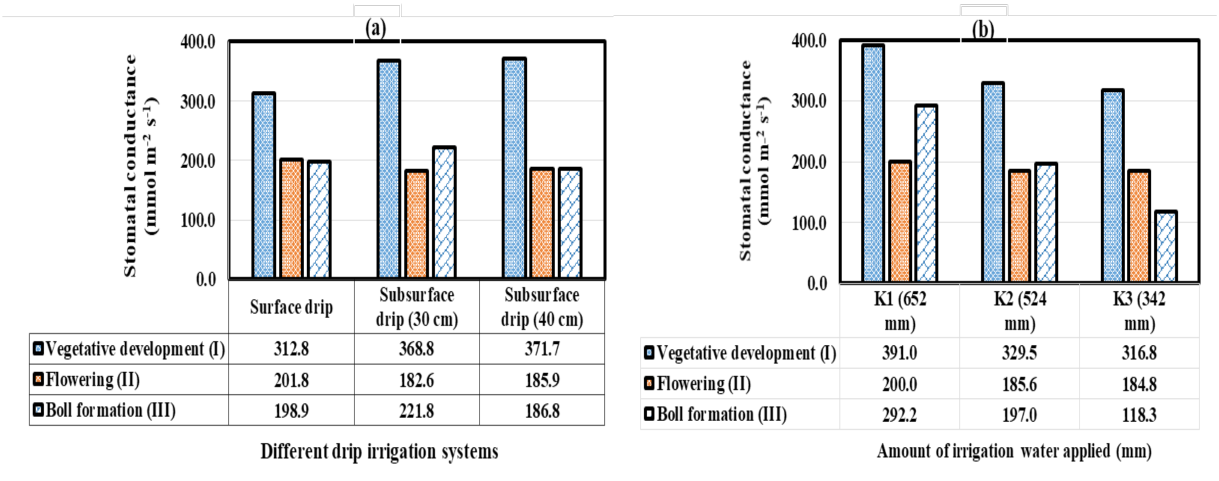

Figure 3. The effects of different drip irrigation systems (a) and different amount of irrigation water (b) on stomatal conductance according to the crop development stages for the average of two experimental years

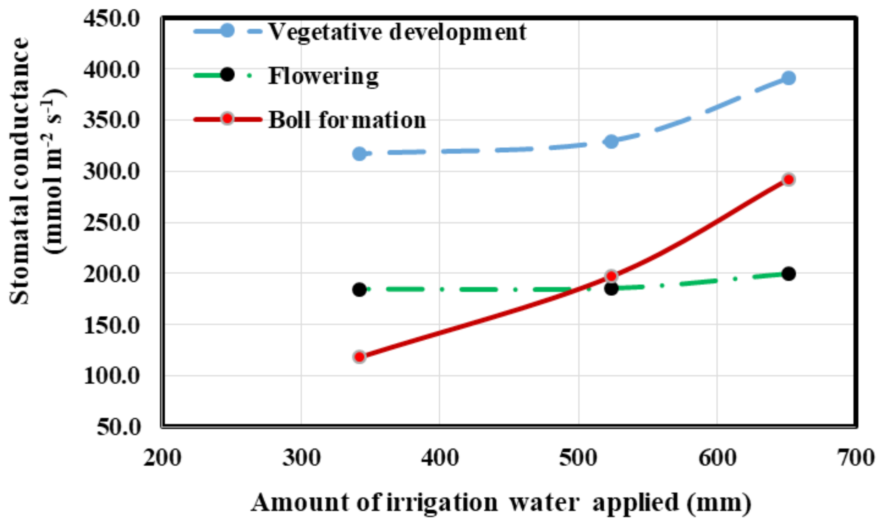

Figure 4. Relationship between stomatal conductance and amount of irrigation water applied regardless SDI and SSDI

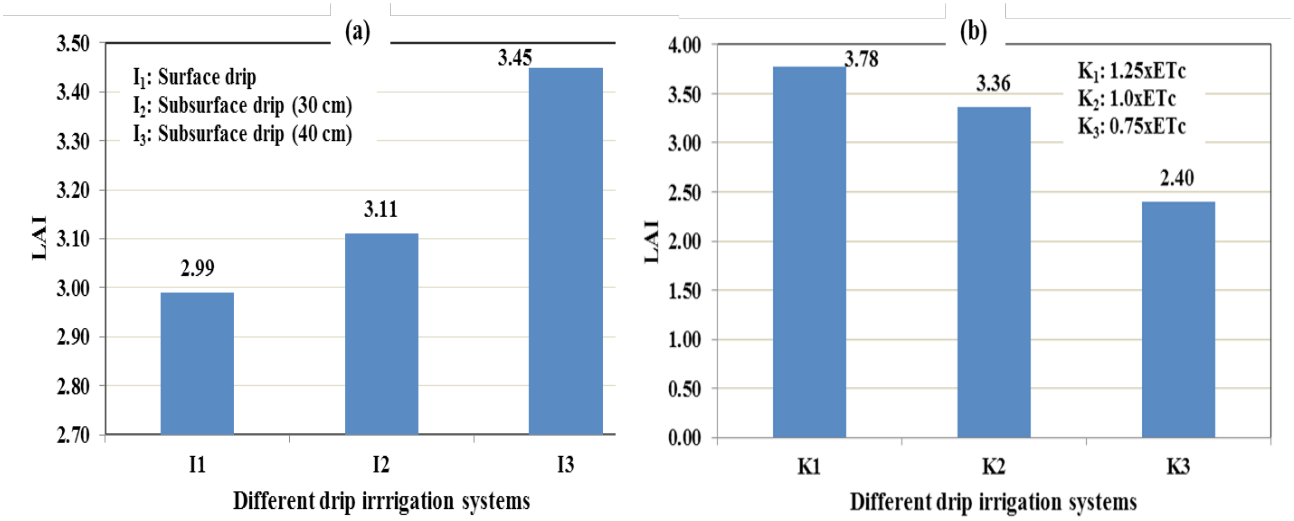

Figure 5. The values of leaf area index according to the different drip irrigation systems (a) and different amount of irrigation water (b)

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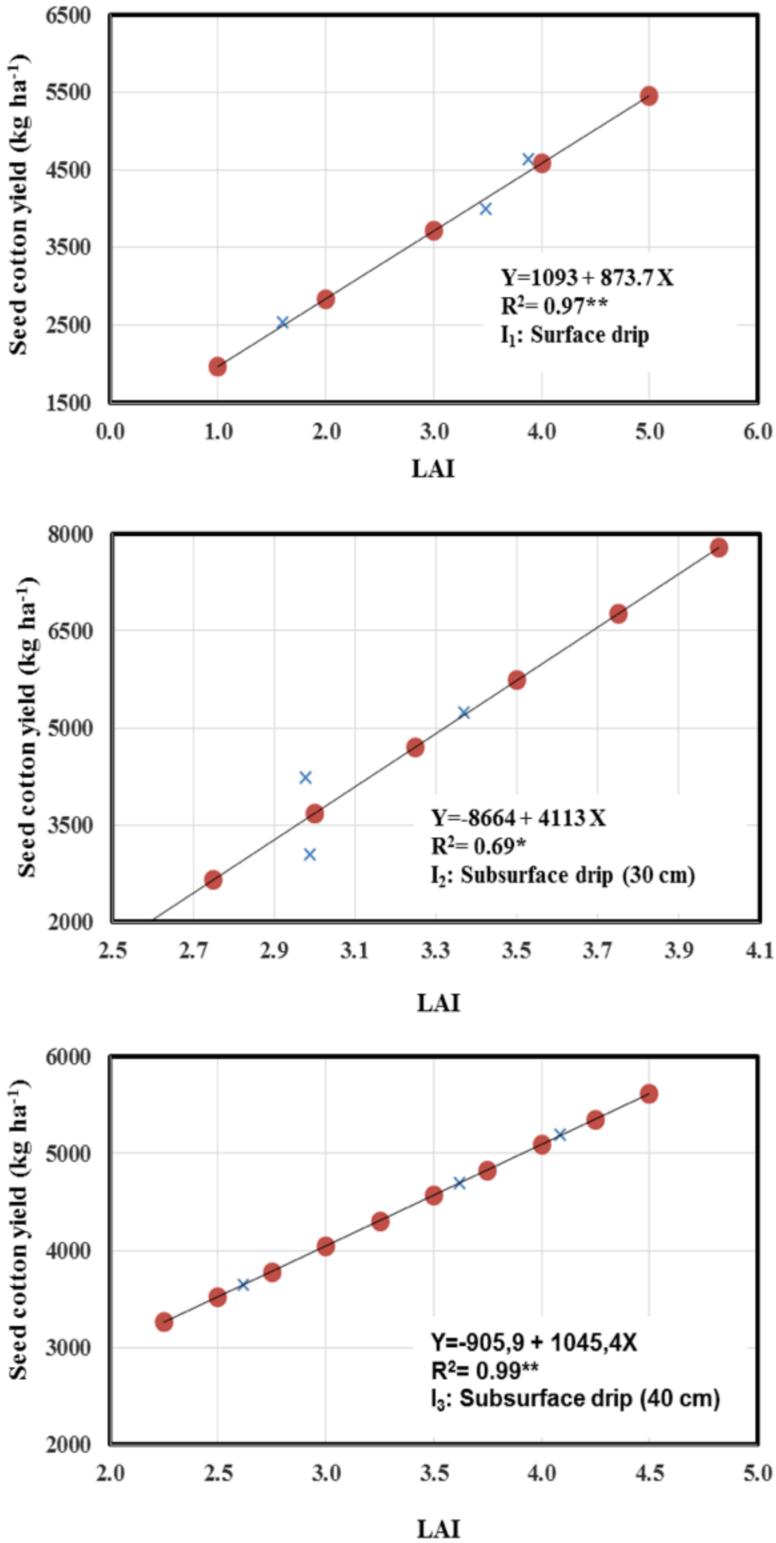

Figure 6. Relationship between LAI and seed cotton yield according to the drip irrigation systems

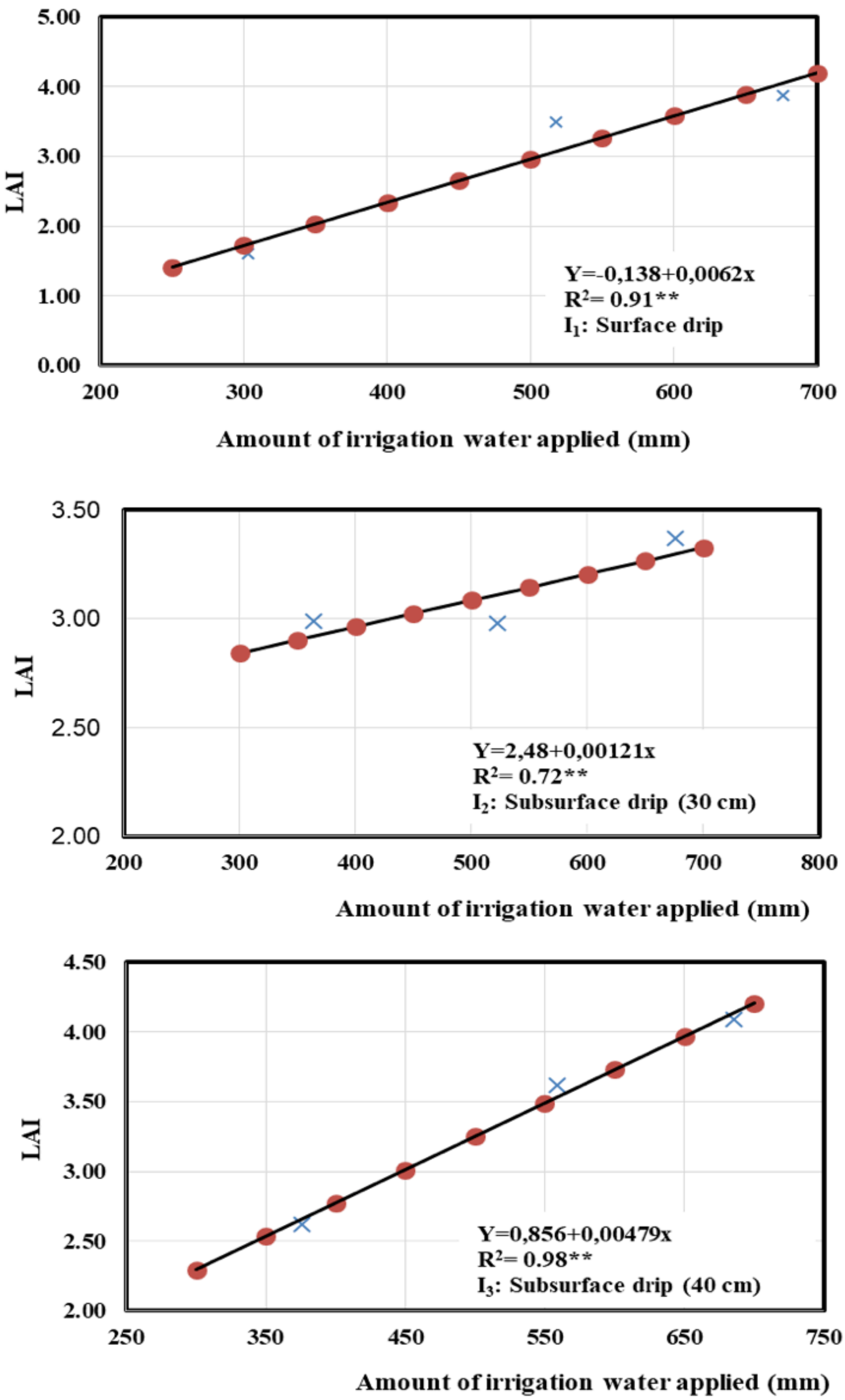

Figure 7. Relationship between amount of irrigation water applied and LAI according to different drip irrigation systems

Conclusion

In this study, the critical LWPs in vegetative period, flowering stage and boll formation stage of cotton in SDI for irrigation time were -24, -23 and -24 bar, respectively. Considering the same putting in order for the crop development stages in SSDI-40 cm, those were -23, -23 and -24 bar, respectively. The values of LWP in SSDI-30 cm were the same levels in SSD-40 cm. LWP in the boll formation stage were, in general, lower (bigger in minus numerical number) compared to the first two development stages, vegetative and flowering stages of the crop.

The critical SCs in vegetative period, flowering stage and boll formation stage in SDI were 312.8, 201.8 and 198.9 mmol m^{-2} s^{-1}, respectively. The values of SC in the same putting in order for the crop development stages in SSDI-30 cm and SSDI-40 cm were 368.8, 182.6 and 221.8 mmol m^{-2} s^{-1}; and 371.7, 185.9 and 186.8 mmol m^{-2} s^{-1}, respectively. SC decreased from the vegetative period through generative period of the crop. The SCs increased together with increasing amount of irrigation water and it decreased with increasing water stress conditions. The LAIs were 2.99, 3.11 and 3.45 in SDI, SSDI-30 cm and SSDI-40 cm, respectively. The values of LAI increased from the surface drip irrigation and lower amount of irrigation water applied through subsurface drip irrigation and highest level of amount of irrigation water. Although some plant physiological indicators such as LWP and SC might be used for irrigation scheduling and irrigation time, these indicators are highly affected by soil water status, light, air humidity, temperature and calibration of the devices used.

As a result, useful parameters for crop irrigation scheduling are provided by measurements of the soil or plant water status. Some physiological criteria such as leaf water potential (LWP) and stomal conductance (SC) might be usefull for irrigation scheduling and/or to show the water stress conditions for plants. LWP is reference measuring of water status of cotton leaves and have enabled solid reference thresholds of cotton plant water status. This data is obtained by measuring the leaf water potential by means of a pressure chamber. The LWP could be used to manage cotton irrigation. It is a useful method for precision irrigation which could help to save water. However, these indicators are highly affected by soil water status, light, air humidity, temperature, atmospheric CO_2 and calibration of the devices used. Thus, the measurement time and the threshold values for critical consideration such as irrigation scheduling might vary depending all these conditions.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Changes in the content of total polyphenols and the antioxidant activity of different beverages obtained by *Kombucha* 'tea fungus'

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Abstract

Kombucha 'tea fungus' is a traditional refreshing drink obtained by fermentation of black tea with sugar as well as a strong symbiosis of acetic bacteria and yeasts. *Kombucha* tea has several health benefits such as antihyperglycemic, antilipidemic, antimicrobial, hepatoprotective, hypocholesterolemic and anticancer effects due to their antioxidant activity. In this study, six *kombucha* beverages were prepared by placing *Kombucha* 'tea fungus' in green, black and Echinacea teas, as well as goat, cow and soy's milk. The fermentation process was monitored by pH, total sugar amount, and titratable activity, as well as their antioxidant activities and total phenolic contents, were analyzed prior to the fermentation process and at the end of fermentation. The results showed that tea-based beverages were fermented for nine days and milk-based beverages were for 6 hours. Their sugar contents were significantly decreased ($p < 0.05$) as depending on their sugar contents. All fermented beverages displayed a statistically significant decrease ($p < 0.05$) in the DPPH and ABTS radical scavenging activity at the end of fermentation, while FRAP assays were displayed a statistically significant increase ($p < 0.05$). Further studies are necessary to the research of nutrients of tea and milk-based beverages on human organs the throughout fermentation.

Keywords: *Kombucha,* Antioxidant activities, Beverages, Functional food

Introduction

Since consumers have become increasingly aware of the role of diet in promoting health and preventing disease, new food products called functional food have been improved. Functional foods have a positive effect on health, physical and mental condition of the human body in addition to the nutritional value and the most famous examples of functional foods are fermented products, especially milk or dairy products (Siró, Kápolna, Kápolna, and Lugasi, 2008).

Kombucha, also named as tea fungus, has been consumed worldwide as a healthy drink for a very long time especially in China, Russia, and Germany (Vázquez-Cabral et al., 2017). Kombucha is a symbiosis of acetic acid bacteria (Acetobacter), including Acetobacter xylinum, A. xylinoides, Bacterium gluconicum, A. suboxydans, Gluconobacter liquefaciens, A. aceti and A. pasteurianus, various yeasts (the genera of Brettanomyces, Zygosaccharomyces, Saccharomyces, and Pichia depending on the source), including Saccharomyces cerevisiae, S. inconspicus, S. ludwigii, Schizosaccharomyces pombe, Candida tropicalis, C. krusei, Debaryomyces hansenii, Brettanomyces spp., Kloeckera spp., Zygosaccharomyces bailii and Z. Kombuchaensis, and lactic acid bacteria (Lactobacillus bulgaricus) (Battikh, Bakhrouf, and Ammar, 2012; Zubaidah, Yurista, and Rahmadani, 2018). Kombucha is composed of a floating cellulosic pellicle layer and the sour liquid broth (De Filippis, Troise, Vitaglione, and Ercolini, 2018) and converted the sugar which is its carbon source into organic acids and ethanol: sucrose into glucose and fructose and produce ethanol, while acetic acid bacteria convert glucose into gluconic acid and fructose to acetic acid (Malbaša, Lončar, Vitas, and

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Čanadanović-Brunet, 2011).

The metabolic activity of kombucha results in a refreshing beverage with the sour taste and many health beneficial compounds. The kombucha beverage can be used to help with headaches, gastric illnesses, diabetes, nervousness, and aging problems. Moreover, it possesses antibiotic properties, relieves rheumatism, gout, and hemorrhoids and has a positive influence on the cholesterol level, arteriosclerosis, toxin excretion, and blood cleansing. The most important effect is that it provides resistance to cancer and increases the immune system performance (Chakravorty et al., 2016).

Black and green teas are the usual and the best substrates known for the preparation of Kombucha drinks (Velićanski, Cvetković, Markov, Tumbas Šaponjac, and Vulić, 2014), but other substrates such as coca-cola, wine, beer, fruit drinks, milk, and herbal teas can be used (Malbaša et al., 2011). Furthermore, kombucha can be cultivated on dark beer, red wine, white wine, whey (Vukic et al., 2014), coffee, Jerusalem artichoke (Helianthus tuberosus L.) (Yang, He, Corscadden, and Udenigwe, 2015) and molasses (Lončar, Malbaša, and Kolarov, 2001). Kombucha constitutes several compounds that are known to be strong antioxidants, such as vitamins C and B2, as well as polyphenols, primarily catechins (Malbaša, Vitas, Lončar, Grahovac, and Milanović, 2014). Application of Kombucha in lactose fermentation is still under investigations and a few investigations have been reported and its technological and nutritional aspects in dairy products have been described recently (Malbaša et al., 2009).

In the present work, black, green and Echinacea teas, as well as goat, cow and soy's milk were used as a medium for Kombucha fermentation. To the author's knowledge, a few previous studies reported the antioxidant activity of other different fermented Kombucha beverages, however, there were no studies about the usage of different kind of milk as an alternative medium besides black and green tea. The aim of this work is to analyze fermentation of sucrose and lactose from three different types of teas, including black, green and Echinacea teas and milk, including goat, cow, and soy inoculated with plain Kombucha 'tea fungus' starter (which is not concentrated before application). Changes in the pH and total sugar of the beverages during fermentation were determined. This different tea and milk-based beverages compared to the traditional kombucha prepared from black tea with regards to colour, total phenolic content and antioxidant activities. In view of this, the recent study showed the antioxidant effect of kombucha application in the development of new fermented beverages.

Materials and Methods

Materials

The plain kombucha culture was cultivated on six different substrates: goat's, cow's, soy's milk as well as black, green, and Echinacea teas.

Milk: Pasteurised, homogenized goat, cow and soy's milk (approximate values of the milk fat content at the local market) were used for the production of fermented milk products. The used goat milk has contained, on average, 2.6-3.5 % protein, 4.5-5.5 % carbohydrate, and 10-13 % dry matter content; cow's milk has 3.1 % protein, 4.5 % carbohydrate, and 11 % dry matter content; soy's milk has 3.3 % protein, 0.2 % carbohydrate, and 6.5-7.0 % dry matter content.

Tea: The substrate from black tea was prepared using four grams of tea leaf and it was added to 1 l boiling water and kept boiling for 5 min. Sucrose (100 g) was added, the tea was then filtered through a stainless sieve and the decoction was boiled for another 5 min. The sugared tea was immediately distributed into several sterilized 250 ml-jars (each contained 150 ml) under aseptic conditions and allowed to cool the tea to room temperature. Each fermentation was repeated three times. The substrate from green tea, echinacea tea was prepared under the same conditions as the substrate from black tea.

Starter culture: Kombucha culture (3 g)

Fermentation Process

Kombucha starter was cultivated in different kinds of milk at room temperature. The inoculum was added to the milk in the amount of 10 % (v/v). The fermentation was performed until the pH 4.5. The obtained milk gel was centrifuged at 10000g for 30 min and stored at -20 ºC for further analysis.

The fermentation was also initiated by adding 3 % (w/v) starter to fresh medium and maintained at room temperature for 7 days for tea samples. Sampling was performed periodically at 0, 3, 7 days and the broth was centrifuged at 10000g for 20 min and stored at -20 ºC for further analyses. The fermentation was done in triplicate.

Methods

Determination of pH: pH of the beverages values was measured using a pH meter (WTW Inolab Level 1, Weilheim, Germany) during fermentation.

Total sugar content: The sugar content was assayed by the phenol-sulfuric acid method (Hall, 2013).

Determination of colour: The colour of the beverages were measured using the Hunter-Lab Colorflex (CFLX 45-2 Model Colorimeter; HunterLab, Reston, VA). The cylindrical quartz cell containing the sample was placed directly into the colourimeter, and L*, a*, and b* values were recorded. L* represented the lightness (L* = 0 yields blackness and L* = 100 indicates diffuse whiteness), a* represented the redness-greenness (negative values indicate greenness while positive values indicate redness/magenta) and b* represented the yellowness-blueness (negative values indicate blueness and positive values indicate yellowness) as index of the CIELAB.

Determination of total titratable acidity: Total titratable acidity was determined according to the potentiometric titration acidity of the beverages. The beverages were diluted 10 fold in deionized water and filtered. The filtrate was titrated up to a pH 8.1 with a solution of 0.1 N NaOH using phenolphthalein as indicator. The results were expressed as g 100 ml-1 with reference to anhydrous citric acid.

Determination of total phenolic content: The total phenolic contents of the beverages were measured according to Folin-Ciocalteu method (Singleton, Rossi, and Jr, 1965). 0.05 ml of the beverages were added to 2 ml of 2 % sodium carbonate. After 2 min, 0.1 ml Folin–Ciocalteu reagent was mixed with the above solution, the absorbance at 750 nm was then measured after 30 min. The total phenolic content was expressed as gallic acid equivalents (GAE, mM) from the calibration curve.

Evaluation of the Antioxidant Capacity: To achieve a more realistic characterization of the antioxidant properties of the beverages, three different antioxidant capacity assays were applied: DPPH, ABTS, and FRAP assays as spectrophotometric assays. Modified versions of the original DPPH, ABTS and FRAP assays were performed to fit the antioxidant capacity analyses according to the procedures described by Brand-Williams et al., 1995 (Brand-Williams, Cuvelier, and Berset, 1995), Re et al., 1999 (Re et al., 1999) and Gou et al., 2003 (Guo et al., 2003), respectively. Trolox standard curves were correlated with the difference in absorbance between a final reading and the reagent blank reading for the spectrophotometric assays. The results were expressed as a mean of three determinations in milligrams of Trolox per gram of beverages.

Statistical Analysis: All experimental results were presented as mean values with their corresponding standard deviations. Significant differences between mean values ($P < 0.05$) were determined by ANOVA using SPSS 16 (SPSS Inc., Chicago, USA). Statistically significant differences were compared between treatment groups.

Results and Discussion

Development of fermentation

The six different kinds of beverages were produced: three milk-based beverages and the three tea-based beverages. The development of all fermentation processes was investigated by measuring pH values in time. Milk-based beverages were incubated until their pH 4.4 and tea-based beverages were incubated for 9 days. pH of these beverages throughout fermentation was presented in Figure 1.

Namely, since investigating the influence of fermentation duration on the milk and tea-based beverages, it has been statistically proven that fermentation duration was a significant variable. As shown in Figure 1, a statistically significant decrease in the pH was observed in all six beverages during the fermentation period ($P < 0.05$). The overall decrease in pH of the beverages would have been due to the increased concentration of organic acids produced during the fermentation process by Kombucha 'tea fungus' as inoculum (Battikh et al., 2012). In milk samples, fermentations were stopped when the pH reached to between 4.4 and 5.7, while these beverages had pH between 6.6 and 7.0 at the beginning of fermentation. Tea-based beverages had the pH between 5.2 and 6.4 prior to the fermentation process. At the end of day 9, the pH of the tea-based beverages varied between 3.4 and 5.6. pH for final product of Echinacea tea-based beverage was observed to be the most acidic, having a final pH of 3.4, while cow's milk-based beverage had the lowest pH of 4.43 at the end of fermentation duration. The pH exchange pattern during fermentation was as expected and it had the same shape for all substrates. Kanuric et al. (2018) investigated the effect of new starter culture on milk fermentation by kombucha and the pH value gradually decreased and after 9 h, fermentation was stopped (Kanurić et al., 2018). This difference between the pH values of the substrates could be the consequence of differences in chemical composition of substrates (Malbaša et al., 2011).

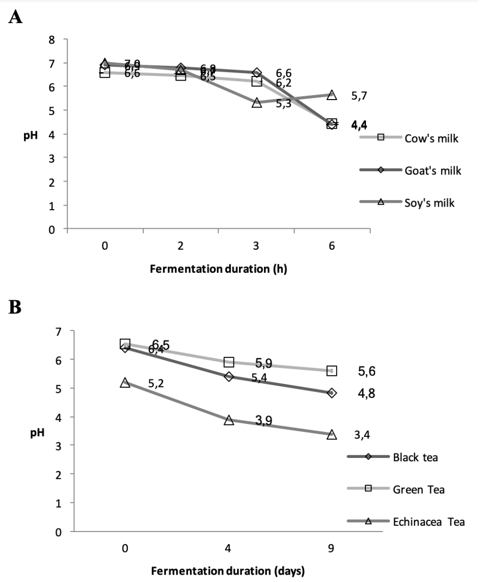

Figure 1. The pH values of milk (A) and tea-based beverages (B) throughout fermentation.

The total sugar content of the beverages is shown in Table 1. Considering these values, the total sugar content of milk and tea-based beverages were lower than unfermented sample (control). Total sugar content decreased Kombucha' tea fungus' consumed glucose in the beverages throughout the fermentation. Soy's milk-based and Echinacea tea-based beverages had the highest reduction in total sugar content while cow's milk-based and black tea-based beverages had the lowest reduction at the end of the fermentation period. Value of pH and amount of total sugar are standard parameters that indicate the success of the production process (Vitas, Cvetanović, Mašković, Švarc-Gajić, and Malbaša, 2018).

Table 1. Total sugar amount values of beverages throughout fermentation (mg l^{-1})

Milk based beverages	Fermentation time (hours)		Tea based beverages	Fermentation time (days)		
	0	6		0	3	9
Goat's milk	$138.74 \pm 2.03^{b, A}$	$88.26 \pm 0.01^{b, B}$	Black tea	155.59 ± 0.81^{c}	155.47 ± 0.93^{b}	154.48 ± 0.023^{a}
Cow's milk	$147.36 \pm 2.1^{a, A}$	$100.47 \pm 3.27^{a, B}$	Green tea	$159.62 \pm 0.031^{a,A}$	$155.63 \pm 0.031^{b,B}$	$155.064 \pm 0.041^{a,C}$
Soy's milk	$78.06 \pm 6.54^{c, A}$	$19.35 \pm 1.01^{c, B}$	Echinachae tea	$157.2 \pm 0.37^{b,A}$	$156.59 \pm 0.23^{a,B}$	$152.87 \pm 2.31^{a,C}$

a-c Different letters in the same column indicate significant differences between means ($p < 0.05$).

A-C Different letters in the same row indicate significant differences between means ($p < 0.05$).

Colour and Titratable activity (TA)

As shown in Table 2, L*, a*, and b* values were measured throughout fermentation. Five beverages except for Echinacea tea-based beverages were observed to get lighter as the fermentation progressed in terms of L* values. Statistically significant changes ($p < 0.05$) in the L* values of all five beverages were observed from beginning point itself as compared with the final products. A sharp increase in the lightness was especially observed in Echinacea tea-based beverage at the end of the fermentation. However, as the L* values, statistically significant changes ($P < 0.05$) in the a* values of all six fermented beverages were observed between the beginning and the end of fermentation. As for the b* values, except for green tea-based beverages other five beverages were observed the increase in the yellowness at the end of the fermentation and statistically significant differences were found ($P < 0.05$) as compared with the beginning of fermentation. Overall, the L*, a* and b* values indicated that the colour value of the beverages changed during fermentation, despite their individual fluctuations. Previous studies showed that the colour changes were associated with the microbial transformation of polyphenols (Jayabalan, Marimuthu, and Swaminathan, 2007).

The statistically significant increase ($p < 0.05$) in the TA in milk and tea-based beverages was observed as compared with the beginning of fermentation. The TA of three milk-based beverages were found between 1.95 and 3.2 at the beginning of fermentation as well as between 8.4 and 12.75 at the end of fermentation. The TA of three tea based beverages varied between 0.45 and 0.65 prior to the fermentation process, while at the end of fermentation, these values varied between 0.65 and 1.55. The statistically significant increase ($P < 0.05$) in the TA in all beverages was observed as compared with the unfermented beverages. These observations were in agreement with the findings of other studies (Xu et al., 2012).

Table 2. The L*, a* and b* values of the milk (A) and tea-based beverages (B) throughout fermentation.

A

Milk based beverages	Fermentation duration (h)	Colour			TA
		L*	a*	b*	
Goat's milk	0	93.1±0.01	-0.88±0.02	8.5±0.01	2.05±0.05
	6	82.72±0.1	0.86±0.03	13.57±0.17	11.9±0.1
Cow's milk	0	91.97±0.02	0.25±0.01	10.64±0.03	1.95±0.05
	6	85.08±0.13	1.12±0.11	13.17±0.47	12.75±0.05
Soy's milk	0	85.77±0.04	1.03±0.11	20.21±0.03	3.2±0.1
	6	78.05±0.44	3.4±0.08	22.24±0.31	8.4±0.1

B

Tea based beverages	Fermentation duration (days)	Colour			TA
		L*	a*	b*	
Black Tea	0	4.62±0.56	2.35±0.16	3.53±0.38	0.45±0.05
	3	4.25±0.05	2.98±0.07	3.4±0.22	0.55±0.05
	9	4.29±0.11	3.61±0.39	4.3±0.37	0.65±0.05
Green Tea	0	6.01±0.2	1.41±0.19	5.25±0.16	0.45±0.05
	3	4.66±0.08	3.78±0.15	4.8±0.23	0.55±0.05
	9	3.12±0.37	2.16±0.8	2.79±0.47	0.65±0.05
Echinacea Tea	0	2.79±0.14	1.47±0.19	3.2±0.12	0.65±0.05
	3	8.09±0.13	4.12±0.1	7.79±0.4	1.05±0.05
	9	7.37±0.02	3.49±0.37	7.73±0.34	1.55±0.05

Determination of Total Phenolic Contents

The total phenolic contents are shown in Table 3. Total phenolic contents of beverages were progressively increased with fermentation duration. Goat's milk-based and green tea-based beverages had the highest total phenolic contents, while soy's milk-based and Echinacea tea-based beverages had the least amount of total phenolic prior to the fermentation. However, following the initiation of the fermentation process, all fermented beverages had a statistically significant increase ($P < 0.05$) in the total phenolics content. Goat's milk based and green tea-based beverages were observed to have the highest total phenolics content by the end of the fermentation since their initial total phenolic contents were high as well. The result of this study were found consistently with other reports (Chakravorty et al., 2016; Jayabalan et al., 2007). De Flippis et al. (2018) determined the total phenolic content of Kombucha tea fermented using ceylon black or bancha green tea. At the beginning of the fermentation, the total phenolic content was found the higher in green tea than black tea. During fermentation, the phenolic content were found stabile in green tea and increase in black tea (De Filippis et al., 2018). Literature data suggest that there were different extraction techniques were performed to extract the phenolic contents (Cvetanovic, Svarc-Gajic, Maskovic, Savic, and Nikolic, 2015). Vitas et al (2018) was found that their Kombucha fermented beverages had higher total phenolic contents as compared to corresponding initial beverages (Vitas et al., 2018). Ayed and Hamdi (2015) used cactus pear as substrate to produce Kombucha-fermented beverages and the results displayed that total phenol content increased with fermentation time (Ayed and Hamdi, 2015). This increase in phenolic components can be explained by the fact that acid hydrolysis and the bioconversion of condensed phenolic components takes place (Jayabalan, Subathradevi, Marimuthu, Sathishkumar, and Swaminathan, 2008).

Table 3. Total phenolic content of milk (A) and tea-based beverages (B) throughout fermentation (µgGAE ml^{-1})

A

Fermentation duration (h)	Milk-based beverages		
	Goat milk	Cow milk	Soy milk
0	$540.65\pm38.6^{a,B}$	$345.15\pm4.12^{b,B}$	$334.8\pm24.46^{b,B}$
6	$1334.43\pm3.88^{a,A}$	$981.52\pm38.6^{b,A}$	$642.19\pm76^{c,A}$

B

Fermentation duration (days)	Tea-based beverages		
	Green Tea	Black Tea	Echinacea Tea
0	$326.5\pm52.68^{a,C}$	$227.78\pm0.88^{b,C}$	$209.65\pm8.87^{b,C}$
3	$380.87\pm28.1^{a,B}$	$261.81\pm0.93^{b,B}$	$256.52\pm9.05^{b,B}$
9	$472.09\pm4.94^{a,A}$	$312.26\pm18.55^{b,A}$	$279.29\pm21.55^{c,A}$

a-c Different letters in the same row indicate significant differences between means ($p < 0.05$).
A-C Different letters in the same column indicate significant differences between means ($p < 0.05$)

Determination of Antioxidant Activity

Total phenolic contents of the beverages are expected to be directly related to their antioxidant capacity (Bravo, Goya, and Lecumberri, 2007). Since no single method can quantify the total antioxidant capacity of the samples, the usage of different antioxidant activity assays is possible. Therefore, three different methods were applied in the this research (DPPH, ABTS and FRAP) in order to achieve a general view of the antioxidant potential of the beverages during fermentation. The antioxidant capacity results of the beverages, measured by the three different methods, were presented in Table 4. Similar behavior patterns were commonly observed for the results of DPPH, ABTS and FRAP assays regardless of their action mechanism. The hypothesis of this study was to investigate the higher antioxidant activity of beverages at the end of fermentation compared to the beginning of fermentation as samples blank.

Results of DPPH assay showed different ability for milk and tea-based beverages (Table 4). Prior to fermentation, soy's milk-based beverage showed minimum DPPH value, while goat's milk based beverage showed minimum DPPH value at the end of fermentation. However, before and after fermentation, maximum DPPH value was observed in cow's milk based beverage. When the different kind of teas were applied as substrates, the highest DPPH value was observed in green tea-based beverages before fermentation while the lowest DPPH value was found in black tea-based beverages. However, at the end of fermentation, black tea-based beverage had the highest DPPH value and Echinacea tea-based beverage had the lowest DPPH value. Amarasinghe et al. (2018) traditionally carried out four kombucha beverages by placing the tea fungal mats in sugared Sri Lankan black tea at varying concentrations for a period of 8 weeks. A statistically significant decrease ($p < 0.05$) in the antioxidant activity was found in these four beverages after fermentation (Amarasinghe, Weerakkody, and Waisundara, 2018).

Table 4. The antioxidant capacity of the beverages measured by DPPH, ABTS, and FRAP (µmol Trolox g $extract^{-1}$).

A

Fermentation duration (h)	Milk based beverages								
	DPPH			ABTS			FRAP		
	Goat milk	Cow milk	Soy milk	Goat milk	Cow milk	Soy milk	Goat milk	Cow milk	Soy milk
0	$2.8\pm0.14^{b,B}$	$3.61\pm0.79^{a,B}$	$0.28\pm0.076^{c,B}$	$1.03\pm0.08^{c,B}$	$1.16\pm0.02^{b,B}$	$1.09\pm0.096^{a,A}$	$1.46\pm0.039^{a,B}$	$0.75\pm0.28^{b,B}$	$0.65\pm0.20^{b,B}$
6	$0.061\pm0.006^{c,A}$	$0.28\pm0.003^{a,A}$	$0.2\pm0.014^{b,A}$	$0.81\pm0.06^{c,A}$	$0.96\pm0.03^{c,A}$	$1.03\pm0.02^{a,A}$	$2.06\pm0.031^{a,A}$	$2.02\pm0.02^{a,A}$	$1.39\pm0.05^{b,A}$

a-c Different letters in the same row indicate significant differences between means ($p < 0.05$).
A-C Different letters in the same column indicate significant differences between means ($p < 0.05$).

B

Fermentation duration (days)	Tea based beverages								
	DPPH			ABTS			FRAP		
	Green Tea	Black Tea	Echinacea Tea	Green Tea	Black Tea	Echinacea Tea	Green Tea	Black Tea	Echinacea Tea
0	$2.32\pm0.04^{b,A}$	$2.18\pm0.01^{c,C}$	$2.26\pm0.04^{b,B}$	$1.32\pm0.00^{c,A}$	$1.30\pm0.02^{c,B}$	$1.27\pm0.013^{b,C}$	$1.54\pm0.011^{b,A}$	$0.61\pm0.013^{c,C}$	$0.91\pm0.24^{b,B}$
3	$2.30\pm0.04^{b,C}$	$2.06\pm0.01^{b,A}$	$2.25\pm0.05^{b,B}$	$1.30\pm0.00^{b,A}$	$1.12\pm0.00^{b,B}$	$0.93\pm0.07^{a,C}$	$1.62\pm0.04^{a,A}$	$1.21\pm0.01^{b,B}$	$1.12\pm0.13^{b,B}$
9	$1.81\pm0.35^{a,AB}$	$2.02\pm0.02^{a,A}$	$1.59\pm0.11^{a,B}$	$1.29\pm0.00^{a,A}$	$1.06\pm0.00^{a,B}$	$0.87\pm0.01^{a,C}$	$1.66\pm0.01^{a,A}$	$1.38\pm0.002^{a,B}$	$1.37\pm0.12^{a,B}$

a-c Different letters in the same column indicate significant differences between means ($p < 0.05$).
A-C Different letters in the same row indicate significant differences between means ($p < 0.05$).

Before and after fermentation, among the all tea substrates green tea was found to be the best substrate which showed higher ABTS value, whereas the least ABTS value was observed in Echinacea tea based beverage. Ayed and Hamdi (2015) performed to determinate the two antioxidant activity in the fermented juice: DPPH and ABTS. The DPPH and ABTS radical scavenging abilities of the fermented juice increased significantly (Ayed and Hamdi, 2015). However, the results in current studies displayed incompatible with that study. This situation may be explained to the utilization of phenolic compounds by the tea fungus. Because, scavenging effects were formed from many of the compounds present in the fruit and their synergistic effects (Amarasinghe et al., 2018).

Among the three tea substrates studied, green tea performed better ability which was similar to those observed in DPPH value. At the beginning of fermentation, the lowest FRAP value was observed in black tea and thisvalue was attained in Echinacea tea at the end of fermentation. Among Kombucha based beverages prepared from three different milk, fermented goat's milk was considered to be the best beverage which had the ability of FRAP reaction, while fermented soy's milk can have the lowest effect on the FRAP value both before fermentation and at the end of fermentation.

A review of the literature concerning antioxidant capacity of kombucha beverages reveals a quite difficult comparison among the reported data, mainly because of the utilization of different analytical methods (ABTS, CUPRAC, DPPH, FRAP, ORAC, or TRAP, among others), standards, reference units (in a wet or dry matter basis). Furthermore, substrates might influence significantly their antioxidant capacity (Ayed, Ben Abid, and Hamdi, 2017; Hrnjez et al., 2014; Malbaša et al., 2014, 2011; Villarreal-Soto, Beaufort, Bouajila, Souchard, and Taillandier, 2018).

Conclusion

The present study demonstrated that milk and tea-based beverages prepared from different substrates have excellent antioxidant activities. It has been interested to note and use kombucha-based beverages in preventing diseases caused by the overproduction of free radicals. Kombucha fermented beverage displayed an increase in antioxidant activities of beverages during fermentation. The extent of the activity depended upon the fermentation time and substrates. However, further investigations are required to investigate these results and the study which carried out with the different kind of substrates can be used as a platform to form more studies in the future based on different starter cultures, microbial compositions, fermentation duration etc. and their combined effects as well as on changing the health effect of kombucha beverages during various periods of fermentation.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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The effect of high hydrostatic pressure on some quality parameters of a traditional tortellini-like Turkish food (mantı) packed with at modified atmosphere

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Abstract

Traditional cereal-based foods need to be thoroughly processed to fulfill the transportation conditions to distant locations. The present study examines the combined use of high hydrostatic pressure (HHP) and modified atmosphere packaging (MAP) processes in mantı, a traditional tortellini-like Turkish food. After the mantı's inner material and dough were separately processed with a HHP treatment at 400 MPa and 600 MPa, the mantı was prepared, and packaged under the modified atmosphere (60% CO_2 + 40% N_2) and stored for 14 days at 4°C. Also, the thermal inactivation was examined of inoculated mantı samples with bacterial cells of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*. The differences of physicochemical and microbial results were found significant ($P < 0.05$) among the samples, as affected by high pressure at the modified atmosphere package. In case of the consumer sensory evaluation regarding cooking, HHP processed samples (HHPR) were more liked than semi-processed (CR) samples (stored for 14 days at 4°C). Inoculated bacterial cells to mantı samples exhibited varying inactivation responds at different temperatures (55 to 75°C) of heat treatment. As a result, it has been found that the high hydrostatic pressure treatment is a promising process for the mantı packaged in the modified atmosphere, extending the 7-day storage time compared to the untreated control sample.

Keywords: Consumer acceptability studies, Non-thermal processing, High hydrostatic pressure, Modified atmosphere packaging, Mantı

Introduction

In the food and packaging industry, there are many attempts to implement new processing methods for food products. Among these are traditional foods that must be industrialized for both of the domestic and export markets. Mantı consists of a wheat dough (75% w/w of mantı) filled with minced beef, onion, and spices (25% w/w of mantı).

If mantı is to be consumed immediately, then it is cooked in boiling water (200 g mantı in 2 liters of water) for 10-12 mins. Before serving, a sauce is prepared by combining and cooking butter, tomato paste and ground red pepper. If desired, yogurt, garlic, sumac, red pepper and mint are added. When there is a need for storage, mantı is frozen or oven baked before packaging in industrial applications. Packaging is mostly applied in plants by using a vacuum or modified atmosphere (Sitti, 2011). Vacuum and modified-atmosphere packaging extends the shelf life of mantı up to four months (Uzunlu and Var, 2016).

Mantı is a highly perishable product due to its high water activity (>0.87), moisture (>40%) and pH content (6.0-7.0), as well as the fact that its nutrients are beneficial for microorganism growth (Uzunlu and Var, 2016). In the context of cereal-based foods, the standards limit the presence of potential food poisoning risk carrying microorganisms, such as *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella*

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spp., pathogenic *Escherichia coli*, and spore forming bacteria (Anonymous, 2011).

Previous research has mainly focused on modified-atmosphere packaging. One such study investigated the effects of both heat treatment and modified-atmosphere packaging (MAP) on mantı. Both the wheat dough and filling were first boiled and then baked (dried), followed by modified atmosphere packaging with different gas compositions. A 70% CO_2 + 25% N_2 + 5% O_2 provided a stable mantı product as judged against microbial, chemical and sensorial parameters for 35 days at 4°C (Yücetepe, 2011). Other research has been carried out to determine the refrigerated storage shelf life of modified-atmosphere-packed mantı. The findings shown that both 60% CO_2 and 80% CO_2 with N_2 led to stable storage at 4°C for 4 months (Uzunlu and Var, 2016). Similarly, Sitti et al. (2009) reported that the modified-atmosphere packaging of mantı was found more effective than vacuum packaging in terms of achieving at least one month of stability during refrigerated storage.

As stated above, depending on the processing conditions, heat treatment or freezing may be applied prior to mantı packaging. However, any heat treatment applied to the filling may adversely affect the overall quality of the mantı. For example, heat treatment may cause the leakage of meat lipids from the dough of the mantı, in addition to browning the meat, which, together, may result in poor sensory quality and reduced consumer purchase intent. High hydrostatic pressure (HHP) treatment has been used for over 20 years as a cold pasteurization or non- thermal processing method (Bermúdez-Aguirre and Barbosa-Cánovas, 2011). This treatment is usually applied in the range of 100 and 1000 MPa (Bárcenas et al., 2010). The use of high hydrostatic pressure permits new process development opportunities both for industry and researches (Rastogi et al., 2007).

Because of the stated reasons, the current research aimed to assess; (a) the combined effect of HHP and MAP for mantı storage at 4°C, and (b) to determine the thermal death responses of some common food-borne bacteria in mantı.

Materials and Methods

Peptone water (0.1%), tryptic soy broth (TSB), tryptic soy agar (TSA), nutrient agar (NA), 1,2-propanediol (used as a pressure medium) were purchased from Sigma-Aldrich (United Kingdom). Minced beef (fat content <20%, collagen/meat protein ratio <25%) and strong white fine wheat flour (Allinson, UK) were purchased from a local market in Reading, Berkshire (UK).

High Hydrostatic Pressure and Modified Atmosphere Packaging Applications

For this purpose, three sample types were prepared and analyzed. (I) High Hydrostatic Pressured (HHPR) samples, (II) Semi-Processed (CR) samples, and (III) Control samples. High hydrostatic pressure was applied at 400 MPa and 600 MPa to the filling material (minced beef) and the wheat dough for 15 mins at room temperature, respectively. The mantı samples which prepared with dough treated with HHP, and minced beef untreated with HHP was defined as Semi-Processed mantı samples (CR). While HHPR mantı samples were prepared by treating both of the dough and minced beef with HHP. Prior to treatment, samples of beef mince and wheat dough was gently placed in polyethylene (PE) bags, double wrapped, and sealed. Stansted Fluid Power Ltd (UK) high pressure rig was used at the food process center of University of Reading Food and Nutritional Sciences Department's (UK).

Mantı was prepared from wheat flour, minced beef and sterilized distilled water. The dough was prepared by mixing wheat flour and water (35-40% w/v, flour basis) followed by the application of high pressure, as described above. It was then sheeted and cut into equal squares (using a kitchen knife), with an average sheet thickness of 0.19mm (±0.02). Each square of dough (1.125±0.1g) was filled with an equal amount (0.375±0.1g) of beef mince (25% w/w of mantı), and the squares were then folded diagonally by hand to form the distinctive mantı shapes under the hygienic procedures. Mantı samples were placed in PE bags, and were filled as to be with with 60% CO_2 + 40% N_2 gas mixture in a 2/1 gas volume to mantı weight ratio in each bag was applied and sealed using a Multivac A 300 packaging unit (under 2 bar pressure and 75L/min gas flow) at they were put in cold storage at 4°C for 14 days (34% relative humidity).

Physicochemical and Microbial Analyses

From each sample types ten grams of mantı were weighed in a sterile stomacher bag containing 90mL peptone water (0.1%) and homogenized in a stomacher (Seward 400, UK) at 230 rpm for 2 mins. Serial decimal dilutions were prepared and spread onto TSA. Plates were incubated for 24–48h at 37°C in terms of pathogen microorganisms. Each three sample types (HHPR, CR, Control) were stored at 4°C for 14 days and analyzed on each sampling days (0, 2, 4, 7, 14). Color analyses were performed using a ColorLite sph850 instrument (ColorLite GmbH, Germany). Illuminant was D_{65} and observation angle was 10°. For measuring, port was applied force onto the samples, and the size of port was 3.5 mm. Triplicate readings were evaluated for C.I.E. L^*, a^*, b^* parameters. While, ten grams of samples were homogenized in 90 mL of distilled water and pH value was recorded by using pH meter (SevenEasy, Mettler Toledo) averaging 3 measurements. The a_w of the mantı samples were measured and recorded using a Pawkit water activity meter (Decagon, WA). Calibration was adjusted to 0.76 a_w with NaCl standard before measurement (Clavero and Beuchat, 1995; AOAC, 2000).

Experimental Design and Protocol of Consumer Study

Sixty healthy consumers were involved in the study. Individual sensory booths at the sensory science center (Department of Food and Nutritional Sciences, University of Reading, UK) were used. All consumers (*N*=60) were recruited from the University campus and provided informed written consent. For both of the high hydrostatic pressured (HHPR) and semi-processed (CR) samples two sample types were prepared and evaluated; raw and cooked mantı. Due to the spoilage on day 7 of the untreated control, this sample was excluded from the consumer study. Raw mantı samples were presented to consumers in their original package, stored for either 4 or 14 days at 4°C. Appearance of raw mantı samples (10g +/- 0.1) was rated using a nine-point hedonic category scale (categories from dislike extremely to like extremely). For cooked samples, packages (10g +/- 0.1) of mantı (stored for 4 or 14 days) were opened and cooked in a boiling water at min. 75°C for 7 minutes. The temperature was continuously monitored via two copper-constant thermocouples inserted into the core of each mantı piece and held for 7 mins after the core temperature reached above 75°C. Samples were kept warm at 50°C for an hour until served to the consumers. Cooked samples were served plain (without yogurt and spices). Overall liking of cooked mantı samples, followed by liking of appearance, taste and texture, were rated using the nine-point hedonic category scale. Free text comments for each samples were collected. Four batches of samples were served in balanced order in a monadic sequential manner; each session lasting no more than forty-five minutes. Testing was held between 15:00 and 19:00 and consumers were scheduled every hour. Data were collected using Compusense (version 5.5, Ontario, Canada).

Bacterial Inoculation and Heat Treatment

The indigenous microbial flora of both minced beef meat and dough were inactivated prior to inoculation of bacterial cultures to mantı pieces by applying HHP at 600 MPa for 15 mins at room temperature, and checked before the inoculation procedure. *Staphylococcus aureus* NCTC 01803, *Escherichia coli* K12 NCTC 10538, and *Bacillus cereus* NCDO 0578 strains were acquired from the National Collection of Type Cultures (NCTC, England). The preserved strains at -80°C in vials containing TSB were thawed at room temperature in a biosafety cabinet. One ml of the cultures was transferred to 50 ml of TSB in 250 ml flasks, where incubated at 37°C for 24h. Following incubation, the freeze-damaged cells were not used in the thermal treatments. Where, 0.1 ml of each culture were pipetted to 10 ml TSB tubes, and incubated at 37°C for 24h. The cultures were prepared daily in a week basis. Each culture was centrifuged at 5000 *g* for 10 mins at 4°C and washed twice in peptone water (PW). The supernatant was discarded, and pellets were diluted to obtain a final concentration of 3 logs CFU/mL.

Three groups of mantı samples for each bacterial culture were prepared. Each group samples were aseptically placed into the stomacher bags. The bags composed of 15 grams of mantı samples (the weight of one piece is 1.5±0.1g). The mantı bags (contains 10 mantı pieces) were then divided into three groups (four packages were used for each group). 500µL of bacterial cultures of *S. aureus*, *E. coli* and *B. cereus* were separately inoculated with either mantı pieces (50 µL per each) for each bag. Bacterial cultures were pipetted into the distinctive shapes of each mantı pieces. The samples were held at 37°C for 4 h to allow the propagation.

Cultures were then aseptically placed onto sterilized glass lenses (85mm in diameter), and heat treated at 55°C to 75°C for 4 mins in an oven (Gallenkamp, UK). The temperature degrees were continuously monitored. Two copper-constant thermocouples inserted into the core of each mantı piece and held for 4 mins after the core temperature reached above the specified degrees. Bags were then cooled in an ice box and homegenised (Seward 400, UK) in a stomacher at 230 rpm for 2 mins. Peptone water (0.1%) was used for homogenization. Serial decimal dilutions were prepared and spread onto NA for *B.cereus*, and TSA for *S. aureus* and *E. coli*. Plates were incubated at 30°C for *B.cereus* and 37°C for *S. aureus* and *E. coli* for 24-48h, prior to counting the colonies.

Statistical Analysis

Each experiment was repeated twice and duplicate (microbiological and a_w analyses) samples were tested at each sampling time. In order to determine the effect of high pressure treatment, ANOVA was used to evaluate the data, using Statistical Package for the Social Sciences (SPSS) software, version 21 (IBM, USA)). Duncan's *post hoc* test was applied at a significance level of P< 0.05. ANOVA was performed to determine the significant differences between samples in consumer liking ratings. To determine whether there were groups of consumers exhibiting similar liking patterns, agglomerative hierarchal cluster analysis (AHC) was carried out using Ward's method and dissimilarity. The mean liking scores of these clusters were then re-analyzed by ANOVA. Multiple pairwise comparisons were determined using Tukey's HSD test. All analysis of consumer data was done using XLStat (AddinSoft, France, 2007).

Results and Discussion

Physicochemical and Microbial Analyses

The semi-processed (CR), pressure processed (HHP) and control group raw samples were analyzed for color at five stages of shelf life (0, 2, 4, 7 and 14 days) and substantially affected by the high pressure; *L** values decreased after day 2, indicating that the samples became darker. *L** values of control and semi-processed samples were lower than for high pressurized samples (Table 1). In terms of *a** and *b** values, high pressurized samples' values were significantly (P< 0.05) lower than semi-processed group samples (Table 1) indicating that the samples became less red and yellow in hue. Decreased *a** values lead to the loss of redness in meat. The largest difference among the high pressurized, control and semi-processed samples were observed for *a** values (Table 1) because the redness of the meat was lost due to high-pressure treatment.

Jung et al. (2003) state that the luminosity of the meat can be heightened via HHP. Also, these increased *L** values become steady for pressures around 300–400 MPa. This can be attributed to the denaturation of globin and heme displacement or release; an increase in drip losses, leading to water content changes in the meat; or damage to porphyric rings and protein coagulation (Jung et al., 2003). In case of the wheat dough, Bárcenas et al. (2010) showed that *L** values were not significantly (P> 0.05) different between the control (untreated dough) and the treated doughs (50 to 250 MPa).

When the shelf life of the meat increased, the meat assumed a more cooked appearance because actomyosin denatures at about 200 MPa and myoglobin denatures and/or co-precipitates with other proteins at about 400 MPa (Ma and Ledward, 2013). In a research project by Bárcenas et al. (2010), *a** values were found to be significantly decreased in wheat dough (without meat) samples exposed to HHP (50 to 250 MPa) as compared to control (untreated dough) samples. The findings of the current study show that the *a** values of the HHP, control and semi-processed samples decreased during storage due to the lack of oxygen in the package atmosphere. It is well-known that oxygen maintains the redness of meat (Narasimha Rao and Sachindra, 2002). Similar to *a** values, *b** values increase during the blooming of meat (Young et al., 1999). Generally, an increase in *b** indicates increased yellowness in foods. *b** values increased from day 0 to day 14 in all sample types (Table 1).

Table 1. pH, a_w, color and microbial changes during 14 days of storage at 4°C [1,2]

	Storage days				
	0	**2**	**4**	**7**	**14**
Parameters					
pH					
HHPR	6.29±0.01ax	6.32±0.03ax	6.45±0.01bx	6.60±0.02cx	6.50±0.03bx
CR	6.19±0.04ay	6.18±0.02ay	5.48±0.04by	5.37±0.02cy	4.71±0.01dy
CONTROL	6.24±0.03	5.45±0.03	5.39±0.01	ND	ND
a_w					
HHPR	0.96±0.01abx	0.98±0.00bx	0.93±0.02ax	0.88±0.01cx	0.90±0.00cx
CR	0.91±0.00ay	0.95±0.00by	0.98±0.00cy	0.91±0.01ay	0.88±0.01dx
CONTROL	0.92±0.01	0.90±0.01	0.88±0.01	ND	ND
L*					
HHPR	49.56±0.89ax	51.90±1.16bx	45.42±1.54cx	44.48±2.38cx	32.68±5.23dx
CR	35.11±1.34ay	37.79±0.73by	28.64±5.65cy	29.43±4.36cy	26.20±5.95dy
CONTROL	27.10±1.24	32.58±1.76	22.50±1.39	ND	ND
a*					
HHPR	4.50±0.56ax	4.53±0.46bx	4.95±1.41cx	3.42±1.23dx	2.92±0.63ex
CR	9.70±1.18ay	8.55±1.97ay	8.47±0.83by	8.13±1.16cy	7.75±2.37dy
CONTROL	10.60±1.69	10.40±0.93	8.97±0.69	ND	ND
b*					
HHPR	18.58±1.88ax	17.51±1.38ax	18.78±1.33ax	20.47±2.61bx	21.35±1.64bx
CR	21.05±2.00ay	21.19±2.21ay	24.51±2.09by	22.77±1.51abx	22.57±2.02abx
CONTROL	22.31±0.92	22.67±1.81	23.44±0.14	ND	ND
TAMB					
HHPR	0.1±0.05ax	4.04±0.02bx	5.23±0.04cx	5.69±0.10cx	5.81±0.00dx
CR	3.29±0.04ay	6.66±0.08by	6.49±0.06cy	6.69±0.00cy	7.41±0.00dy
CONTROL	3.73±0.44	5.76±0.9	7.22±0.19	ND	ND

[1]TAMB= total aerobic mesophilic bacteria; ND= not determined owing to the spoilage.
[2] Mean data and standard errors in the same raw bearing different lower-case letters (a, b, c, d) are significantly different (P<0.05) from each other. Mean data and standard errors in the same column bearing different lower-case letters (x, y, z) are significantly different (P <0.05) among each process.

According to the microbiological analyses, total aerobic mesophilic bacteria counts increased over storage for all the studied samples. As expected, this increase was lower in the high pressurized samples compared to the control and semi-processed samples (Table 1). In fact, high pressurized sample's microbial load was 3 log lower than control and semi-processed samples on day 0. The microbial counts increased in all sample groups, while high pressurized sample's load was 1.6 log colony forming unit/g lower than semi-processed sample on the 14th day. The control sample spoiled after day four, resulting in no available data for days seven and fourteen.

The pH of the high pressurized samples was slightly increased compared to neutral values; however, the pH of the control and semi-processed samples decreased during storage. This finding was correlated with an increase in microbial growth in the semi-processed samples. Microbial cell growth leads to decreased pH values in some foodstuffs (Jay et al., 2005). There were significant (P< 0.05) but slight differences in pH between the high pressurized samples. The increase from HHPR0 to HHPR14 was 0.31 (Table 1). However, pH of the control and semi-processed samples decreased from day 0 to day 14 (Table 1). Therefore, differences of the samples were significant (P <0.05). The a_w value is a reliable standard parameter that affects the microbial stability of fresh foods (Kong and Singh, 2011). The findings show that a_w values ranged between 0.88 and 0.96, however differences were found significant (P <0.05) between semi-processed and high pressurized samples (Table 1). Such values are optimal for the growth of spoilage and pathogenic bacteria (Jay et al., 2005).

As is already well-known, microbial growth limits the shelf life of foods (Nychas and Panagou, 2011). Such previous research used HHP on cookie dough for preservation and cookie quality. Cookie dough, which had 6 log colony forming unit per gram total aerobic mesophilic bacteria in control samples (untreated with HHP) were subjected to HHP at 100 and 200 MPa for 2 and 4 min. and 400 MPa for 15 min. resulted in 4 log reduction. After HHP treatment in heat-sealed bags, the cookie dough stored at ambient temperature for 7 days did not increase in total aerobic mesophilic counts, while counts of the control samples increased 5 times (Aguirre et al., 2018). Hence, in our study the dough in heat-sealed PE bags after HHP treatment were opened, and immediately used in mantı preparation. The microbial counts were, therefore, increased during cold storage (Table 1).

Microbiological safety of the raw materials to be used in food recipes should be high. One such study applied high pressure to provide microbiological safety to the meat used to prepare Çiğ Köfte, and found a six decimal microbial reduction at 300 MPa at room temperature treatment (Uzunlu, 2019).

Once the meat became discolored, at pressures higher than at 325 MPa, microbiological improvement was achieved. If an improvement in meat color redness for only a few days is needed, then 130 MPa is required. However, this level of pressure is not sufficient to modify the microbial load of the meat (Jung et al., 2003). Taking into account both the meat's location (inside of mantı) and its package without oxygen suggested the use of 400 MPa in the current study, because pressures of ~300–600 MPa are sufficient to inactivate the vegetative cells of microorganisms (Rastogi et al., 2007). Moreover, pressures above 300 MPa cause the irreversible denaturation of enzymes and proteins, leading to the rupturing of the cell membrane and the excretion of internal substances, ultimately causing bacterial death (Huang et al., 2014). It is well-known that MAP extends the shelf life of foods (Narasimha Rao and Sachindra, 2002; Zhang et al., 2016). Using CO_2-enriched atmospheres also extends the shelf life of mantı in refrigerated storage, as previously reported (Yücetepe, 2011; Uzunlu and Var, 2016). While, the use of high pressure and modified-atmosphere packaging provided microbial stability to mantı for 14 days of refrigerated storage in the current findings.

Thermal Inactivation of Inoculated Bacteria

The effect of high hydrostatic pressure on the indigenous microbial flora of raw materials of mantı were as follows.

Total microbial cells in dough and minced beef were inactivated in 15 mins at 600 MPa. N_0 (initial load) was 4 log CFU/g in dough and 3.65 log CFU/g in minced beef. In terms of the thermal treatment in mantı samples, no inactivation was recorded at 55°C for all the inoculated bacterial cells (*S.aureus*, *E.coli*, *B.cereus*). *S.aureus* was inactivated by 1.1 log CFU/g to 1.81 log CFU/g at 60°C to 75°C. No inactivation at 60°C was recorded for *E.coli*. And the cells were inactivated by 0.23 log CFU/g to 0.51 log CFU/g at 65°C to 75°C in mantı samples. This was similar for *B.cereus*. No inactivation was observed at 60°C. 0.3 log CFU/g inactivation was recorded at 65°C, while a total inactivation was recorded at 70°C and 75°C.

As a sum, *E.coli* was found more resistant than *S.aureus* and *B.cereus*. Heat treatment at 75°C for 4 mins to inactivate 3 log of the *S.aureus* and *B.cereus* cells was found adequate, while this was found inadequate for *E.coli* cells. This finding should be taken into account for providing food safety of this product. Because, currently industry is using heat or freeze treatment in mantı production. We studied several combinations of heat (55 to 75°C) and time (1 to 6 mins) treatments in mantı samples (Uzunlu, 2013; Uzunlu and Var, 2014).

When mantı held in the oven in increasing periods, it resulted in leaking meat lipids from the dough of mantı and browning the mantı pieces. For this reason, the mantı retention time in the oven was determined optimum 4 minutes at max. 75°C.

As stated by Rastogi et al. (2007) HHP should be combined with heat treatment, to avoid the risk of spore germination in foods. Because, evaluating HHP as an alternative to heat treatment might lead to have the risk of spore resistant bacterial presence in the products. Where, very high resistance to increased levels of high pressure, such as 1000 MPa, was reported for bacterial spores (Rastogi et al., 2007).

Consumer Acceptability

When the consumer acceptability of high hydrostatic pressure processed mantı was evaluated we could say that, there was no significant difference between for appearance liking scores of the high hydrostatic pressured raw samples (HHPR) and semi-processed raw samples (CR) of mantı whether stored for 4 or 14 days at 4°C. There was a significant difference in taste liking scores between the cooked high pressure processed (HHPC14) and semi-processed samples CC14 (P=0.001), with the HHPC14 samples being more liked than semi-processed samples CC14, where both had been stored for 14 days at 4°C. However, consumers found no significant difference in taste liking between the high pressure processed (HHPC4) and semi-processed samples (CC4) when cooked after storage for 4 days at 4°C. In addition, there was no significant difference between the processed and semi-processed samples, whether for 4 or 14 days of storage at 4°C, for the parameters of overall liking, liking of appearance and texture (Table 2).

Taking the consumer evaluation into account, the consumers preferred raw HHPR14 to CR14 in terms of appearance (Figure 1A). Therefore, consumers preferred high pressurized samples stored for 14 days at 4°C to semi-processed samples stored in the same way. Similar results were found for the cooked mantı samples. Consumers preferred HHPC14 and HHPC4, shown at Figure 1B, 1C, 1D, and 1E, to CC14 and CC4 regarding all of the tested parameters (overall liking, taste, texture and appearance). Although not all consumers had the same liking trends (as shown by the cluster analysis), the overall conclusion is that at both 4 and 14 days of storage at 4°C, the high pressurized samples were preferred to the semi-processed samples.

There were three patterns of overall liking of the cooked mantı; the largest two clusters were of similar size (35% and 38.3%). Cluster 1 liked both high pressure processed (HHPC) mantı samples significantly more than semi-processed (CC) samples, however cluster 2 gave lower scores to all cooked mantı and particularly disliked the HHPC sample that had been stored for 4 days. When comments of cluster 1 were analyzed, consumers encountered that the dough was opened and the meat had come out in some of the samples, for both HHPC and CC. The lower scores of the CC samples compared to the HHPC samples were due to shape, tastelessness and moist texture. However, cluster 2 consumers mostly gave lower scores to HHPC4 samples, similarly for the reason that filling had come out of the dough when cooked.

Table 2. Mean liking scores for clusters of consumers following hierarchical cluster analysis [1,2]

Appearance liking scores of raw mantı						
Cluster	N (%)	Samples				
		CR4	CR14	HHPR14	HHPR4	Significance of sample (P-value)
1	5 (8.3)	2.6	2.2	2.4	3.0	n/a
2	23 (38.3)	6.3	5.9	6.3	5.7	<0.0001
3	32 (53.4)	4.6	4.3	4.2	5.3	<0.0001
Overall liking scores of cooked mantı						
Cluster	N (%)	Samples				Significance of sample (P-value)
		CC4	CC14	HHPC14	HHPC4	
1	21 (35.0)	5.6	5.4	6.0	6.2	<0.0001
2	23 (38.3)	4.2	4.8	4.7	3.3	<0.0001
3	16 (26.7)	2.5	2.6	3.2	3.6	n/a
Appearance liking scores of cooked mantı						
Cluster	N (%)	Samples				Significance of sample (P-value)
		CC4	CC14	HHPC14	HHPC4	
1	19 (31.7)	4.8	3.5	5.1	4.2	<0.0001
2	17 (28.3)	3.0	6.6	4.9	3.9	<0.0001
3	11 (18.3)	2.0	2.5	2.7	2.6	n/a
4	13 (21.7)	6.3	5.9	5.3	6.3	<0.0001
Taste scores of cooked mantı						
Cluster	N (%)	Samples				Significance of sample (P-value)
		CC4	CC14	HHPC14	HHPC4	
1	39 (65)	3.9 ^{a,b}	3.6 ^{a}	4.6 ^{b}	4.3 ^{a,b}	0.001
2	9 (15)	2.3	1.7	2.4	2.1	n/a
3	12 (20)	6.8 ^{a,b}	6.0 ^{a}	6.9 ^{b}	6.0 ^{a,b}	<0.0001
Texture scores of cooked mantı						
Cluster	N (%)	Samples				Significance of sample (P-value)
		CC4	CC14	HHPC14	HHPC4	
1	16 (26.7)	5.1	5.7	3.6	5.3	<0.0001
2	31 (51.7)	3.3	2.9	4.4	3.4	<0.0001
3	13 (21.6)	6.3	6.1	6.5	6.1	<0.0001

[1] CR4= semi-processed (dough treated with HHP, but with untreated filling) raw samples stored for 4 days; CR14= semi-processed raw samples stored for 14 days; HHPR4= high hydrostatic pressured raw samples stored for 4 days; HHPR14= high hydrostatic pressured raw samples stored for 14 days; CC4= semi-processed samples stored for 4 days served as cooked; CC14= semi-processed samples stored for 14 days served as cooked; HHPC4= high hydrostatic pressured samples stored for 4 days served as cooked; HHPC14= high hydrostatic pressured samples stored for 14 days served as cooked; n/a= ANOVA was not analyzed on these clusters due to their small size.

[2] Data was analyzed by one-way ANOVA for comparisons between mantı samples of within each cluster of consumers followed by Tukey's post hoc test. Different superscript letters within the same row indicate significant differences between mantı samples at $P < 0.05$.

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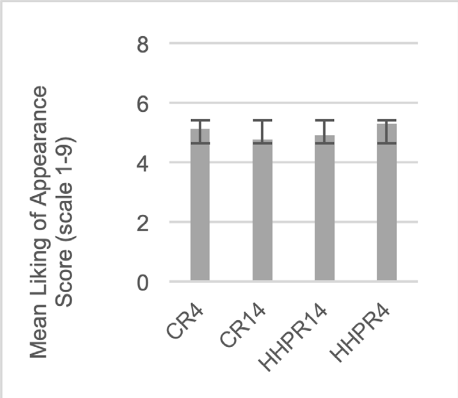
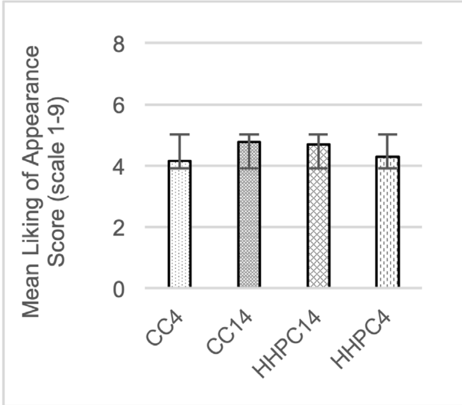
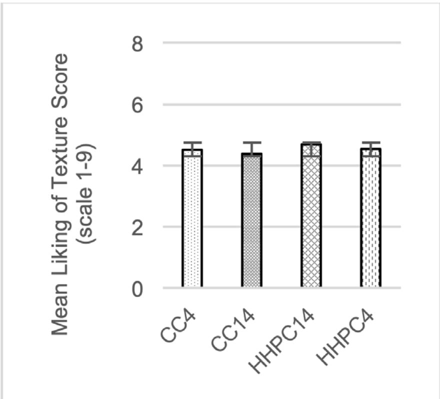
1A. Raw Mantı Appearance Liking



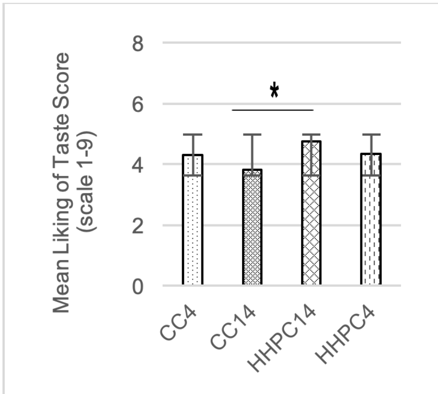
1B. Cooked Mantı Appearance Liking



1C. Cooked Mantı Texture



1D. Cooked Mantı Taste



1E. Cooked Mantı Overall Liking

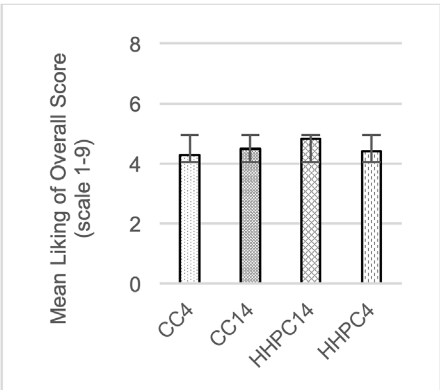


Figure 1. Mean liking scores for clusters of panelists following hierarchical cluster analysis of liking scores.[1,2]

[1] CR4= semi-processed (dough treated with HHP, but with untreated filling) raw samples stored for 4 days; CR14= semi-processed raw samples stored for 14 days; HHPR4= high hydrostatic pressured raw samples stored for 4 days; HHPR14= high hydrostatic pressured raw samples stored for 14 days; CC4= semi-processed samples stored for 4 days served as cooked; CC14= semi-processed samples stored for 14 days served as cooked; HHPC4= high hydrostatic pressured samples stored for 4 days served as cooked; HHPC14= high hydrostatic pressured samples stored for 14 days served as cooked.

[2] Values are means ± SD. * indicates significant difference at $P < 0.05$.

Conclusion

Combined use of HHP and MAP substantially improved the microbiological and sensorial parameters of both raw and cooked mantı samples compared to semi-processed and control untreated samples, and was extended refrigerated storage from 7 days to 14 days. However, the need for studying rheology of the HHP processed dough is apparent, in further studies. Because, for instance, while serving cooked mantı the important problem was filling material had come out of the dough. As a conclusion, when all data evaluated it can be said that the results can contribute to new processing conditions of high hydrostatic pressure and modified atmosphere packaging treatments.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Growth and minituber yield response of potato plantlets in micropropagation to different plant spacing under greenhouse conditions

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Abstract

The present research work was conducted to find out the most suitable inter and intra spacing for Greenhouse transplantation of *in vitro* grown potato plantlets in peat moss soil in order to get maximum seed potato yield. There were nine different combinations of inter and intra row spacing (30x15, 30x10, 10x15, 20x5, 30x5, 10x5, 20x15, 10x10, 20x10cm) in completely randomized design. Regarding vegetative growth, maximum plant height i.e 59.01 cm, 58.92 cm, 57.01 cm was observed at 30cmx5cm, 20cmx5cm, and 10cmx5cm, respectively. Greater number of minitubers in each tuber category i.e. large, medium and small were obtained at greater inter and intra plant spacing , whereas the maximum average tubers yield per plant (165.0g) were recorded at spacing of 30cm x15cm followed by the spacing 20cm x10cm where the average yield per plant was 145.38g. It was concluded that the plant spacing of 30x15cm is the best for potato plantlets transplantation in the greenhouse benches in order to get maximum minituber yield.

Keywords: Inter and intra plant spacing, Potato, Growth parameters, Minituber yield

Introduction

Vegetables are rich and comparatively cheaper source of vitamins. Potato (*Solanum tuberosum*) is one of them. It is a starchy, tuberous crop from the perennial night shade family *Solanaceae.* Potato plays an important role to mitigate the food and nutritional requirements of the fast growing population of developing countries of the world. The balance of protein to calories and the composition of minerals make potato second only to eggs in nutritional value as a single source (Swaminatha and Sawyer, 1983).

Among the root and tuber crops, it ranks first followed by cassava, sweet potatoes and yams (Hawkes, 1990; FAO, 2008). There are a number of constraints that negatively affect potato production in the world and these include lack of quality seed potato, declining soil fertility and structure due to poor management practice, diseases etc (Demo *et al*., 2007, 2009).

In Pakistan, it is also an important vegetable with an area and production of 177.3 thousands hectares and 3977.6 thousand tons, respectively during 2015-16 (GOP 2016). Although potato production in Pakistan has increased many folds but it's per acre yield is far less than in other parts of the world (Malik, 1995).

Among the various factors responsible for low per acre potato production, potato diseases are considered to be the most important. More than 18 potato diseases are reported in Pakistan, of which 13 are of common occurrence. Their importance, however, varies considerably in different potato growing areas (Ahmad *et al.,* 1991).

Tissue culture helps in the production of disease free potato because shortage of good quality seed has been recognized as the single most important factor limiting potato production in the developing countries. Production of quality planting material is essential not only for improving domestic potato productivity but also to ensure commercial quality as required under

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international agreements.

Plant tissue culture is widely used to produce clones of a plant through micro propagation. In vitro micro propagation is an alternative to conventional propagation of potatoes (Chandra and Birhman, 1994). In vitro propagation methods using meristem tips, nodal cuttings and micro tubers are more reliable for maintaining genetic integrity of the multiplied clones. In vitro produced disease free potato clones combined with conventional multiplication methods have become an integral part of seed production in many countries (Naik and Sarkar, 2000). Mini tubers can be produced throughout the year and are principally used for the production of pre-basic or basic seed by direct field planting (Lommen, 1999, Ritter *et al*., 2001).

Appropriate plant density is a key for gainful production of potato in various environments. The possibility of securing high yield depends much upon maintenance of optimum number of plants per unit area and their spatial arrangement in the field. Many studies have been conducted to establish the optimal combination of planting distance for a certain environment not only for potato but many other plants as well (Zerga *et al* 2017,Idoko *et al* 2017, Sultana and Shahiduzzaman 2016, Mahmood *et al,* 2015) etc.

It is well known that plant density (inter and intra-row spacing) is very important aspect of potato production since it significantly affects number of tubers per plant, tuber weight, tuber yield and size (Arega et al, 2018). According to Hani et al., (2018) increase in plant density decreases the mean tuber size due to increased inter plant competition for light, water and nutrients. However, the optimal planting density differs depending on the environmental conditions and cultivars. According to Gulluoglu and Arioglu (2009) closer spacing reduced tuber number per hill, average tuber weight, tuber yield per hill.

Since, quality of potato seed is a key factor in profitable potato production, it is imperative to identify appropriate spacing for seed potato productions. Previous studies were conducted in the field or uncontrolled environment and no work has been done so far on adequate plant spacing for tissue cultured plantlets transplantation in green house to obtain maximum mini-tubers production. The present study was conducted to optimize a protocol for best inter and intra row spacing for tissue cultured potato plantlets transplantation on greenhouse tables in order to get maximum mini tubers yield and size.

Materials and Methods

The present research work was performed at tissue culture laboratory and greenhouse, Hazara Agriculture Research Station, Abbottabad in 2018. Five liters of MS media (Murashige and Skoog media 1962) was prepared from the stock solutions (Table 1) of macro and micro salts with 1 mgl^{-1}Ca-pentothenate, 100mgl^{-1} Myoinositol and 30gl^{-1} sucrose. The pH was adjusted to 5.8 with either 0.1N HCl or 0.1N NaOH. The media was poured in to the test tubes @ 10ml per test tube and were autoclaved at 121°C at 15 psi for 15 minutes.

About 30 days old disease free plants of potato cultivar Desiree were selected from the Tissue culture laboratory as explants (fig. 1). The explants were cut in to nodal segments and inoculated into test tubes. These cultured test tubes were incubated into growth chamber for 30 days at 18°C under photoperiod of 16h light and 8 hours dark (Fig. 1).

After 30 days the plantlets were transplanted in peat moss soil on greenhouse tables. There were nine different combinations of inter and intra row spacing. Plants of S1 having distance of row-row 30cm x plant-plant 15cm, S2(30cm x10cm), S3 (10cm x15cm), S4 (20cm x5cm) , S5(30cm x5cm) , S6(10cm x5cm), S7(20cm x15cm) , S8(10cm x10cm) and S9 having distance of (20cm x10cm) respectively.

The data on growth parameters were recorded for the each spacing after every 20 days. The study parameters were shoot length, number of leaves, inter nodal distance, number of nodes, number of branches, number of leaves per branch, tuber weight, tuber diameter, number of eyes and average tuber yield per plant.

The experiment was conducted using completely randomized design (CRD). The data were analyzed by Analysis of Variance (ANOVA) and Least Significance Difference test was used for significance of results at 95% level of confidence. All the statistical analysis were done using computer software Statistix 8.1.

Figure 1. Plantlets of potato variety Desiree are ready to be transplanted in peat moss on the greenhouse tables.

Results

Each treatment showed different results regarding vegetative growth parameters at different spacing.

Shoot length

The data regarding shoot length in all nine inter and intra row spacing combinations were differed significantly (P≤0.05) after 10 days of transplantation (Table 1). The plants of spacing treatments 20 x 5 cm and 30 x 5 cm showed higher shoot length (9.26 cm) and (9.27 cm), respectively, whereas the spacing 20 x 10 cm showed significantly minimum shoot length i.e 6.77 cm (Table 1).

Table 1. Effect of different inter (r-r) and intra (p-p) plant spacing on various growth parameters of potato plantlets Desiree after 10 days of transplantation in peat moss on Green house tables.

Plant spacing (r-r x p-p)* (cm)	Shoot length (cm)	No of leaves	No of nodes
S1(30 x 15)	8.27 abc	12.37 ab	10.0 ab
S2 (30 x 10)	7.26 cd	11.87 ab	9.37 ab
S3(10 x 15)	8.87 ab	14.12 a	10.2 ab
S4(20 x 5)	9.26 a	13.25 ab	11.0 a
S5(30 x 5)	9.27 a	13.12 ab	10.3 ab
S6(10 x 5)	7.63 bcd	11.37 b	8.87 b
S7(20 x 15)	7.00 cd	12.00 ab	9.25ab
S8(10 x 10)	7.26 cd	12.50 ab	9.87ab
S9(20 x 10)	6.77 d	12.00 ab	9.37ab

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05. *(r-r = row to row; p-p = plant to plant distance).

After 30 days of transplantation a significant (P≤0.05) increase in shoot length was found at spacing 20x5cm; 10x15 cm and 30x5cm (Table 2). Whereas the lowest shoot length was recorded at spacing 20 x 10cm,, 20x15cm 20x15cm and 30 x 10cm i.e 11.53cm, 12.17cm, 12.17cm and 11.45cm respectively. After 50 and 60 days of transplantation, a similar trend in increase in shoot length was observed in the plants of spacing 20x5cm and 30x5cm (Table 3 & 4) however on 60 days of transplantation the minimum growth was recorded only at spacing 30x 15cm (49.9cm). After 60 days no further increase in shoot length was observed in any spacing combination.

Table 2. Effect of spacing on various growth parameters of potato plantlets (Desiree) after 30 days of transplantation.

Spacing (cm)	Shoot length (cm)	No of leaves per plant	No of nodes	No of branches	No of leaves per branch
S1(30 x 15)	12.63bc	16.25abc	14.25abcd	7.25 a	4.50 cde
S2(30 x 10)	11.45 c	15.25 c	13.87 bcd	6.75ab	5.5 bc
S3(10 x 15)	14.08ab	17.00abc	15.00abc	4.52 d	5.25bcd
S4(20 x 5)	14.36 a	18.12 a	15.75 a	5.00cd	6.62 a
S5(30 x 5)	14.01ab	17.62ab	15.62ab	4.75cd	5.75ab
S6(10 x 5)	12.81abc	15.62 c	13.62 cd	5.62 bc	4.00 e
S7(20 x 15)	12.17 c	16.25abc	13.62 cd	5.87 abc	5.50bc
S8(10 x 10)	12.51bc	15.62 c	13.00 d	6.00abc	4.37 de
S9(20 x 10)	11.53 c	16.25abc	13.87bcd	4.12 d	5.50bc

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05

Numbers of Leaves per plant

The data pertaining to numbers of leaves presented in Table 1 revealed no significant difference (P>0.05 in number of leaves among various spacing. After 30 days (Table 2) showed that leaves emerge in maximum number at spacing 20x5cm that have a mean value of 18.12, followed as 17.62 cm from 30x5cm spacing.

The data pertaining to number of leaves after 50 days of transplantation showed a significantly higher average numbers of leaves at 30x15cm spacing i.e 24.62. (Table 3). A similar trend in average lead number was observed in plants of various spacing after 60 days (Table 4) and no further increase in leaves number was recorded after this period.

Number of nodes

No significant difference (P>0.05 was present in average number of nodes after 10 days of transplantation. However, highest mean no of nodes were recorded in spacing 20x5cm (11.0) (Table 1). After 30 days (Table 3) of transplantation similar trend in mean average number of nodes was observed.

Data after 50 days recorded for number of nodes revealed that the plants of spacing 30x15cm and 20x5cm have maximum number of nodes i.e 21.87 & 20.25, respectively. Minimum numbers were recorded in 10x10cm spacing i.e 13.00 mean nodes per plant (Table 3).

At about 60 days of transplantation, maximum mean numbers of nodes was observed in 30x15cm spacing i.e 25.37 nodes (Table 4). After 60 days no change in the mean number of nodes were recorded

Number of branches

Branches emerge after 30 days of transplantation and the data pertaining to number of branches showed that the maximum mean number of branches were present in 30x15cm spacing (7.25). Minimum average number of branches i.e 4.125 were recorded at spacing 20x10cm (Table 2).

After 50 days the data regarding number of branches per plant (Table 3) showed the maximum average value i.e 9.25 and 8.00 at spacing 30x15cm and 30x10cm respectively. Whereas the minimum mean numbers of branches (4.896) were observed at spacing 10x5cm.

No significant difference was recorded in mean branch number after 60 days of transplantation except at spacing 30x15 cm which showed significantly maximum branches (14.75) (Table 4).

Table 3. Effect of spacing on various growth parameters of potato plantlets (Desiree) after 50 days of transplantation.

Spacing (cm)	Shoot length (cm)	No of leaves	No of nodes	No of branches	No of leaves per branch
S1(30 x 15)	35.66 de	24.62 a	21.87 a	9.250 a	6.500
S2(30 x 10)	37.68 bcde	21.50 b	18.87 b	8.000 b	5.250
S3(10 x 15	40.90abc	21.00 ab	18.37 b	6.875 b	6.625
S4(20 x 5)	43.96 a	23.00ab	20.25ab	5.875 b	6.125
S5(30 x 5)	42.92ab	21.25 b	18.62 b	6.000 b	5.875
S6(10 x 5)	41.70abcd	23.12 b	20.25 ab	4.896 c	6.000
S7(20 x 15)	33.21 e	21.75 b	19.25 b	7.000 b	6.500
S8(10 x 10)	35.91cde	21.37 b	18.75 b	6.375 b	6.250
S9(20 x 10)	35.40 de	21.87 b	19.25 b	5.960 b	6.125

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05

Number of leaves per branch:

Data pertaining to number of leaves per branch after 30 days of transplantation showed maximum average leaves per branch (6.62) were recorded at spacing 20x5cm followed by (5.75) at spacing 30x5cm. Whereas at spacing 30x10cm, 20x15cm and 20x10cm the same number of leaves per branch was recorded i.e 5.50 (Table 2).

Mean number of leaves per branch increases with time in all the spacing but no significant difference (P>0.05) was found after 50 days among all nine space combinations (Table 3). After 60 days of transplantation the data pertaining to number of leaves per branch revealed maximum average leaf number per branch at spacing 30x15cm i.e 10.12 (Table 4) while minimum average value (6.000) was recorded at spacing 10x5cm . After 60 days further growth was not observed in plants at any spacing.

Table 4. Effect of spacing on various growth parameters of potato plantlets (Desiree) after 60 days of transplantation.

Spacing (cm)	Shoot length (cm)	No of leaves	No of nodes	No of branches	No of leaves per branch
S1(30 x 15)	49.90 d	29.00 a	25.37 a	14.75 a	10.12a
S2(30 x 10)	53.28bcd	24.25 b	21.12 b	11.00 b	8.50 abc
S3(10 x 15	54.85 ab	24.87 b	22.62 b	10.00 b	6.25 ab
S4(20 x 5)	58.92 a	26.00 b	22.62 b	10.12 b	9.12 d
S5(30 x 5)	59.01 a	25.12 b	22.50 b	10.50 b	7.12 cd
S6(10 x 5)	57.01 ab	26.25 b	23.12 b	9. 12 b	6.00 d
S7(20 x 15)	54.55bc	24.62 b	21.37 b	9.12b	9.25ab
S8(10 x 10)	53.92bcd	24.37 b	21.25 b	10.7 b	9.75ab
S9(20 x 10)	50.50 cd	25.00 b	22.00 b	9. 75 b	8.37bc

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05.

Regarding tuber yield, the tubers were categorized into three types on the basis of weight i.e large > 20g; medium 10-20g; small < 10g (Fig. 2).

Tubers Numbers

Data regarding average tuber number revealed that large size tubers are formed in small number as compared to medium and small size tubers among all nine plant spacing combinations (Table 5a) (Fig.2). The highest average tuber number (1.28; 2.25; 8.87) of all the three tuber size categories i.e large, medium and small tubers, respectively were recorded at spacing 30x15cm and 20x10cm (Table 5).

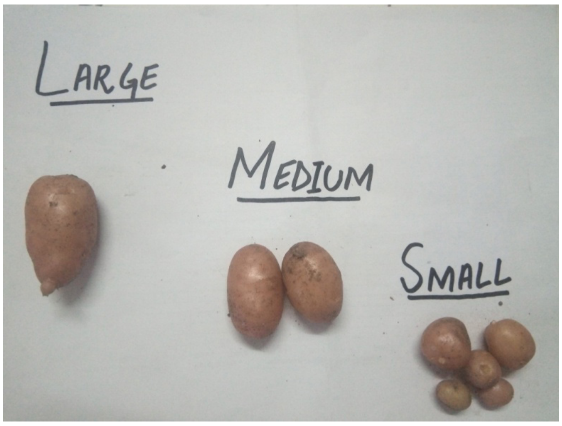

Figure 2. Tuber categories on the basis of weight i.e large > 20g; medium 10-20g; small < 10g.

Tubers Weight

No significant difference(P>0.05) was observed in all nine plant spacing combinations in case of average weight of large tubers (Table 5a). The highest average weight of large size tubers (28.7g) were recorded at spacing 30x15cm followed by 20x10cm i.e 27.54g. Analysis of variance revealed maximum average weight of medium size tubers (31.45g) at inter and intra row spacing of 20x15cm, followed by (27.87g) at 30x15cm (Table 5). In case of small size tubers the greatest mean average weight was recorded at spacing 20x10cm (33.49g) and 30x15cm (32.26g). However, in all the three tubers categories the lowest mean tuber weight was recorded at the plant spacing 10x5cm (Table 5a).

Tubers Diameter

The data for mean tuber diameter revealed that the maximum mean value (28.33 mm and 27.04mm) was obtained at spacing 30x15cm and 20x10cm, respectively for large size tuber category (Table 5b). Similar trend of maximum tuber diameter was also recorded in case of medium and small tuber categories. Whereas least average tuber diameter was recorded at spacing 10x5cm in all the three tuber categories (Table 5b).

Number of Eyes on tubers

Mean number of eyes per large tuber presented in Table 5 showed maximum numbers 5.62 at spacing 20x10cm followed by 30x15cm (5.50). No significant difference (P>0.05) was observed in average number of eyes on medium and small size tubers (Table 5b). Relatively tubers of all spacing showed average 4-5 eyes per tuber for medium and small tubers, respectively.

Average tuber yield per plant

A significant difference (P≤0.05) is noted on the average yield obtained in all nine spacing combination (Table 5b). The highest average tuber yield per plant (165.0g) was recorded at inter and intra row spacing of 30x 15cm (Table 5b), followed by spacing 20x10cm in which the mean total yield was 145.38g. The lowest yield (58.62g) was recorded at row and plant spacing of 10x5cm (Table 5b).

Table 5a. Effect of spacing on tuber size and yield of potato variety Desiree after harvesting.

Spacing (cm)	Tubers weight (g)			Tuber numbers		
	large	medium	small	large	Medium	small
S1(30X15)	28.71 a	27.87 ab	32.26 a	1.28 a	2.12 a	8.87 a
S2(30X10)	25.45 a	19.35 bc	29.10 ab	1.00 a	1.37 b	6.87 abc
S3(10X15)	27.54 a	18.06 c	27.97 ab	1.14 a	1.16 b	5.87 bc
S4(20X5)	22.70 a	19.95 bc	25.63 ab	1.00 a	1.42 b	6.50 abc
S5(30X5)	23.10 a	18.33 bc	21.63 ab	1.00 a	1.42 b	6.12 bc
S6(10X5)	21.95 a	15.78 c	19.70 b	1.00 a	1.12 b	4.75 c
S7(20X15)	24.25 a	31.45 a	26.14 ab	1.00 a	2.25 a	7.25 abc
S8(10X10)	23.13 a	20.75 bc	25.63 ab	1.00 a	1.37 b	6.50 abc
S9(20X10)	26.07 a	21.95 bc	33.49 a	1.20 a	1.42 b	7.75 ab

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05.

Table 5b. Effect of spacing on tuber size and yield of potato variety Desiree after harvesting.

Spacing (cm)	Tuber diameter (mm)			Number of eyes per tuber			Av. yield per plant (g)
	large	medium	small	large	medium	small	
S1(30X15)	28.33 a	22.97 abc	16.52 a	5.50 a	5.37 a	4.00 a	165.00 a
S2(30X10)	26.87abc	25.05 a	15.68 ab	1.87 b	5.87 a	4.25 a	100.63 cde
S3(10X15)	25.50abc	22.61 abc	15.55 ab	4.62ab	5.50 a	4.00 a	101.88 cd
S4(20X5)	25.15abc	22.27 abc	15.13 bc	1.87 b	5.12 a	4.12 a	77.875 de
S5(30X5)	25.30abc	21.62 bc	14.67 bc	2.37ab	5.62a	4.37 a	84.500 de
S6(10X5)	24.15 bc	20.26 c	15.31 bc	1.75 b	5.00 a	4.12 a	58.625 e
S7(20X15)	25.97abc	22.27 abc	14.33 c	3.25ab	5.37 a	3.87 a	128.88 abc
S8(10X10)	23.96 c	22.38 abc	15.12 bc	5.62 a	4.62 a	4.00 a	109.00 bcd
S9(20X10)	27.04 ab	23.91 ab	15.36 b	5.00ab	4.87 a	4.00 a	145.38 ab

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05.

Discussion

In potato crop, plants inter and intra spacing plays an important role on crop growth and productivity. An inappropriate spacing may lead to either too dense or too thin plant population resulting in yield reduction. In this study very densely populated plants (10 x 5, 20x5 and 30x5 cm) in terms of plant to plant distance with in a row (intra plant spacing) which was 5cm showed good vegetative growth in terms of shoot length, number of leaves etc. The result of the experiment was in line with the findings of Kifle Zerga *et al.,* (2017) who reported that densely populated plants showed intensive competition which leads to increase in plant height.

The effect of plant spacing observed on number of leaves and nodes was that maximum numbers appeared in widest spacing within plants. This may be due to availability of better nutritional components. This sought of information was reported by Idoko *et al*., (2017) in which the maximum number of leaves were seen at widest spacing, which gave it the ability to initiate more leaves as a result of enough above and below ground natural resources available to it. As the number of leaves increases, number of nodes also increased.

The number of branches per plant were considerably affected by spacing in our experiment. It was noticed that with the increase in spacing, increases the number of branches (14.75) at inter row spacing of 30 cm and intra plant spacing 15 cm, but at closer spacing, the mean number of branches per plants were found decreased. This may be due to the fact that plants transplanted at wider spacing have relatively less competition and they found more space to grow and produce more branches for absorption of light that enhances photosynthetic activity which resulted in good vegetative growth of the plants.

Regarding number of large size tubers maximum number was recorded at wider spacing 30 x 15 cm. This may be due to less competition within plants at wider space. Plants can absorb the sufficient nutrients and utilized them in producing the large size tuber.

This result is in consistency with the work of Tesfaey Getachew (2013) who confirmed that maximum number of large tubers in total tuber yield was increased with wider spacing among the plants. Similarly Yenagi *et al*., (2004), Essah *et al*., (2004) and Tafi *et al*., (2010) also confirmed the increased percentage of large size tubers at wider spacing as compared to narrow spacing. Narrow spacing leads toward decrease in numbers of large size tubers. Gulluoglu and Arioglu (2009) also have reported that mean tubers weight and tuber yield per plant significantly decreased as plants were planted closer due to increase in inter plant competition.

Regarding small size tubers category (small < 10g) more mean tubers number was observed at narrow spacing. This is because of high competition among the plants, plants were may be unable to get the required amount of nutrients from the soil due to dense vegetation and they were competing to attain height rather than storing the food in the tubers.

Mean average tubers yield per plant is also influenced by the plant spacing. By decreasing plant space, a significant reduction in yield was observed and lowest mean tuber yield per plant was recorded at 10 × 5 cm. Bhagirath (2013) also reported that plant spacing influence the vegetative growth and yield of potato tubers; wider the plants spaced, maximum will be the average yield.

Conclusion and Recommendations

It has been concluded from the results that inter and intra spacing of 30x15cm comparatively produces the highest yield. In the experiment, the spacing of 30x5cm, 20x5cm and 10x5cm lead to the maximum vegetative growth whereas the wider spacing 30x15cm leads to the maximum tuber yield.

Therefore, it has been recommended that the spacing of 30 cm between rows and 15 cm between plants is the best space combination for the transplantation of tissue cultured potato plantlets in the green house for maximum mini-tubers production. It has also been recommended that this plant spacing can also be tested for other plants produced through tissue culture for their transplantation on greenhouse benches.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Data availability

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Consent for publication

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Effects of bio-fertilizers (*Bacillus lentus – Pseudomonas putida*) and different rates of Triple Superphosphate fertilizers on some attributes of sugar beet (*Beta Vulgaris* L.)

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Abstract

The aim of this study was to determine both combined and individual effects of phosphorus bio-fertilizers (*Bacillus lentus – Pseudomonas putida*). Additionally, the study also monitored the different effects of triple superphosphate fertilizers on agronomic and quantitative characteristics of sugar beet. The factorial experiment approach (RCB design) was used to conduct this experiment. Furthermore, cultivars 7233 and BR_1 were used as experimental materials to enhance the evaluation of the different fertilizer treatment. An evaluation of the experiments result showed minimal adverse effect, of application of phosphorous fertilizers to phosphorous rich soils. In particular, continued application of phosphorous fertilizer despite its improved values, will results into plant nutritional stress. In conclusion, this experiment results indicated that BR_1 cultivar in comparison to 7233 cultivar BR_1 had improved qualitative and quantitative characteristics like dry weight, leaf area, high sugar content and better tolerance to increased application of phosphorus fertilizer. Consequently, increased application of phosphorus fertilizers to phosphorus rich soils resulted to decreased yield output. However, biological fertilizer in comparison to its mineral counterpart had reduced impacts on the traits

Keywords: Bio-fertilizer, Seed, Sugar beet, Triple superphosphate

Introduction

It is important to note that various agricultural soils in the world, in one way or another lack the key nutrients required to support the growth of healthy plants. Consequently, this necessitates application of fertilizers to promote sufficient fertilizer supply and boost yield output. Efficient use of chemical fertilizers is arguably lowers the base on the reduced ratio uptake by plants (Adesemoye et al., 2009). The heavy use of chemical fertilizers over a long period of time results into adverse structural defects on both plant and soil. These adverse impacts on both plant and soil by chemical fertilizers have encouraged the alternative use of organic fertilizers in the recent years (Nur et al., 2016). It is noteworthy that fertilizer used efficiently, can be an improvement and by fertilizer management where application rate, time and place are carefully considered for both organic and conventional farming. Research has indicated that for efficient crop production to be achieved a balance between nutrient use efficiency and optimal crop production must be undertaken (Roberts, 2008). Intensive farming results into high demand for chemical fertilizers which then result into adverse environmental impacts. Additional, these adverse impacts are associated with negatively altered biogeochemical cycles. Therefore, attention has shifted to organic fertilizers which are cost effective, environmental friendly (Ehteshamiet al., 2007). In an attempt to manage negative environmental impacts from chemical fertilizers, inoculation with plant growth promoting rhizobacteria (PGPR) has been encouraged. One of the advantages of the bacteria is that they assist plants in bet-

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ter growth and development thus making them suitable to be used as bio-fertilizers (Adesemoye et al., 2009). Many tests have proved that plant rhizosphere have boosted crop quality, yield output and general plant growth. Ideally, this study was conducted to determine the individual and combined effect of phosphorous bio-fertilizer (*Bacillus lentus – Pseudomonas putida*) and different rates of triple superphosphate fertilizers on agronomic, quantitative traits of sugar beet. Cultivars 7233 and BR_1 were used as experimental materials in this study to evaluate the effects of different fertilizers.

Materials and Methods

Experimental Details and Treatments

The experiment was carried out in sandy loam soils with EC of 0.72 dS m^{-1} and pH of 7.8 at the Agriculture Research Station of the Islamic Azad University, Tabriz Branch, Iran. Tabriz is located at 38º5'N and Long 46º17'E about 1360 m above sea level and its annual mean temperature is 13.04ºC. The region is classified as cold and semi-arid. Its annual precipitation is 271.3 mm (physical and chemical properties of soil in experimental field were presented in table 1).

Seed material:

Two sugar beet varieties (BR_1-7233) were used in the experiment. The seed material was obtained from the Sugar Beet Seed Institute Research Center at Karaj, Iran.

Table 1. Soil analysis result for physical and chemical characteristics

Characteristic	Soil depth (cm)	Soil texture	pH	P	K	N
Value	0-30	Sandy-loam	7.8-8.9	8/39	513	059/0 %

The study was performed as a factorial experiment based on randomized complete block design with three replications and 16 treatments. The fertilizer levels were presented in Table 2.

Table 2. The levels of fertilizers

Control
Only impregnated seed with organic biological phosphorus (phosphorous biofertilizer)
50kg/ha triple super phosphate
100kg/ha triple super phosphate
150kg/ha triple super phosphate
25kg/ha triple super phosphate + impregnated seed with organic biological phosphorus
50kg/ha triple super phosphate + impregnated seed with organic biological phosphorus
75kg/ha triple super phosphate + impregnated seed with organic biological phosphorus

After measuring the shoot fresh weight and LAI (leaf area index), 20 kg sample from each plot was obtained randomly. About 150 g of pulp from each plot was prepared by Venema apparatus and kept in a freezer until analysis. Frozen sugar beet pulp samples were analyzed in sugar technology laboratory in Sugar Beet Seed Preparing and Breeding Center at Karaj of Iran for purity parameters with Betalyser (model OR-KERNCHEN). Betalyser is a computer controlled system for automated routine analysis of sugar beet on sugar content and impurities including Na^+, K^+ and NH_4^+-N. Sugar content (SC) was measured by polarimetr, $Na^+$and $K^+$by flame bemission photometry and NH_4^+-N by double beam filter photometry using the blue number method (Sheikh-Aleslami, 1997). The combined effect of Na^+, $K^+$and NH_4^+-N on the amount of sugar lost to molasses in the factory process was determined following the Reinfeld et al.(1974) method.

Molasses sugar (MS) = 0.343 * ($K^\pm$ + $Na^\pm$) + 0.094 * NH_4^+ -N 0.31.

[Na^+, K^+ and NH_4^+-N in meq (100 g^{-1} beet).

Standard factory loss (SFL = 0.6).

White sugar contents (recovered sugar content) were calculated using the formula of Reinefeld et al. (1974):

WSC = SC– MS– SFL

White sugar yield (WSY) = root yield (RY) * WSC.

An alkalinity coefficient (AC) was determined from the major non-sugars $K^\pm$, $Na^\pm$ and NH_4^+ -N, as follows (Sheikh-Aleslami, 1997):

AC = (K^++ Na^+)/ NH_4^+ - N

Gross sugar yield and white sugar yield were obtained multiplying sugar content (SC) and white sugar content (WSC) by root yield. Statistical data analysis was done by using SAS software. The ANOVA test was used to determine significant ($p \leq 0.01$ or $p \leq 0.05$) treatment effect and Duncan Multiple Range Test to determine significant difference between individual means.

Results and Discussion

Variance analysis revealed that differences in leaf area index (LAI), total dry weight, shoot fresh weigh, shoot dry weight, single root weight, Na (%), Molasses sugar (%) and harvest index values of the cultivars were found to be significant at 1% level and the differences in root fresh weight, white sugar content and extraction sugar content (%) of the cultivars were found to be significant at 5% level. Effects of different fertilizers on LAI, total dry weight and Na contents were found to be significant at 1% level and the effects on shoot dry weight were found to be significant at 5% level. On the other hand, effects of cultivar x fertilizer interactions were on LAI, Na and Molasses Sugar Content (MS) were found to be significant at 1% level and the effects of interactions on N (%) and extraction sugar content (%) were found to be significant at 5% level (Table 4 and 5).

Table 3. Technical terms of sugar beet yield and quality (Abdollahi Noghabi et al., 2005)

No	Title: English	Title: Symbol	Definition		English
1	Root yield	RY	Root yield of sugar beet per area unit (root wet weight	Weight of harvested roots in area unit after rinsing (net weight)	$t.ha^{-1}$
2	Sugar content	SC or (Pol)	Sugar content in wet root of sugar beet	Polarimetric method	% in beet or g sugar.100g beet-1
3	- Potassium - Sodium - Amino-nitrogen	K Na α-N	Amount of health threatening potassium, sodium, amino nitrogen	Potassium and sodium were measured through photometric film Nitrogen was measured using chromometry (blue number)	meq.100g beet-1 or mmol. 100g beet-1
4	Molasses sugar	MS	Amount of extractable sugar from root of sugar beet (molasses/sugar beet rate)	Based on volume of health threatening potassium, sodium, and nitrogen and using a standard experimental formula	% in beet or g sugar.100g beet
5	White sugar content or - Recoverable white sugar	WSC RWS	Amount of extractable white sugar content of sugar beet in mill Among of extractable sugar	WSC = SC - (MS + 0.6*) Sugar waste in the mill (set to 0.6)*	% in beet
6	Sugar yield	SY	Amount of produced sugar in area unit (sucrose content of sugar beet root)	SY = SC × RY	$t. ha^{-1}$
7	White sugar yield	WSY	Extractable while sugar content of white beet per area unit	WSY = WSC × RY	$t. ha^{-1}$
8	Extraction coefficient of sugar (Purity)	ECS (Yield)	Content of extractable white sugar from sucrose content in sugar beet root	ECS = (WSC ÷ SC) ×100	% in sugar
9	Alkalinity coefficient	Alc or AC	Health threatening sodium/potassium to nitrogen ratio in sugar beet	Alc=(K+Na) ÷ (α-N)	-
10	Brix	Brix	Density of roughage in extract of sugar beet root	Refrectometry method	% in extract

* Terms in the parentheses are wrong commonly used terms which are not recommended.

Table 4. Anova of effect of fertilizer and cultivar on different characters of sugar beet

ANOVA	df	Mean squares: LAI (leaf area index)	Total Dry Weight Kg/m^2	Shoot Dry Weight Kg/m^2	Root Fresh Weight Kg/m^2	Shoot Dry Weight Kg/m^2	Root Dry Weight Kg/m^2	Single Root Weight Kg/m^2	LAR (leaf are ratio)	Sugar Contents %	Na (%)
Replication	2	0.557**	1.058**	23.689**	2.036**	0.551**	0.056^{ns}	0.752*	0.373**	5.000**	2.067**
Cultivar	1	2.460**	1.026**	35.432**	1.922*	0.707**	0.058^{ns}	1.245**	0.028^{ns}	1.505^{ns}	4.332**
Fertilizer	7	0.122**	0.112**	1.518^{ns}	0.362^{ns}	0.052*	0.009^{ns}	0.112^{ns}	0.042^{ns}	0.521^{ns}	0.797**
Cultivar x Fertilizer	7	0.134**	0.039^{ns}	1.266^{ns}	0.219^{ns}	0.014^{ns}	0.008^{ns}	0.003^{ns}	0.016^{ns}	0.783^{ns}	0.834**
Error	7	0.030	0.026	0.827	0.346	0.020	0.0022	0.164	0.025^{ns}	0.461	0.228
CV	30	4.05	7.14	32.07	8.14	30.23	8.41	32.33	8.23	4.03	26.39

ns = Non significant, ** = $p < 0.01$ and * = $p < 0.05$

Table5. Anova of effect of fertilizer and cultivar on different characters of sugar beet

ANOVA	df	Mean squares										
		K (%)	N(%)	Alc	White Sugar Content (WSC)	Extraction Sugar Content (ESC)	Mollase Sugar (MS) %	Quality	Brix	Harvest Index	Sugar Contents Yield	White Sugar Yield (ton/ha)
Replication	2	1.772**	1.307*	2.718^{ns}	10.470**	85.088**	1.055**	2.8723^{ns}	6.772**	0.059**	28.834^{ns}	29.031^{ns}
Cultivar	1	0.018^{ns}	0.517^{ns}	0.780^{ns}	4.219*	47.661*	0.684**	0.0008^{ns}	3.101*	0.082**	0.450^{ns}	0.838^{ns}
Fertilizer	7	0.164^{ns}	0.441^{ns}	1.0774^{ns}	0.950^{ns}	8/894^{ns}	0.148^{ns}	0.0006^{ns}	0.945^{ns}	0.005^{ns}	9.325^{ns}	6.081^{ns}
Cultivar x Fertilizer	7	0.389^{ns}	0.668*	0.953^{ns}	1.864^{ns}	18.685*	0.317**	0.0002^{ns}	1.261^{ns}	0.002^{ns}	7.822^{ns}	9.169^{ns}
Error	7	0.247	0.255	0.940	0.829	6.852	0.083	0.017	0.585	0.003	11.023	9.614
CV	30	5.95	23.02	19.82	7.09	3.44	8.53	3.36	3.24	6.57	11.18	13.65

ns = Non significant, ** = $p < 0.01$ and * = $p < 0.05$

Table 6. Mean comparison of effect of biological and chemical phosphorus fertilizer on total dry weight and shoot dry weight of sugar beet

	Total dry weight	Shoot dry weight
Control	2.488 a	0.5866 ab
Only impregnated seed with organic biological phosphorus (phosphorous bio fertilizer)	2.362 ab	0.6188 a
50kg/ha triple super phosphate	2.297 ab	0.4943 abc
100kg/ha triple super phosphate	2.101 b	0.3908 c
150kg/ha triple super phosphate	2.102 b	0.3625 c
25kg/ha triple super phosphate + impregnated seed with organic biological phosphorus	2.195 b	0.4648 abc
50kg/ha triple super phosphate + impregnated seed withorganic biological phosphorus	2.151 b	0.4093 bc
75kg/ha triple super phosphate + impregnated seed withorganic biological phosphorus	2.189 b	0.4213 bc

Columns means followed by the same letter are not significantly different at 0.05 probability level.or 0.01 probability level.

Table 7. Means comparison of effects of biological and chemical phosphorus fertilizer on LAI, Na, N (%), ECS and MS (%) of sugar beet

Cultivar	Fertilizers	LAI	Na	N (%)	ECS	MS (%)
BR_1	Control	4.401ab	1.930 bc	1.700 abcd	77.29 abc	3.220 abcd
BR_1	Only impregnated seed with organic biological phosphorus (phosphorous bio fertilizer)	4.593 ab	2.707 ab	2.703 a	73.26 c	3.727 abc
BR_1	50kg/ha triple super phosphate	4.682 a	2.250 bc	2.080 abc	75.61 abc	3.490 abcd
BR_1	100kg/ha triple super phosphate	4.448 ab	2.020 bc	1.633 abcd	76.31 abc	3.353 abcd
BR_1	150kg/ha triple super phosphate	4.376 abc	2.600 abc	2.627 ab	74.70 bc	3.627 abcd
BR_1	25kg/ha triple super phosphate + impregnated seed with organic biological phosphorus	4.269 abcd	2.213 bc	1.470 abcd	77.31 abc	3.313 abcd
BR_1	50kg/ha triple super phosphate + impregnated seed with organic biological phosphorus	4.555 ab	2.417 abc	2.660 ab	73.11 c	3.763 ab
BR_1	75kg/ha triple super phosphate + impregnated seed with organic biological phosphorus	4.622 ab	2.250 bc	1.993 abcd	73.86 bc	3.517 abcd
7233	Control	4.451 ab	3.273 a	2.360 abc	72,87 c	3.930 a
7233	Only impregnated seed with organic biological phosphorus (phosphorous biofertilizer)	4.323 abc	2.527 abc	2.313 abc	74.45 bc	3.583 abcd
7233	50kg/ha triple super phosphate	3.879 de	1.943 bc	1.227 cd	78.66 ab	3.100 bcd
7233	100kg/ha triple super phosphate	3.879de	1.730 bc	1.387 cd	76.99 abc	3.253 abcd
7233	150kg/ha triple super phosphate	3.837 de	1.663 c	0.8367 d	80.41 a	2.900 d
7233	25kg/ha triple super phosphate + impregnated seed with organic biological phosphorus	3.959 cde	1.877bc	1.327 cd	76.54 abc	3.243 abcd
7233	50kg/ha triple super phosphate + impregnated seed with organic biological phosphorus	4.227 bcd	1.930 bc	1.427 bcd	78.53 ab	3.117 bc
7233	75kg/ha triple super phosphate + impregnated seed with organic biological phosphorus	3.769 e	1.783 bc	1.183 cd	78.93 ab	2.973 cd

Columns means followed by the same letter are not significantly different at 0.05 or 0.01 probability level.

Table 8. Means comparison of effects of cultivar on Brix, Harvest Single root weight (Kg/m^2) index, Total dry weight (Kg/m^2), Shoot fresh weight (Kg/m^2), Root fresh weight (Kg/m^2), Shoot dry weight (Kg/m^2), Single root weight (Kg/m^2) and WSC

Cultivar	Brix	Harvest index	Total dry weight(Kg/m^2)	Shoot fresh weight(Kg/m^2)	Root fresh weight (Kg/m^2)	Shoot dry weight (Kg/m^2)	Single root weight (Kg/m^2)	WSC
BR_1	23.376	0.795	2.382	3.696	7.423	0.590	1.387	12.551
7233	23.884	0.841	2.090	1.977	7.023	0/374	1.056	13.144

Columns means followed by the same letter are not significantly different at 0.05 or 0.01 probability level.

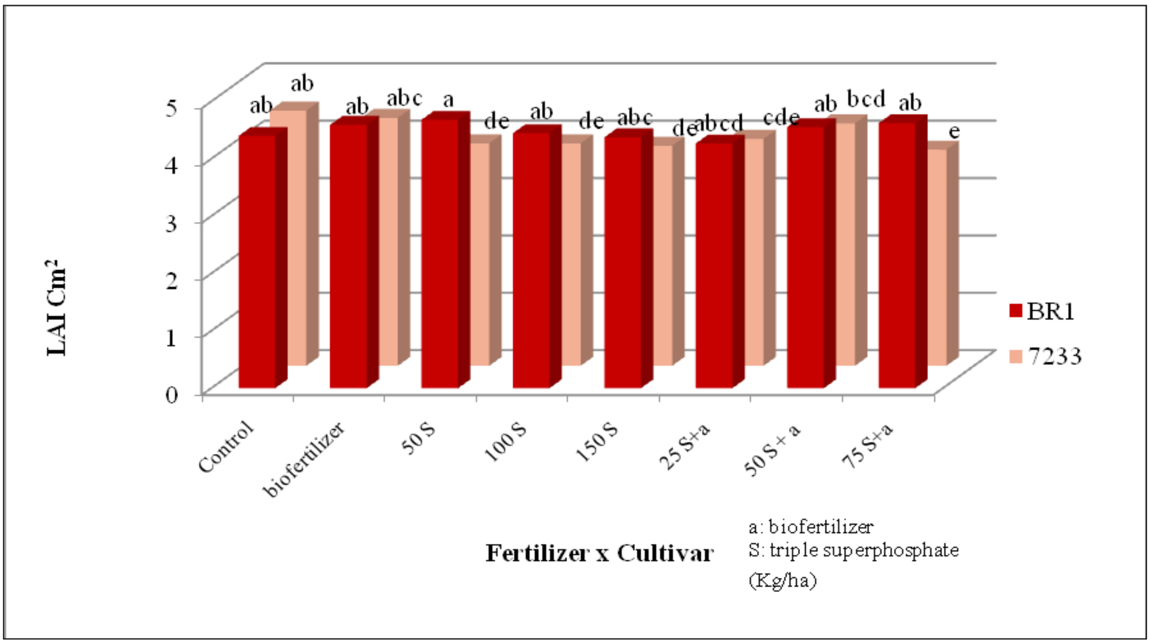

Figure 1. LAI of sugar beet due to the combined effect of cultivars and fertilizers.

LAI (Leaf Area Index): With regard to LAI, cultivar x fertilizer interaction was found to be significant at 1% level (Table 4). Considering the effects of fertilizers on cultivars, the greatest LAI (4.682 cm^2) was obtained from 50 kg/ha triple super phosphate treatments of BR_1cultivar. As compared to 7233 cultivar, BR_1 exhibited more constant respond to different fertilizer doses and placed within the same statistical group with the control plants. Except for the control and biological fertilizer treatments, other fertilizers had negative effects on LAI of 7233 cultivar and decreased LAI values. Biological fertilizers reduced the negative effects of excessive soil phosphorus levels in 7233 cultivar. As compared to 7233 cultivar, biological fertilizers yielded more positive outcomes in BR_1cultivar. In other words, cultivar x fertilizer interaction was found to be significant since the cultivars had different responds to biological fertilizers and doses (Table 7). Excessive soil phosphorus levels reduced LAI values of sugar beet plants in different ways. Excess phosphorus resulted in Fe and Zn deficiency, thus restricted leaf growth and development and decreased LAI values (Marschner, 1995). Burnett et al. (2008) carried out a study with Fan flower plants and indicated that soil phosphorus levels over 40 mg/l reduced LAI values. Similarly, Zhang et al. (2004) also worked with Fan flower plants, but indicated this time that soil phosphorus levels over 14.5 mg/l reduced LAI values.

Total dry weight (kg/m^2): Effects of cultivars and fertilizers on total dry weights were found to be significant at 1% level (Table 4). In general, BR_1cultivar (2.382 kg/m^2) had greater total dry weights than 7233 cultivar (2.090 kg/m^2). Since BR_1 cultivar had greater LAI (leaf area index) values, it produced greater quantities of dry matter (Table 8). Effects of different fertilizers (bio-fertilizers and chemical) on total dry weights are presented in Figure 2. Although phosphorous bio-fertilizers and 50 kg/ha triple super phosphate treatments were placed in the same statistical group with the control plants, the greatest total dry weight was obtained from the control plants. In other words, in case of sufficient soil phosphorus levels, supplementary phosphorus fertilizers (either biological or chemical) had negative effects on plant total dry weights. Over-treatments may alleviate toxic impacts of phosphorus in soils, imbalance soil Fe, Cu, Zn, Mn and B microelements and ultimately result in yield losses. Excessive phosphorus in soil also result in toxic accumulation of B, alleviated Cd pollution in soil, thus reduced quality and dry matter yields (Marschner, 1995). Excessive phosphorus reduce Fe ratio and thus decrease dry matter and LAI values. Excess phosphorus resulted in Mn deficiency in spinach plants and thus reduced photosynthesis rate by 28% and dry mater yield by 20%.

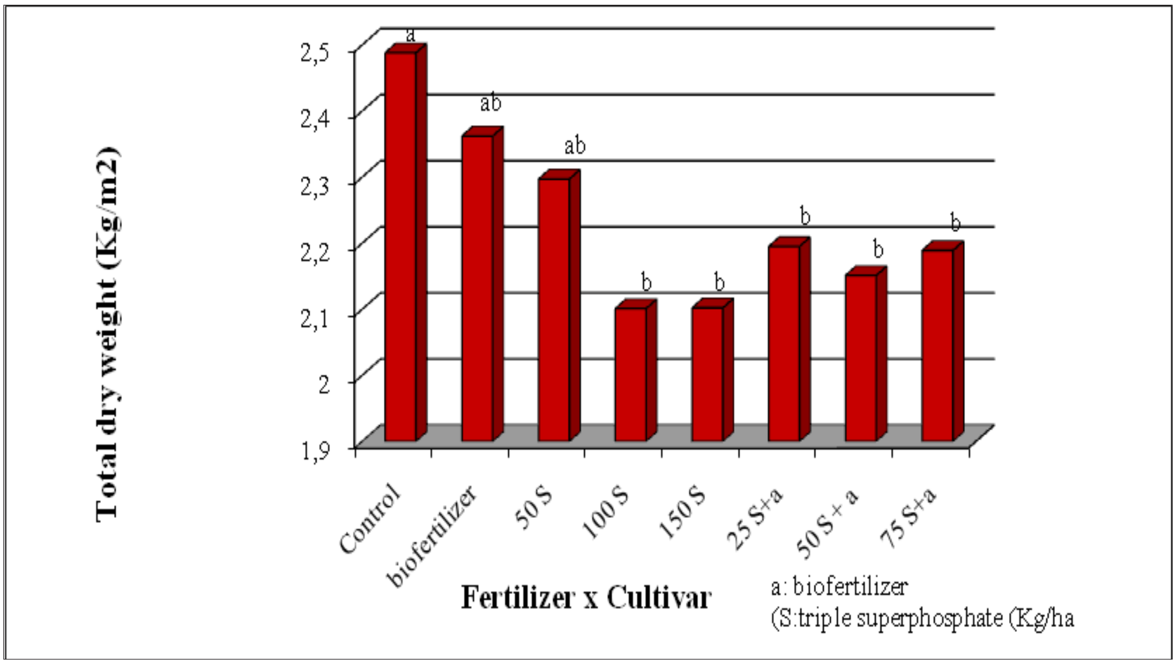

Figure 2. Total dry weight (Kg/m^2) of sugar beet due to the effect of fertilizers.

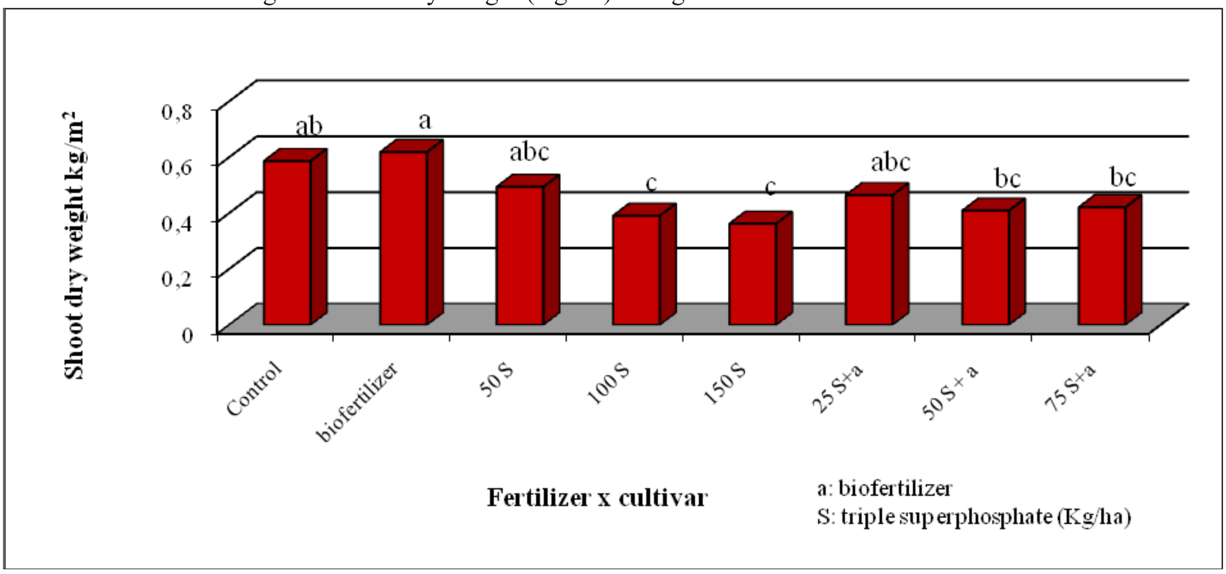

Figure3. Shoot dry weight (Kg/m^2) of sugar beet due to the combined effect of cultivars and fertilizers.

Shoot Dry Weight: According to variance analysis, fertilizers had significant effects on shoot dry weights at 5% level (Table 4). As can be seen in Figure 3, cultivars had different responds to different fertilizer treatments. Considering shoot dry weight and total dry weight data together, it was observed that plants had similar responds. The greatest shoot dry weight was obtained from single biological fertilizer treatments. Such a case indicated that the difference in total dry weight was resulted from shoot dry weight. As can be seen in Table 4, fertilizer factor did not have significant effects on root fresh and dry weights. Again, according to variance analysis, there were significant differences in shoot dry weights of the cultivars at 1% level. BR_1cultivar (0.590 kg/m^2) had about 41% greater shoot dry weight than 7233 cultivar (0.347 kg/m^2). High soil phosphorus levels indirectly influence soil microelements and thus reduce shoot dry weights. Rumheld and Marschner (1991) indicated that excessive soil phosphorus negatively influenced manganese (Mn) quantities absorbed by the soils and reduced shoot dry weight. According to Marschner (1995), high soil phosphorus levels decreased plant IAA (Indole acetic acid) contents, increased tryptophan quantities and such a case then decreased leaf dimensions and thus leaf areas. Burnett et al. (2008) indicated that increasing soil phosphorus levels to a certain level resulted in increased shoot dry weights, but further increases resulted in decreasing shoot dry weights. Zhang et al. (2004) reported for fan flower plants that soil phosphorus levels over 14.5 mg/l reduced shoot dry weights, Shan et al. (2004) indicated for *Hakea prostrata* plants that phosphorus quantities over 30 µmol reduced leaf dry weights.

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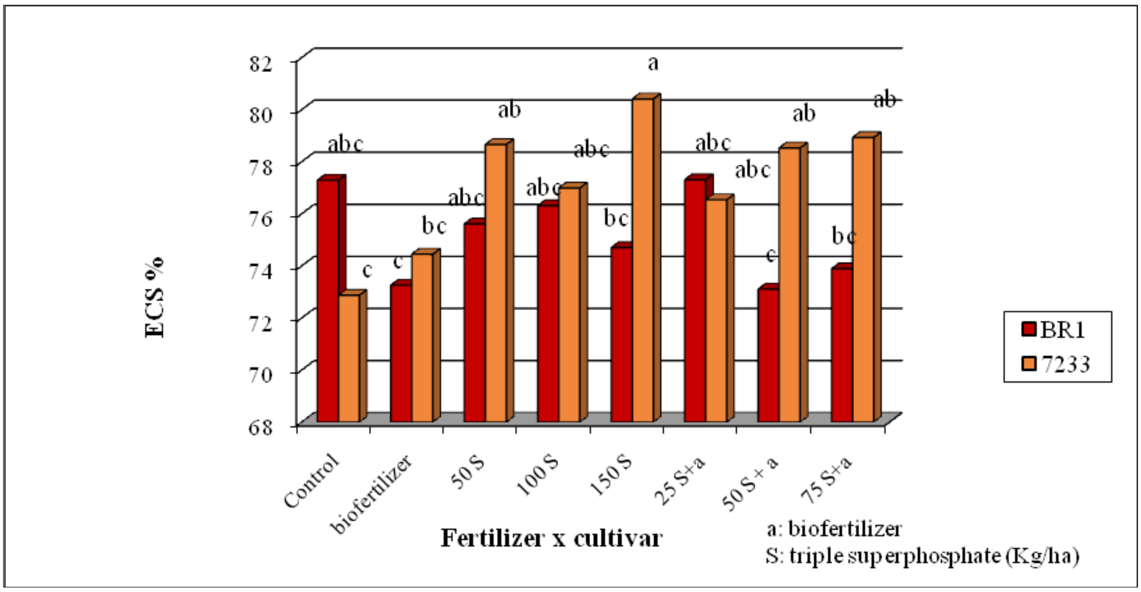

Figure4. Extraction sugar (ESC) of sugar beet due to the combined effect of cultivars and fertilizers.

Extraction coefficient of sugar (ECS): As can be seen in Table 5, fertilizer x cultivar interactions was found to be significant at 1% level with regard to extraction coefficient of sugar (ECS). The greatest ECS was obtained from 150 kg/ha phosphorus treatment of 7233 cultivar and the lowest value was obtained from the control plants. In BR_1 cultivar, the greatest ECS value was obtained from the control plants (Table 7). Draycott and Christenson (2003) carried out a research in Minnesota of the USA and reported that high soil phosphorus levels had greater impacts on sugar content (%) than on ECS ratio of sugar beet plants. It was indicated in another research that increasing phosphorus contents of phosphorus-deficient soils increased ECS ratios, but increasing phosphorus quantities did not have significant effects on ECS ratios. In other words, phosphorus fertilizer did not have significant effects on ECS.

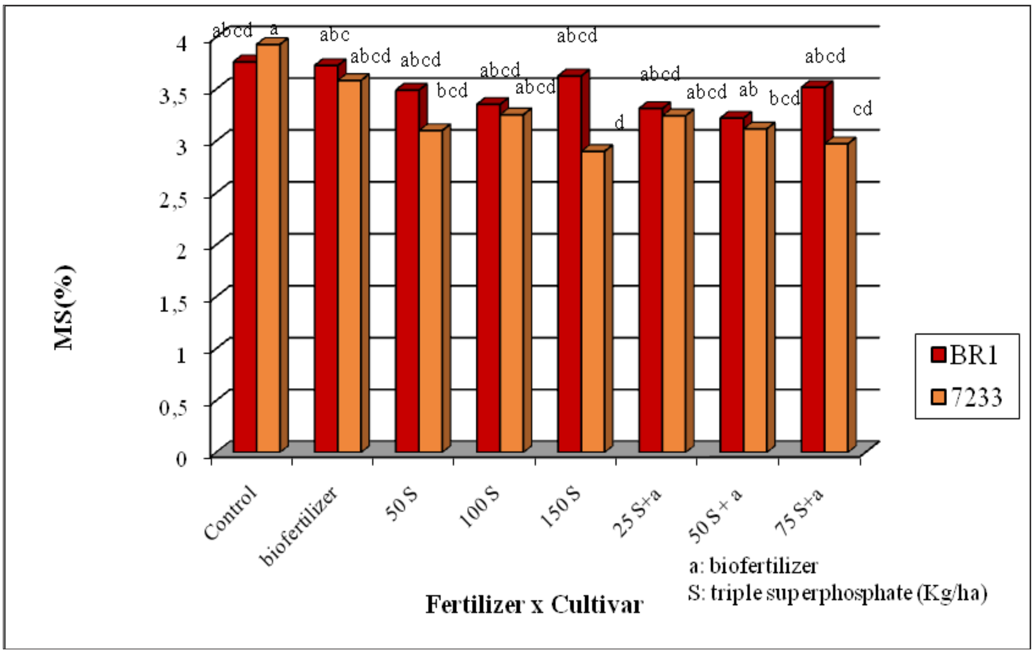

Figure 5. Molasses sugar of sugar beet due to the combined effect of cultivars and fertilizers.

Molasses sugar (MS): In sugar production from sugar beet, the economically non-valuable portion of the sugar, so called as molasses, is used as a byproduct of the sugar facility. Since the molasses sugar is not able to be converted into white sugar, there is a loss in sugar content. With regard to molasses sugar, cultivar x fertilizer interactions was found to be significant at 1% level (Table 5). As can be seen in Table 7, the greatest molasses sugar in 7233 cultivar was obtained from the control group and the other fertilizer treatments reduced molasses sugar ratios. In BR_1 cultivar, different fertilizer doses did not created significant differences in molasses sugar and fertilizer treatments yielded similar results with the control plants. As it was in 7233 cultivar, the greatest molasses sugar in BR_1 cultivar was also obtained from the control plants.

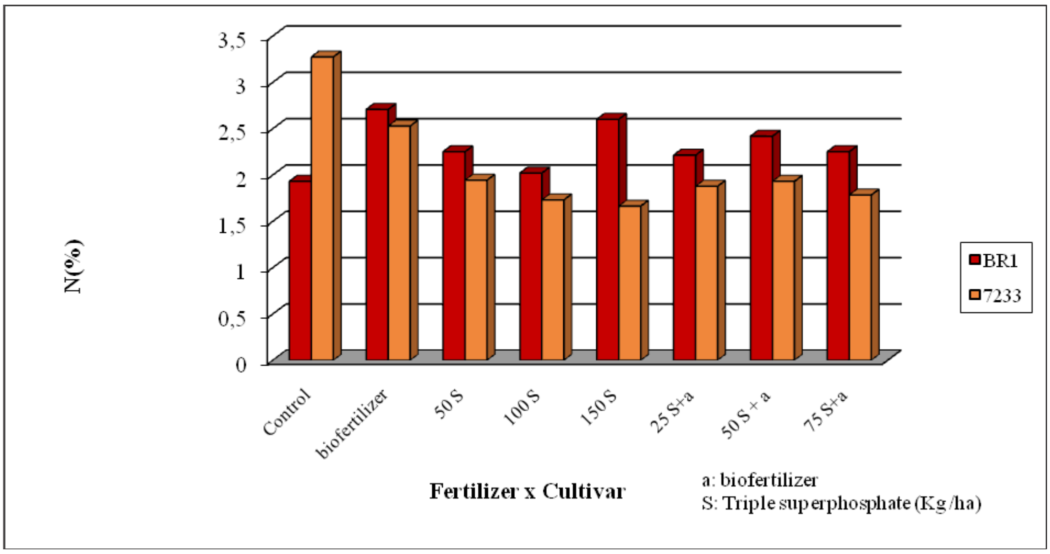

Figure 6. N (%) of sugar beet due to the combined effect of cultivars and fertilizers.

Alpha-amino nitrogen **(N):** As can be seen in Table 4, cultivar x fertilizer interactions was found to be significant at 5% level with regard to N (%) content. Present findings revealed that with regard to N (%), cultivars had different responses to phosphorus fertilizer. In 7233 cultivar, the greatest N (%) content was obtained from the control and bio-fertilizertreatments. Although different phosphorus treatments did not have significant differences, N (%) contents decreased with increasing phosphorus contents. In BR_1 cultivar, control and fertilizer treatments yielded similar results and the greatest N (%) content was obtained from phosphorus bio-fertilizer treatments. In general, 7233 cultivar was found to be more sensitive to fertilizer quantities than BR_1 cultivar. Kuang et al. (2005) reported increasing N quantities in soybean plants with increasing phosphorus quantities. Groot et al. (2003) also indicated increasing plant N quantities with increasing soil phosphorus quantities.

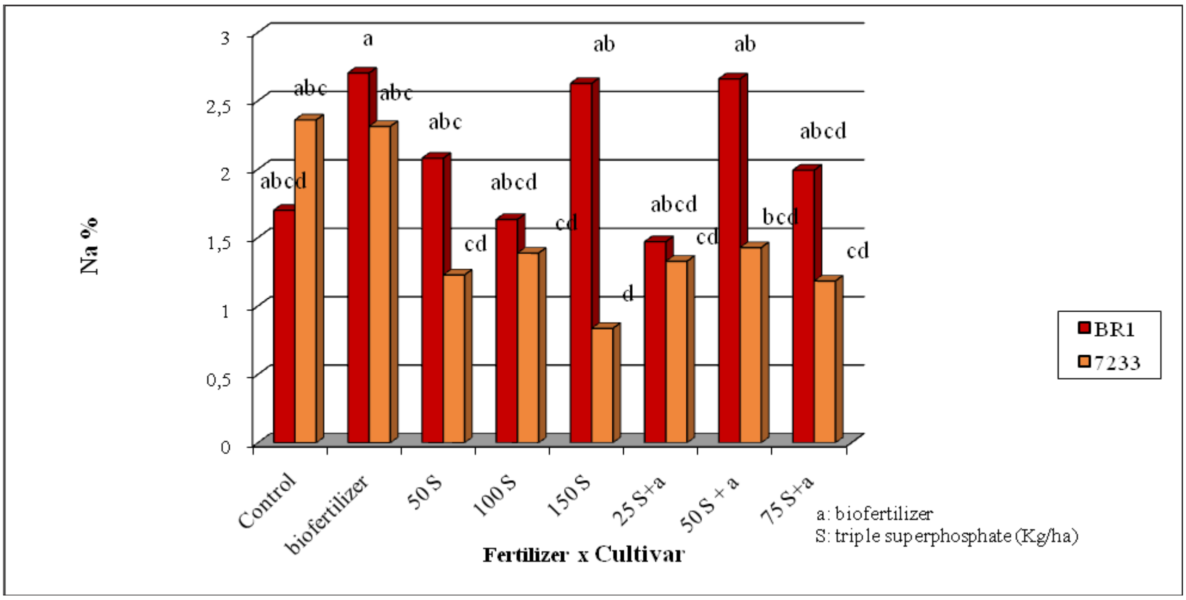

Figure7. Na (%) of sugar beet due to the combined effect of cultivars and fertilizers.

Na (%): Na, K and*alpha-amino nitrogen* (N) substances increase molasses sugar, thus reduce white sugar content of sugar beet. Variance analysis revealed that cultivar x fertilizer interactions was significant for Na (%) factor at 1% level. In 7233 cultivar, the lowest Na ratios (1.663%) was obtained from 150 kg/ha triple super phosphate treatments and the greatest Na (%) ratio was obtained from control plots and bio-fertilizertreatments. Increasing phosphorus quantities reduced Na (%)

ratio and positively influenced sugar quality. In BR_1 cultivar, the lowest Na ratio (1.930%) was obtained from the control plots and the greatest Na ratios were obtained from biological fertilizer, 50 kg/ha triple super phosphate and 50 kg/ha triple super phosphate + impregnated seed with organic biological phosphorus treatments. Different responds of two cultivars with regard to Na (%) may be resulted from own characteristics of the cultivars. It was reported in a previous study carried out with chickpeas that decreasing soil phosphorus levels increased plant NA (%) contents (Das and Sen, 1981).

Conclusions

Excess soil phosphorus may have indirect negative impacts on sugar beet yield and quality. Excess phosphorus fertilizer treatments reduce plant Fe, Zn and Mn uptake from the soils, thus negatively influence plant growth and development. Such negative impacts are not solely attributed to chemical phosphorus fertilizers. In case of excessive soil phosphorus contents, even biological phosphorus fertilizers do not have positive impacts on sugar beet quality. Present findings revealed decreasing LAI and dry weight values with increasing phosphorus fertilizer quantities. While biological phosphorus fertilizer did not have any significant effects on shoot dry weight, chemical phosphorus fertilizer significantly decreased shoot dry weights even with the lowest dose. Sugar beet cultivars had different responses to phosphorus quantities. In present study, BR_1has better quality than 7233 cultivar and exhibited more stable respond to excess phosphorus fertilizers. In other words, BR_1 was found to be more tolerant to negative conditions than 7233 cultivar, thus it is recommended to be cultivated under adverse conditions.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Investigation of the changes in bran mineral content according to the years and growth conditions in bread wheat genotypes

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Abstract

Bran is not only the raw material of animal feed or functional products; it has also become a natural additive used in the other industrial fields. For this reason, in this study, it has been aimed to reveal to what extent the minerals (K, P, S, Mg, Ca, Zn, Fe, Mn, Cu, and Na) are affected by genotypes, growth seasons (years) and growth conditions and their interactions, rather than how much and what kind of minerals the wheat bran contains. For this purpose, in the study, in the growth seasons of 2013-2014 and 2014-2015, five bread wheat genotypes were carried out as three replicated under organic and conventional growth conditions according to the trial design of split plots in random blocks. At the end of the study, all minerals except Cu were affected by at least one of the variation sources; in other words, although it varies according to minerals, wheat bran minerals were significantly affected by wheat genotypes, growth years and growth conditions and their interactions. In addition, with this study, significant correlations were found between some minerals in organic and conventional conditions.

Keywords: Minerals, Nutrition, Organic farming, Wheat bran

Introduction

Wheat grain is composed of three parts as coat (bran), endosperm and embryo (germ). Endosperm is made of aleurone and starchy endosperm. Germ consists of embryonic part and scutellum. Germ occupies the percent of 2.5 to 3.5 of wheat grain and is rich for protein, sugar, fat and ash (Delcour and Hoseney, 2010). Bran is composed of pericarp, testa and aleurone. It contains non-starchy polysaccharide (40%), starch (34%), lignin (5%), and protein (13.5%) (Palmarola-Adrados et al., 2005). Pericarp is rich interms of insoluble diet fiber, ferulic acid, bioactive compunds, and vitamins (Hemery et al., 2007). Aleuron, the outer layer of the endosperm, is included in the bran by milling. However, it is rich in minerals and B vitamins and constitutes 7% of the grain (Antoine et al., 2002). Testa, rich in alkylresorcinol, a phenolic lipid, is located between aleurone and pericarp (Landberg et al., 2008). Wheat bran is considered brown gold by some researchers and its enormous applications and high market value make it very important. Wheat bran is used in many fields of the industry such as fermentation, water retaining ability, complex substrate and nitrogen source in media for enzyme production, metabolite production, bioremediation, health aspects, food and feed additive, etc. (Javed et al., 2012).

It is seen that the wheat bran ingredients used in bakery products are very high. The wheat bran content used in biscuit in the study of El-Sharnouby et al. (2012) can be given as an example which were included crude protein (14.0%), crude fiber (15.4%), ash (4.8%), and carbohydrate (75.0%) and also high mineral contents such as calcium, sodium, potassium, iron, phosphorus, zinc, magnesium, manganese, and copper (76.0, 2.0, 1182, 10.6, 1013, 7.3, 611, 11.5, 1.0 mg kg^{-1}). Similar values are confirmed by USDA (2020). Sudha et al. (2011)

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reported the ash, crude protein and fat content in wheat bran used in making pasta as 5.99, 15.1 and 5.83%, respectively. They stated that the total dietary fiber content of pasta was increased 5.2 times with the participation of 40% wheat bran. Hell et al. (2014) stated that wheat bran consists of 3-8% minerals as dry matter. The oil to be used in fried cereal products will be reduced while the dietary fiber content of the product will be increased by the bran additive (Onipe et al., 2015). Wheat bran usage has been increasing year by year and the amount of product obtained from wheat bran was 52 in 2001; however, this number reached 800 in 2011 (Curti et al., 2013). Wheat bran is rich for mineral contents like iron, zinc, manganese, magnesium and phosphorus as mentioned before, but 80% of phosphorus is kept in the phytate forms of iron, zinc and magnesium, and these forms of the elements decrease their intakes (Anderson et al., 2014). Mineral elements have the vital importance for humans, animals and plants. Indeed, they have important biochemical functions; for examples, Ca and P join the structure of bones and teeth; P also functions in the nucleotid, ATP. Na is the cation, provides fluidity outside the cell while K is a cation, provides intracellular fluidity. Also, Mg, Cu, Fe, Mn, Mo and Zn functions some enzymes; S is in the structure of some aminoacids (Soetan et al., 2010). For these responsibilities of the elements, they have been the subjected to the researhes in foods, feeds, and their organic and conventional additives.

This study aimed to investigate the change of some nutritionally important minerals in bread wheat brans according to years and organic and conventional growth conditions.

Materials and Methods

Material

In the study, five hopeful winter bread wheat lines, which were grown in Siran district of Gumushane province in 2013-2014 and 2014-2015 seasons and whose pedigrees and origins are given below, are used as materials (Table 1). Some physical and chemical properties of the trial soils are also given in Table 2.

Table 1. Numbers, pedigrees and origins of the bread wheat genotypes used in the trial

No	Pedigree	Origin
1	KARL/NIOBRARA//TAM200/KAUZ/3/TAM200/KAUZ	Turkey/CIMMYT/ICARDA
2	PYN*2/CO725052/4/PASTOR/3/KAUZ*2/OPATA//KAUZ	Mexica -Turkey/CIMMYT/ICARDA
3	OK98649/TX95V6608/3/ID#840335//PIN39/PEW	USA-Turkey/CIMMYT/ICARDA
4	ST.ERYHTR 1287-08	Ukraine
5	TX98D1170*2/TTCC365	USA

Table 2. Some physical and chemical properties of the trial soils

Analyze	Saturation (%)	Total salt (%)	pH	$CaC0_3$ (%)	P_2O_5 (kg ha^{-1})	K_20 (kg ha^{-1})	Organic matter (%)
Value	68.0	0.15	7.99	10.66	10.95	1490.1	1.63
Evaluation	clayey	saltless	slightly alkaline	calcareous	poor	sufficient	low

Climatic data for 2013-2014 and 2014-2015 growth seasons were given in Table 3. According to the table, although there were not many differences between the years in terms of average temperature. June, which corresponds to the flowering and grain filling period, was more wet (+ 9.4 mm) and more humid (+ 9.1%) in the second growth season (2014-2015).

Table 3. Climatic data of Gumushane province between 2013-2015 and long years average (1950-2015)

Months	Temperature (°C)			Precipitation (mm)			Relative humidity (%)		
	2013-2014	2014-2015	Long years	2013-2014	2014-2015	Longyears	2013-2014	2014-2015	Longyears
October	12.3	14.4	13.8	28.2	61.4	34.3	53.5	64.6	54.0
November	8.7	7.2	7.0	19.6	51.6	45.2	63.4	64.5	48.7
December	-2.2	6.2	-1.3	31.3	14.2	45.7	65.0	63.0	66.3
January	2.1	0.8	0.0	28.5	55.5	47.8	62.9	62.0	59.9
February	3.3	3.3	4.6	22.1	34.4	33.4	54.3	59.5	54.3
March	8.9	7.3	7.3	45.3	67.4	64.2	55.7	55.9	50.6
April	13.5	9.6	12.0	38.1	46.8	47.2	53.8	57.4	44.6
May	17.1	15.9	16.0	66.7	45.3	95.8	58.5	55.1	50.2
June	20.8	20.5	20.5	31.0	40.4	69.1	51.5	60.6	51.5
July	26.0	24.5	24.1	19.3	2.8	5.8	48.7	48.8	41.4
Mean/Total	11.05	10.97	10.40	330.1	419.8	488.5	56.73	59.14	52.15

Method

The trials were planted in the last week of October during the 2013-2014 and 2014-2015 growth seasons, with three replicates by drill, with 500 seeds per square meter. Each plot was the area of 1.05 m (17.5 cm × 6 rows) × 5 m = 5.25 m^2. Twenty tons of organic farm fertilizer (0.32% N, 0.16% P_2O_5, 0.12% K_2O) and 5 t organic poultry manure (3% pure nitrogen) were used per hectare before sowing time. In the conventional plots, 120 kg of pure nitrogen and 60 kg of P_2O_5 per hectare, half of the nitrogen and all of the phosphorus were given together with 20.20.0 commercial fertilizer and half of the remaining nitrogen with ammonium nitrate (26% N) during the tillering period. During the growth period, the weeds in the plots were removed by hand; no herbicides were used. After the maturation is completed, the plots were harvested by sickles and threshed by the Monomak (MNHR- CD0025-BAH type) laboratory thresher machine.

Element contents (K, P, S, Mg, Ca, Zn, Fe, Mn, Cu, Na) of the bread wheat genotypes which were observed from the organic and conventional cultivation between 2013-2015 years were determined according to EPA 6020 method in flour samples milled in laboratory type roller mill of seeds. Accordingly, 0.5 g of each flour sample was burned with 4 ml of 65% HNO_3 and 6ml of H_2O_2 in the microwave (Milestone Start D) and diluted with 50 mL of ultrapure water. The elemental content of the samples was analyzed by inductively coupled plasma-mass spectrometry (inductively coupled plasma mass spectrometry: ICP-MS; Agilent brand ICP-MS 7700e series) technique. In analyzes, Agilent mix 2a standard was used.

The data were analyzed by JMP (2007) according to the split plot trial design in randomized blocks. The differences between the mean values were determined in the same package program by LSD test, and the relationships between the features examined were also determined in the same package program.

Results and Discussion

F values and coefficient variations related with variance analyzis of bread wheat genotypes in terms of bran mineral content (K, P, S, Mg, Ca, Zn, Fe, Mn, Cu, and Na) were given by Table 4. Also, mean values of these elements for years, growth conditions, genotypes, and interactions were shown by the Tables 5-10.

Potassium (K)

Bran K content of bread wheat genotypes for only the interaction of year×growth condition (Y×GC) showed significantly variations. It is understood that the interaction is due to the fact that K content of bran in organic conditions was in the lower group with 3413 mg kg^{-1} in 2013-2014 growth season and 3634 mg kg^{-1} in the upper group in 2014-2015 growth season (Table 7). Although not statistically significant (Table 4); first year and second year (3472 and 3500 mg kg^{-1}, respectively), organic and conventional growth conditions (3524 and 3448 mg kg^{-1}, respectively) (Table 5) and genotypes (3393 to 3606 mg kg^{-1}) (Table 6) showed minor variations. USDA (2020) has reported the lower values for K content of wheat bran.

Phosphorus (P)

Bran P content of bread wheat genotypes for only the interaction of year×genotype (Y×G) and growth condition×genotype (GC×G) showed significantly variations. When Table 8 is investigated, it will be seen that Y×G interaction is caused by the fact that genotype 4 was in the upper group (with 2198 mg kg^{-1}) in the first year, while it was in the lower group (1962 mg kg^{-1}) in the second year. Also, the GC×G interaction was due to the fact that genotypes 1 and genotype 3 were in different groups in organic and conventional conditions (Table 9). Eventhough statistically non-significant (Table 4); first year and second year (1982 and 1929 mg kg^{-1}, respectively), organic and conventional growth conditions (1967 and 1945 mg kg^{-1}, respectively) (Table 5) and genotypes (1895 to 2080 mg kg^{-1}) showed a slight variation. El-Sharnouby et al. (2012) and USDA (2020) have stated the close results (1013 mg kg^{-1}); but Fardet (2010) and Brouns et al. (2012) have reported much higher values which ranged between 900 to 1500 mg $100g^{-1}$ bran.

Sulphur (S)

Bran S content of bread wheat genotypes changed significantly except years, Y×GC, and GC×G (Table 4). Thus, conventional conditions (1522 mg kg^{-1}) for S content were higher than organic conditions (1291 mg kg^{-1}) (Table 5). In addition, bran S contents of the genotypes changed between 1275 mg kg^{-1} (genotype 3) and 1511 mg kg^{-1} (genotype 4) (Table 6). Y×G interaction was significantly effective on the bran S content, and this interaction was sourced from genotype 4. Because, genotype 4 was significantly higher S values in the second year (1640 mg kg^{-1}) than the first year (1382 mg kg^{-1}) (Table 8). Also, Y×GC×G interaction was significantly effect on the bran S content (Table 4). Because, genotype 2 under conventional conditions was in the upper statistical group with 1610 mg kg^{-1} in 2013-2014 season while it was in the lower group with 1291 mg kg^{-1} in 2014-2015 season. Also, genotype 3 under organic conditions in 2013-2014 was in upper group with 1329 mg kg^{-1} while it was in lower group with 1018 mg kg^{-1} in 2014-2015 (Table 10).

Magnesium (Mg)

Bran Mg content significantly varied according to the years, genotypes, GC×G, and Y×GC×G (Table 4). For bran Mg content, second year (443.8 mg kg^{-1}) was statistically higher than the first year (441.7 mg kg^{-1}) (Table 5). Genotype 4 had the highest Mg content (457.7 mg kg^{-1}) while the genotype 1 (420.4 mg kg^{-1}) had the lowest Mg content (Table 6). Babu et al. (2018) has reported lower values for Mg content of wheat bran (12 mg 100 g^{-1} bran). Also, Mg content of the genotypes'bran was affected from the interaction of GC×G (Table 4). It is understood that this interaction is originated from genotype 1 and genotype 5. Because, these genotypes were in different statistical groups in different growth conditions (Table 9). Also, the interaction of Y×GC×G affected the bran Mg content (Table 4). As a reason of this trio interaction, genotype 1 under conventional conditions was in upper statistical group in 2013-2014 while it was in lower group in 2014-2015. Another reason of the trio interaction was from genotype 5 which was in a low group under organic conditions in 2013-2014 while it was in the highest group under the same growth conditions in 2014-2015 (Table 10).

Calcium (Ca)

Bran Ca content statistically differed for years, genotypes, GC×G, and Y×GC×G (Table 1). Growth season of 2014-2015 had higher for Ca content (374.1 mg kg^{-1}) than the season of 2013-2014 (354.5 mg kg^{-1}) (Table 5). According to the genotypes, range of Ca content were 349.3 mg kg^{-1} (genotype 4) to 373.2 mg kg^{-1} (genotype 5). The lower value for K content of wheat bran has been stated by USDA (2020). GC×G interaction was arised from genotype 5; because, this genotype was in different statistical groups in different growth conditions (Table 9). Also, genotypes 2 and 5 caused to the interaction of Y×GC×G. Genotype 2 under organic conditions was in the lowest statistical group in 2013-2014 while it was in the higher group in 2014-2015. In addition, genotype 5 which was in a low group under conventional conditions in 2013-2014 while it was in upper group under the same growth conditions in 2014-2015 (Table 10).

Zinc (Zn)

Bran Zn content was significantly different for genotypes, Y×G, and Y×GC×G (Table 4). Thus, the highest values for Zn in the genotypes were found in genotype 1 (40.41 mg kg^{-1}) and genotype 4 (40.41 mg kg^{-1}) while the lowest value was in genotype 3 (38.66 mg kg^{-1}) and genotype 5 (38.36 mg kg^{-1}) (Table 6). Y×G interaction is due to the fact that genotype 3 is in the upper group (with 39.71 mg kg^{-1}) in the first year and in the lower group (37.61 mg kg^{-1}) in the second year (Table 8). Some researchers have reported lower values (El-Sharnouby et al., 2012), while others have stated higher values (Brouns et al., 2012; Babu et al., 2018) for bran Zn. Also, Y×GC×G interaction was from the genotypes 3 and 4. These genotypes under organic growth conditions were at different statistical groups according to the years (Table 10).

Table 4. *F* values and coefficient variations (CV) related with variance analyzis of bread wheat genotypes for bran mineral content

Minerals	CV (%)	F value						
		Years (Y)	Growth Conditions (GC)	Genotypes (G)	Y×GC	Y×G	GC×G	Y×GC×G
K	7.64	1.888	1.215	1.327	7.929**	1.236	1.160	1.167
P	8.84	0.679	0.230	2.236	0.080	2.535*	3.797*	1.234
S	12.82	5.149	24.654**	4.407**	3.833	3.395*	2.314	8.678**
Mg	3.87	9.797*	1.354	7.708**	1.210	1.985	3.227**	3.657**
Ca	4.78	43.855**	0.229	3.275*	0.014	2.445	2.937*	3.800*
Zn	4.39	0.753	0.047	3.866*	0.027	2.565*	2.246	6.228**
Fe	3.43	0.499	6.499*	4.563**	1.106	2.035	4.694**	1.948
Mn	9.25	1.299	0.948	2.654*	0.018	2.373	0.805	0.775
Cu	4.76	0.230	0.071	1.877	0.002	0.947	0.853	1.904
Na	1.02	46.416**	4.218*	3.028*	25.970**	3.643**	9.363**	3.324*

*, ** shows significance level of *F* value at the probability of 0.05 and 0.01, respectively.

Iron (Fe)

Growth conditions, genotypes, and GC×G interaction significantly differed for bran Fe content of bread wheat genotypes (Table 4). In this context, conventional conditions had higher bran Fe content (36.71 mg kg^{-1}) than organic growth conditions (35.89 mg kg^{-1}) (Table 5). Also, the genotypes changed between 35.72 mg kg^{-1} (genotype 1) and 37.60 mg kg^{-1} (genotype 3) for Fe content (Table 6). A wide range for bran Fe content have been presented by some researchers (Fardet, 2010; Brouns et al., 2012). Also, the GC×G interaction results from genotypes 1, 2 and 4 which are in different groups under different growth conditions (Table 9).

Manganese (Mn)

Bran Mn content showed statistical difference for only genotypes (Table 4). Thus, the highest values were from genotype 1 (24.46 mg kg^{-1}) and genotype 3 (24.64 mg kg^{-1}) while the lowest value was from genotype 4 (22.21 mg kg^{-1}) (Table 6). Mn content of wheat bran shows an extensively range like Fe content. So and so, Fardet (2010) and Brouns et al. (2012) reported on these contents which were 0.9 to 10.1 mg 100 g^{-1} bran.

Copper (Cu)

All variation sources did not show statistically significant differences in bran Cu content in bread wheat genotypes (Table 4). But anyway, it has determined that range for Cu content of wheat bran were 4.94 to 5.19 mg kg^{-1} (Table 6). USDA (2020) presents lower value for bran Cu.

Sodium (Na)

Bran Na content of the genotypes significantly varied for all variation sources (Table 4). For years, the second growth season (2014-2015) (5.05 mg kg^{-1}) was higher than the first season (2013-2014) (4.89 mg kg^{-1}) for bran Na content. Conventional conditions also showed higher Na values than organic conditions (Table 5). Na contents of the genotypes ranged 4.94 mg kg^{-1} (genotype 1) to 5.00 mg kg^{-1} (genotype 4) (Table 6). USDA (2020)'s Na values of wheat bran are under this study's findings. Also, Y×GC interaction was statistically significant for bran Na content. Thus, all years and all growth conditions were in different statistical groups as shown in Table 4. In addition, wheat bran Na contents were effected from Y×G interaction; because, all genotypes were in different statistical groups in different growth seasons (Table 8). Interaction of

GC×G resulted from genotypes 1, 2 and 5 showing different Na values according to the growth conditions (Table 8). Bran Na content was also affected from the interaction of Y×GC×G (Table 4, 10).

Table 5. Changes of bran mineral content in bread wheat genotypes according to the years and growth conditions (mg kg^{-1})

Minerals	Years		Growth Conditions	
	2013-2014	2014-2015	Organic	Conventional
K	3472	3500	3524	3448
P	1982	1929	1967	1945
S	1391	1423	1291 b	1522 a
Mg	441.7 b*	443.8 a	440.1	445.3
Ca	354.5 b	374.1 a	365.4	363.2
Zn	39.19	39.73	39.41	39.51
Fe	36.11	36.49	35.89 b	36.71 a
Mn	23.98	23.26	23.35	23.89
Cu	5.05	5.02	5.03	5.04
Na	4.89 b	5.05 a	4.96 b	4.98 a

* Values in each column showed by the same letter are not different according to LSD test at 0.05 of significance level

Table 6. Mean values of the bran mineral content of the bread wheat genotypes (mg kg^{-1})

Minerals	Genotypes				
	1	2	3	4	5
K	3393	3456	3606	3549	3426
P	1965	1935	1895	2080	1905
S	1475 a*	1302 b	1275 b	1511 a	1470 a
Mg	420.4 c	442.9 b	449.3 ab	457.7 a	443.4 b
Ca	369.1 a	364.1 a	366.0 a	349.3 b	373.2 a
Zn	40.41 a	39.38 ab	38.66 b	40.51 a	38.36 b
Fe	35.72 b	36.30 b	37.60 a	36.16 b	35.73 b
Mn	24.46 a	23.85 ab	24.64 a	22.21 b	22.96 ab
Cu	4.94	5.05	5.02	5.19	4.98
Na	4.94 c	4.99 ab	4.96 abc	5.00 a	4.95 bc

* Values in each column showed by the same letter are not different according to LSD test at 5 % of significance level

Table 7. Changes of bran mineral content in bread wheat genotypes according to the interaction of year×growth condition (mg kg^{-1})

Minerals	2013-2014		2014-2015	
	Organic	Conventional	Organic	Conventional
K	3413 b*	3531 ab	3634 a	3365 b
P	1987	1978	1946	1912
S	1229	1552	1353	1493
Mg	436.7	446.7	443.6	443.9
Ca	355.9	353.2	374.9	373.3
Zn	39.18	39.21	39.65	39.82
Fe	35.53	36.69	36.25	36.73
Mn	23.67	24.30	23.03	23.50
Cu	5.04	5.05	5.02	5.03
Na	4.85 d	4.94 c	5.07 a	5.03 b

* Values in each column showed by the same letter are not different according to LSD test at 5 % of significance level

Table 8. Changes of bran mineral content in bread wheat genotypes according to the interaction of year×genotype (mg kg^{-1})

Minerals		Genotypes 1	2	3	4	5
K	2013-2014	3427	3570	2522	3481	3360
P		2030 ab*	1982 bc	1803 c	2198 a	1900 bc
S		1387 bc	1376 bc	1363 bc	1382 bc	1445 ab
Mg		428.1	447.2	443.9	448.4	440.8
Ca		354.0	346.9	354.6	352.1	365.1
Zn		40.04 abc	38.51 bcd	39.71 abc	39.57 a-d	38.14 cd
Fe		36.34	35.63	37.10	36.17	35.33
Mn		26.14	23.23	24.07	22.96	23.54
Cu		5.00	5.01	5.03	5.13	5.08
Na		4.87 d	4.95 c	4.89 d	4.89 d	4.87 d
		1	2	3	4	5
K	2014-2015	3358	3342	3689	3617	3492
P		1900 bc	1887 bc	1987 bc	1962 bc	1909 bc
S		1562 ab	1229 c	1187 c	1640 a	1495 ab
Mg		412.7	438.6	454.6	467.0	446.0
Ca		384.2	381.2	377.4	346.4	381.2
Zn		40.78 a	40.25 ab	37.61 d	41.45 a	38.57 bcd
Fe		35.10	36.96	38.09	36.16	36.13
Mn		22.78	24.47	25.22	21.46	22.38
Cu		4.88	5.10	5.02	5.25	4.89
Na		5.01 bc	5.03 b	5.04 b	5.11 a	5.04 b

* Values in each column showed by the same letter are not different according to LSD test at 5 % of significance level

Table 9. Changes of bran mineral content in bread wheat genotypes according to the interaction of growth condition×genotype (mg kg^{-1})

Minerals		Genotypes 1	2	3	4	5
K	Organic conditions	3460	3601	3629	3586	3342
P		2081 a*	1915 a-d	1775 d	2059 a	2003 abc
S		1491	1154	1174	1345	1292
Mg		409.6 d	440.6 bc	446.4 bc	448.8 abc	455.3 ab
Ca		366.5 ab	356.3 bc	362.0 bc	355.5 abc	386.6 ab
Zn		39.98	40.00	39.56	39.80	37.74
Fe		34.66 e	35.28 de	37.87 a	35.32 cde	36.32 bcd
Mn		23.96	22.94	24.76	21.64	23.45
Cu		4.97	5.10	4.94	5.12	5.01
Na		4.90 cd	5.03 a	4.99 ab	4.97 ab	4.89 d
		1	2	3	4	5
K	Conventional conditions	3326	3311	3582	3511	3510
P		1849 bcd	1954 a-d	2015ab	2101 a	1806 cd
S		1459	1450	1377	1678	1648
Mg		431.2 c	445.1 bc	452.1 ab	466.5 a	431.5 c
Ca		371.8 ab	371.8 ab	369.9 ab	343.0 c	359.7 bc
Zn		40.84	38.76	37.76	41.22	38.98
Fe		36.77 abc	37.32 ab	37.33 ab	37.00 ab	35.14 de
Mn		24.96	24.76	24.53	22.79	22.47
Cu		4.91	5.00	5.10	5.26	4.95
Na		4.98 ab	4.95 bc	4.94 bcd	5.03 a	5.02 a

* Values in each column showed by the same letter are not different according to LSD test at 5 % of significance level.

Table 10. Changes of bran mineral content in bread wheat genotypes according to the interaction of year×growth condition×genotype (mg kg-1)

Minerals	Genotip	2013-2014		2014-2015	
		Organic	Conventional	Organic	Conventional
S	1	1111 hi	1663 a-d	1870 a	1255 f-i
	2	1141 hi	1610 a-e	1167 hi	1291 f-i
	3	1329 e-h	1398 d-h	1018 i	1356 e-h
	4	1212 ghi	1553 b-f	1477 c-g	1804 ab
	5	1353 e-h	1536 b-f	1231 ghi	1759 abc
Mg	1	410.1 fg	446.1 a-d	409.1 g	416.2 efg
	2	453.8 abc	440.6b-e	427.4 c-g	449.7 abc
	3	445.7 a-d	442.1b-e	447.1 a-d	462.1 ab
	4	435.9 b-g	460.8ab	461.7 ab	472.3 a
	5	437.8 b-f	443.9b-e	472.8 a	419.2 d-g
Ca	1	347.3 cde	360.8 bcd	385.7 ab	382.7 ab
	2	327.8 e	366.1 a-d	384.9 ab	377.6 ab
	3	346.8 cde	362.4 a-d	377.3 ab	377.5 ab
	4	366.6 abc	337.6 de	344.5 cde	348.3 cde
	5	391.0 a	339.2 cde	382.2 ab	380.3 ab
Zn	1	39.58 b-h	40.50 a-e	40.39 a-f	41.18 abc
	2	40.27 a-g	36.75 h	39.73 b-g	40.78 a-d
	3	41.33 ab	38.10 d-h	37.79 e-h	37.43 gh
	4	36.80 h	42.35 ab	42.80 a	40.10 a-g
	5	37.94 d-h	38.35 c-h	37.53 fgh	39.61 b-h
Na	1	4.78 h	4.97 cde	5.02 bc	4.99 cd
	2	4.94 def	4.97 cde	5.12 a	4.93 def
	3	4.87 fg	4.91 ef	5.12 a	4.97 cde
	4	4.86 fgh	4.92 def	5.09 ab	5.14 a
	5	4.80 gh	4.94 def	4.98 cde	5.11 a

* Values in each column showed by the same letter are not different according to LSD test at 5 % of significance level

Table 11. Correlation coefficients (r) among the bran minerals of the bread wheat genotypes under organic and conventional conditions (n=30)

	K	P	S	Mg	Ca	Zn	Fe	Mn	Cu
P §	**-0.133** 0.057								
S	**-0.108** 0.130	**-0.217** -0.010							
Mg	**0.310** 0.340	**-0.379*** 0.549**	**-0.113** 0.135						
Ca	**-0.295** -0.133	**0.140** -0.182	**0.005** -0.318	**-0.073** -0.257					
Zn	**0.352** 0.082	**-0.279** 0.086	**0.296** -0.209	**-0.092** 0.251	**-0.514**** 0.092				
Fe	**0.039** -0.468**	**-0.035** 0.255	**-0.371*** 0.195	**0.132** 0.336	**0.344** 0.061	**-0.454*** -0.193			
Mn	**0.190** -0.239	**-0.144** 0.286	**-0.299** -0.287	**-0.103** 0.290	**0.166** -0.142	**-0.328** 0.068	**0.380*** 0.295		
Cu	**-0.248** 0.288	**-0.003** -0.044	**0.021** 0.080	**-0.141** 0.133	**-0.109** -0.059	**0.326** 0.032	**-0.110** -0.128	**0.060** -0.299	
Na	**0.508**** -0.034	**-0.212** -0.222	**0.166** 0.446*	**0.144** -0.106	**0.131** -0.010	**0.247** 0.056	**0.338** -0.263	**-0.091** -0.325	**0.122** 0.026

§ In the same line, the upper values in bold indicate the coefficients in organic conditions and the lower values in conventional conditions. *, ** show the significance levels at the probability of $P<0.05$ and $P<0.01$, respectively.

Relationships between Bran Minerals of the Bread Wheat Genotypes

Correlation coefficients of the bran mineral contents of the bread wheat genotypes under organic and conventional conditions were given in Table 11. Thus, there was a negative significant correlation (r=-0.379, P<0.01) between Mg and P under organic growth conditions while there was a positive significant correlation (r=0.549, P<0.01) between them under conventional conditions. Zn and Ca were negatively significantly correlated each other (r=-0.514, P<0.01). Fe was negatively significantly correlated with K (r=-0.468, P<0.01) under conventional conditions while it was negatively correlated with S (r=-0.371, P<0.05) under organic conditions. There was also negative significant correlation between Fe and Zn (r=-0.454, P<0.05) under organic growth conditions. Mn and Fe showed positive significant correlation (r=0.380, P<0.05) under organic conditions. Na presented positive significant correlations with K (r=0.508, P<0.01) under organic conditions, and with S (r=0.446, P<0.05) under conventional conditions.

Conclusion

Like all cereal products; bran, which is a fraction of grain, does not make sense by itself and its source is important. In other words, the conditions in which these genotypes are grown (including the growth seasons and growth conditions) have an effect on the nutritional contents of wheat bran, especially the genotype from which bran is obtained; in this study, it was concluded that bran minerals are significantly affected by these variation sources and their interactions, so that genotype and growth conditions should be considered in bran studies, and even location studies should be added to these factors.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Behavioral variations due to effects of day length on yield and related characteristics of some *Brassica juncea* L. genotypes under hot humid continental conditions

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Abstract

The search for new oil seed crops in the low rainfall drought Central Anatolia has attracted researchers to profitable alternative crops like *Brassica* species in recent years. Breeding of a suitable oilseed crop for the Central Anatolia will provide a new rotational break for controlling soil borne diseases, weeds and drought stress that are very difficult to manage with prevailing agricultural practices. *Brassica juncea* L. is an important oilseed crop and is newly introduced in Turkey. This study was conducted to evaluate the developmental responses to 4 different day lengths and cosequently climatic conditions to screen 38 *B. juncea* genotypes for some oil yield related agronomic charachterisitics under hot humid climatic conditions of Yenimahalle, Ankara, Turkey. It was noted that early and delayed sown genotypes had synchronization towards flowering in the month of June with 15 h 01 min to 15 h to 39 min day length photoperiod. The results showed a significant effects of sowing dates day lengths and consequently climates upon genotypes for oil yield and agronomic traits. The maximum mean crude oil yield (38.4 g m^{-2}) was obtained from AK genotype. The results clearly describe that early sowing provides long period of vegetative growth with increased intake of photosynthates to plant sinks; that is not possible under late sowings.

Keywords: Minerals, Nutrition, Organic farming, Wheat bran

Introduction

Brassica juncea L. (brown mustard) is extensively used for extraction of industrial oils. They are also used as vegetable, spice, and production of pharmaceutical formulations (Kokcu et al., 2015; Kayacetin, 2020). *B. juncea* have an annual growth habit, herbaceous, yellow flowering ensued by seedset and are deep rooted (Guner et al., 2012). Occurrence of phenological events and yield of Brassica species are strongly related to day length related ontogenetic changes and climatic variations (Kar and Chakravarty, 2000).

B. juncea require a period of short, intermediate or neutral, short-long and long days for gradual moving from vegetative phase to flowering phase (Kumar et al., 2018). A delay in sowing dates (with increase or decrease in day length) can decrease seed yield from about 10 to 50% in different Brassica species and influence phenological development of crop plants (Shargi et al., 2011; Shekhawat et al., 2012). Phenological development in Brassica species change significantly by photoperied and temperature (Robertson et al., 2002). Sowing dates can have a significant and variable impact on different genotypes depending on their genetic backgrounds. No information is available on the response of *B. juncea* to spring sowing dates (long day length) for the genotypes grown in Turkey.

The target of the present study was to determine the optimum spring and mild summer sowing time or day lengths with gradual changing day length from short to long days + temperature on growth, development and yield performance of 38 improved *B. juncea* genotypes under hot humid continental

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climatic conditions.

Materials and Methods

The experiments were conducted during the growing seasons of 2017 at at the experimental fields located at Yenimahalle Ankara, Turkey (39°12′ - 43°6′ N, 35°58′ - 37°44′ E, and 925 m altitude), with hot humid continental conditions classified as Dsa type in the Koppen-Geiger climate classification system. The study compared performance of 38 *B. juncea* genotypes obtained from USDA gene bank and obtained from Konya, Turkey. These were multiplied and selected for use in the experiment in Central Research Institute for Field Crops during 2012-2017. All genotypes were grown under natural conditions without using any fertilizer or pesticides. Each genotype was sown manually in plots having three m long three rows for each genotype with 30 cm long unreplicated row spacings and sowing depth of ~1.0-1.5 cm and seed rate of 6000 kg ha^{-1} (Kayacetin, 2019). Sowing was done on four different spring dates (25th March–11 h 27 min to 13 h 26 min or short day length, 10th April-13 h 01 min to 14 h 37 min short to short–long day length, 25th April–13 h 01 min to 14 h 37 min short–long day length to intermediate day length and 10th May–14 h 02 min to 15 h 35 min long day length) and temperature.

Meteorological data pertaining to vegetation period (from March to August) of long term and 2017 climatic conditions of Yenimahalle, was obtained from the Meteorology Stations of the Central Field Crops Research Institute, Ankara, Turkey. There was total precipitation of 194.9 and 277.9 mm, average temperature of 17.0 and 17.6 ºC, and an average humidity of 53.5 and 51.3%, respectively during the experimental period (Table 1).

Table 1. Monthly meteorological data of mustard during growing season at the experimental area and photoperiod (h)

Climatic factors		Months					Total/Mean/ Max./Min.
		March	April	May	June	July	
Total precipitation (mm)	Long years	36.7	46.7	49.9	34.2	14.3	181.8
	2017	46.1	19.8	96.2	102.8	0.0	264.9
Mean relative humidity (%)	Long years	63.2	59.0	56.5	52.1	45.1	55.2
	2017	59.6	49.8	55.7	58.3	38.4	52.4
Mean temperature (ºC)	Long years	6.4	11.5	16.2	20.3	23.8	15.6
	2017	8.1	11.2	15.7	20.3	25.5	16.2
Maximum temperature (ºC)	Long years	21.4	25.7	29.3	33.6	36.2	36.2
	2017	19.9	27.2	29.2	35.8	38.3	38.3
Minimum temperature (ºC)	Long years	-5.9	-0.8	4.1	8.1	11.4	-5.9
	2017	-1.5	-1.0	5.0	9.0	14.2	-1.5
Photoperiod (h)	Max.	13 h 26 min	14 h 37 min	15 h 35 min	15 h 39 min	15 h 38 min	
	Min.	11 h 27 min	13 h 01 min	14 h 02 min	15 h 01 min	15 h 00 min	

Data were obtained from Meteorology Stations of the Central Field Crops Research Institute, Ankara, Turkey

The soil analysis during 2017, from the soil taken at a depth of 0-20, 21-40 cm showed low organic matter (1.32%), in alkaline (pH 7.81), limey (5.3%), and clay-loamy soils of the experimental plots (Table 2). The soil analysis was carried out at The Soil, Fertilizer and Water Resources Institute, Ankara, Turkey.

Table 2. Soil sample features belonging to the experimental area

Depth (cm)	Texture	Saturation content (%)	Total salt (%)	pH	Lime (%)	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Organic Substance (%)
0-20	Clay loamy	56.0	0.025	7.81	5.3	93	1260	1.35
21-40	Clay loamy	56.0	0.025	7.81	5.2	105	2400	1.28
Mean		56.0	0.025	7.81	5.3	99	1830	1.32

Data were obtained from Soil, Fertilizer and Water Resources Institute

The data about growth parameters including seedling emergence, 50% flowering and 90% physiological maturity, plant height, number of branches, number of capsules, and thousand seed weight were measured manualy; whereas, seed yield, seed weight and yield of individual genotypes was measured at about 8.5% moisture content (CFIA, 1999) on middle single rows as an average of 5 randomly selected plants for each genotype (Yousaf et al., 2002).

The oil content was determined by grinding 10 g of powdered samples of each genotype and hexane extracted using Gerhard 2000 apparatus for determining the crude oil content.

The mean, maximum and minimum values were calculated for all investigated parameters.

Results and Discussion

The days to emergence (8.3-14.9 d), days to 50% flowering (29.5-57.5 d), days to maturity (26.5-48.5 d), plant height (25.7-124.6 cm), number of branches (2.9-5.3 branch $plant^{-1}$), number of capsules (46.2-113.8 capsul $plant^{-1}$), thousand seed weight (0.8-1.8 g), seed yield (5.9-101.0 g m^{-2}), crude oil content (11.9-25.0%) and crude oil yield (0.7-25.8 g m^{-2}) showed differences among genotypes and the effects of varying environmental conditions at the time of 4 sowing dates used in the study (Table 3).

Among 38 genotypes the days to emergence, days to 50%

flowering, days to maturity, plant height, number of lateral branches, number of capsules, thousand seed weight, seed yield, crude oil content and crude oil yield changed between 10.3-19.3 d, 44.8-55.3 d, 31.0-38.3 d, 40.5-168.2 cm, 3.0-5.8 branch plant^{-1}, 54.9-118.3 capsul plant^{-1}, 0.5-2.2 g, 6.8-122.4 g m^{-2}, 19.2-27.7% and 1.7-38.4 g m^{-2}, in the same order for all genotypes (Table 3). Day length changed from 11 h 27 min to 13 h 26 min, 13 h 01 min to 14 h 37 min, 14 h 02 min to 15 h 35 min, 15 h 01 min to 15 h to 39 min and 15 h 00 min to 15 h 38 min during March, April, May, June and July 2017 respectively.

Days to emergence (d): Average of the genotypes relating to days to emergence showed maximum number of 14.9 d on 10th May or long days sowing date and the minimum days to emergence (8.3 d) on 25th March (short days) sowing date showed the effects of day length on seed germination (Table 3). Long day length with increased temprature, low soil moisture and relative humidity and precipittion induced poor seed germination taking more days to emerge from soil alongwith poor plant growth. Contrarily, short day length (March sowing) with appropriate low temperature, appropriate soil moisture, relative humidity and precipitation induced rapid seed germination and growth in agreement with (Angadi et al., 2003, Kayacetin et al., 2019). Taking an average of sowing dates the maximum days to emergence was determined as 13.0 d for B18 genotype while the minimum days to emergence was 10.3 d noted with number of other genotypes. This might be due to day length interaction with the genetic potential of the respective *B. juncea* genotypes.

Days to 50% flowering (d): As a mean of genotypes, the average longest days to 50% flowering (57.5 d) was obtained on 25th March sowing date while the mean earliest days to 50% flowering (29.5 d) was noted on 10th May (long days) sowing date (Table 3). This showed that first sowing on 25th March short day sowing helped the genotypes to to take the longest vegetative period with CO_2 assimilation and before transforming them for reproductive or generative phase; whereas, the condition reversed for 10th May long day sowing. Environmental conditions were harsh but day length conditions were appropriate for going of the genotypes from vegetative to generative phase reproductive phase. Tobe et al. (2013) evaluated early sown *B. napus* on 30th March short day conditions and found delayed 50% flowering and capsules induction compared to the late sown genotypes on 14th April 29th April and 14th May under temperate conditions at Ardabil (Iran). Similar findings were noted by Kumar et al. (2004) under semi arid tropic conditions. This study determined minimum days (44.3 d) to 50% flowering for AC1 genotype and the maximum days (48.3 d) to 50% flowering for B24-25-26 genotype.

Days to maturity (d): As a mean of genotypes; the maximum days to maturity (48.5 d) was obtained on 25th March short day sowing date, while the minimum days to maturity (26.5 d) was observed on 10th May long day sowing date (Table 3). Again, the time sowing was very effective and the prevailing environmental conditions (temperature, precipitation and humidity) and day length at earlier sown 25th March short day sowing had comulative positive effects at the time of harvesting. Therefore, 25th March short day sowing date was evaluated as the best and most appropriate sowing condition for these genotypes. Whereas 10th May long day sowing condition in interaction with prevailing environmental conditions was inapropriate and failed to induce the required positive generative stimulus to the plants of respective genotypes. Similar results were reported by Tobe et al., 2013. The maximum days to maturity were determined as 38.3 d for B20 genotype while the minimum days to maturity (d) were noted as 35.0 d for B16-17-18-21-24-25-26 genotypes. This could be attributed to different genetic potentials and backgrounds of the studied genotypes as affected by day length or 4 different photoperiods.

Plant height (cm): Mean of genotypes for plant height was the maximum for the genotypes sown on 25th March short days (113.8 cm) while the minimum height (46.2 cm) was noted on 10th May (long days) sowing. The results revealed that plant height decreased with delaying sowing dates. Plant height was maximum on 25th March (short days) sowings. This can be due to sowing date day length and prevailing environmental conditions during sowing. The day length changed from 11 hours 27 minutes to 15 hours 38 minutes in April to July before flowering. They had maximum time to grow and induce leaves with increased photosynthesis and CO_2 assimilation. This resulted in better plant length and plant yield on earlier sown genotypes. The results confirm earlier findings of Jat (2014) and Gawariya et al. (2015). The maximum plant height was noted as 118.3 cm for B20 genotype; whereas, the minimum plant height was obtained as 68.4 cm for B26 genotype. Variations in plant height at each sowing of different genotypes could be due to genetic backgrounds of the species included in this study.

Long days photoperiod and sub-optimal temperature conditions which prevailed during the emergence and subsequent vegetative growth inhibited vegetative growth on the late sown genotypes that forced the respective genotypes to mature earlier with earlier generative phase induction in late sown genotypes on 10th May (long days) sowing. March sown genotypes had long period of shoot growth necessary for improved plant height. This is desired for appropriate vegetative and reproductive growth. May sown genotypes received long day length of 14 h 02 min to 15 h 35 min; however on reaching optimum day length, the genotypes bolted and flowered precociously with reduced cell elongation and division and nutrient accumulation irrespective of their growth period. These results are in conformity to those reported by Thurling and Das (1980).

Number of branches (branche plant^{-1}): The significant differences among different sowing dates was determined for each genotype. The maximum number of branches (5.3 branch plant^{-1}) was obtained on 25th March (short days) sowing date while the minimum or least number of branches (2.9 branch plant^{-1}) was noted on the genotypes sown on 10th May (long days) sowing date. Induction of maximum number of branches plant^{-1} was noted on all genotypes on 25th March (short days) sowing date due to long period of growth and CO_2 assimilation before enetering of the genotypes into generative phase. Similar results have also been reported by Gawariya et al. (2015); Muhal and Solanki (2016). Average sowing dates showed the maximum number of 5.8 branches plant^{-1} for AC1 genotype;

whereas, the minimum number of 3.0 branches plant^{-1} were obtained for A10 genotype. This could also be due to variable genetic potential of the genotypes under consideration for each sowing and the prevaling environmental conditions at the time of sowing and harvest.

Table 3. Effect of sowing dates on growth, development and yield of the brown mustard genotypes used

Treatments	Days to emergen-ce (d)	Days to 50% flowe-ring (d)	Days to maturity (d)	Plant height (cm)	Number of branches (branch plant^{-1})	Number of capsu-les (capsul plant^{-1})	Thousand seed wei-ght (g)	Seed yield (g m^{-2})	Crude oil content (%)	Crude oil yield (g m^{-2})
Sowing dates										
25th March	8.3	57.5	48.5	113.8	5.3	113.8	1.8	101.0	24.8	25.8
10th April	10.7	55.0	37.8	110.8	4.3	103.8	1.6	82.6	25.0	21.5
25th April	13.9	49.3	28.2	100.0	3.5	100.0	1.5	57.8	22.0	13.3
10th May	14.9	29.5	26.5	46.2	2.9	46.2	0.8	5.9	11.9	0.7
Average	11.9	47.8	35.3	92.7	4.0	91.0	1.4	61.8	18.3	15.1
Max.	14.9	57.5	48.5	113.8	5.3	113.8	1.8	101.0	25.0	25.8
Min.	8.3	29.5	26.5	46.2	2.9	46.2	0.8	5.9	11.9	0.7
Genotypes										
A2-Turkey, İzmir	10.3	48.0	35.3	92.2	4.8	92.2	1.3	122.4	23.2	37.2
A3-Turkey	10.3	48.0	35.3	101.3	3.9	101.3	1.5	84.5	24.2	27.3
A4-Turkey	10.3	48.0	35.3	95.4	4.2	95.4	1.5	72.4	23.5	22.5
A5-Turkey, Tekirdag	10.3	47.0	36.3	98.9	4.0	98.9	1.7	84.0	25.6	27.9
A6-Turkey, Kayseri	10.3	47.0	36.3	94.0	3.8	94.0	1.6	81.3	23.5	25.0
A7-Turkey, Tekirdag	10.3	47.0	36.3	95.5	3.8	95.5	1.6	76.2	25.8	25.3
A9-Turkey, Tekirdag	10.3	48.0	35.3	99.8	3.8	99.8	1.6	84.6	26.0	28.4
A10-Turkey, Kirklareli	10.3	48.0	35.3	100.4	3.0	100.4	1.4	79.5	23.9	25.0
A11-Turkey, Edirne	10.3	48.0	35.3	95.4	3.4	95.4	1.4	76.1	26.6	26.2
B4-Turkey	10.3	48.0	35.3	99.0	3.9	99.0	1.6	72.6	23.3	22.2
B5- Turkey, Tekirdag	10.3	48.0	35.3	103.1	4.8	103.1	1.6	69.1	24.8	22.7
B6-India	10.3	47.0	36.3	108.0	4.2	108.0	1.7	72.8	24.6	23.2
B7-India, Rajasthan	10.3	46.5	36.8	109.2	4.1	109.2	1.9	84.3	26.0	29.0
B8-Pakistan	10.3	47.0	36.3	100.8	3.8	100.8	2.2	65.6	24.2	20.6
B10-India	10.3	46.5	36.8	109.7	3.7	109.7	1.6	72.9	24.4	23.5
B12-Pakistan	10.3	47.0	36.3	104.4	3.8	104.4	1.8	80.1	26.0	27.7
B13-China	10.3	47.0	36.3	113.6	3.7	113.6	1.6	46.9	23.2	14.6
B14-China	10.3	48.3	35.0	115.1	3.8	115.1	1.5	89.2	25.3	29.4
B15-Pakistan	10.3	48.0	35.3	110.6	4.5	110.6	1.4	80.3	25.7	26.9
B16-Canada	10.3	48.3	35.0	115.2	3.8	115.2	1.3	82.0	23.6	24.8
B17-Canada	10.3	48.3	35.0	116.7	3.6	116.7	1.5	56.7	26.4	19.9
B18-United States, California	13.0	45.5	35.0	69.7	4.1	69.7	1.7	49.3	24.6	15.7
B20-Russian Federation	10.3	48.3	38.3	118.3	4.1	118.3	1.8	85.0	27.4	31.1
B21-Russian Federation	10.3	48.3	35.0	115.4	3.8	115.4	2.0	67.9	26.3	23.6
B22-China, Xizang	10.3	46.0	37.3	87.2	4.1	87.2	1.6	69.6	25.4	23.2
B23-Pakistan	10.3	46.0	37.3	100.0	3.8	100.0	2.2	102.5	26.8	35.2
B24-Germany	10.3	48.3	35.0	111.2	3.5	111.2	1.8	60.4	26.8	21.4
B25-Germany	10.3	48.3	35.0	111.8	4.2	111.8	1.5	87.8	25.4	29.2
B26-Italy, Calabria	10.3	48.3	35.0	68.4	4.0	68.4	1.4	53.3	24.4	17.2
B27-United States, Minnesota	10.3	46.0	37.3	106.8	4.1	106.8	1.7	90.6	24.5	28.9
B28-United States, Minnesota	10.3	46.0	37.3	106.4	4.3	106.4	1.4	93.0	25.5	31.8
BJ99-India	10.3	47.8	35.5	107.4	4.3	107.4	1.4	69.1	24.8	22.6
BJ20-India	10.3	48.0	35.3	108.9	4.2	108.9	1.6	95.3	25.9	32.4
AC1-India	10.8	44.8	38.0	78.3	5.8	78.3	1.5	109.0	24.9	36.5
AC2-India	11.5	46.5	35.5	81.0	5.3	81.0	1.6	41.5	24.1	13.4
B30-India	10.3	45.3	38.0	86.6	3.9	86.6	1.9	74.2	27.7	26.3
B33- India	10.3	46.0	37.3	85.2	4.2	85.2	2.0	40.6	25.6	13.6
AK-Turkey, Konya	10.3	47.8	35.5	106.2	3.9	106.2	1.8	114.2	25.2	38.4
Average	10.4	47.3	36.0	100.7	4.1	100.7	1.6	77.3	25.1	25.5
Max.	13.0	48.3	38.3	118.3	5.8	118.3	2.2	122.4	27.7	38.4
Min.	10.3	44.8	35.0	68.4	3.0	68.4	1.3	40.6	23.2	13.4

Number of capsules (capsule plant^{-1}): The maximum number of capsules (113.8 capsul plant^{-1}) were obtained on 25th March (short days) sowing date while the minimum number of capsules (46.2 capsul plant^{-1}) were obtained on 10th May (long days) sowing date. The plant needs long period of vegetation to grow and mature for higher yield. This is possible if vegetative period of growth is prolonged. This condition was provided by the e 25th March short days sowing of 38 genotypes ending up with maximum yield. The highest number of capsules plant^{-1} were noted due to this effect i.e. long period of vegetative growth. As expected precocious induction of generative phase had negative effects on growth and yield number of capsules plant^{-1} due to shorter period of growth to reach generative phase. The genotypes sown on during subsequent dates had shorter periods of vegetative growth generative phase ended up with reduced number of capsules leading to minimum number of capsules plant^{-1} on 10th May (long days) sowing. These plants from each of 38 genotypes were smaller in height and ultimately ended up with less number of branches and capsules plant^{-1}. The results are approved and confirmed by Gawariya et al., 2015. The maximum number of capsules was determined with 118.3 capsule plant^{-1} for B20 genotype; whereas, the minimum number of capsules (54.9 capsul plant^{-1}) were noted on B26 genotype. This might be primarily due to the environmental and photoperiod effects aided with genetic background in agreement with Kumar et al., 2008).

Thousand seed weight (g): Comparing average thousand seed weight of genotypes; the maximum thousand seed weight (1.8 g) was detected on genotypes sown on 25th March (short days). The minimum thousand seed weight (0.8 g) was recorded on genotypes sown on 10th May (long days). The plant needs long period of vegetation to grow before entering maturity. On account of favourable weather conditions, improvement in growth and yield up with the maximum seed weight gain in all genotypes of the 25th March short days sowings as described in this section. Precocious maturing due to 10th May sowing had negative effects on growth and thousand seed weight of genotypes as they had less time to transform from vegetative to generative phase. Higher temperature, drought and changed photoperiod during later phases of growth shortened the crop growth period and forced earlier maturity that resulted in reduced thousand seed weight and ultimately the minimum seed yield under subsequent sowings. High temperatures at flowering phase also cause reduction in seed yield leading to pollen sterility. Similar results were reported by Singh and Lallu-Singh (2014); Muhal and Solanki (2016). The maximum thousand-seed weight was determined with 2.2 g B8 and B23 genotypes. As a average of sowing dates; the minimum thousand seed weight was obtained (1.3 g) for B16 genotype (Table 3). The differences in the thousand-seed weight could be due to plastic genetic potential of the genotypes under 4 different environmental conditions induced by the effects of photoperiod for the genotypes in this study (Yousaf et al., 2013).

Seed yield (g m^{-2}): The maximum seed yield (101.0 g m^{-2}) was determined on 25th. March short days sowing date while the minimum seed yield (5.9 g m^{-2}) was noted on 10th May (long days) sowing date. The seed yield losses occured due to 10th May based late sowing date that decreased precocious entering into generative phase and ended up with low seed yield. The findings of Kar and Chakravarty (2000), also supported that precocious induction of generative phase in plants along with delayed sowing affected completation of seed formation, yield and decreased. This, with ultimate consequence of weak rudimentary and feeble seed induction. It is supposed that differences among sowing dates have plastic genetic potential under different photoperiod and environmental conditions like varying air temperatures, precipitation, humidity and the genetic potential of the genotypes in agreement with the results of Tripathi et al. (2005) and Tobe et al. (2013). Neog et al. (2013) also said that delayed sowing decreased the dry matter production. Angadi et al. (2003) showed that, *B. juncea*, *B. napus* and *B. rapa* significantly differed in optimum day/night temperatures; High temperatures, low humidity and precipitation and photoperiod at sowing and flowering also affected capsule development and yield. Every genotype needed two types of photoperiods necessary for vegetative and generative growth. A disruption in any of these, resulted in serious losses to yield. The results showed that the genotypes sown on 10th March, received appropriate photoperiod for vegetative growth ensued by optimum generative photoperiod to induce flowering. This emphasise that the flowering of the plant must be synchronized to an appropriate length of day light for CO_2 assimilation. March sowing allowed plants belonging to all genotypes have longer vegetative growing phase followed by entering in to generative phase. That was inverse in case of May sown genotypes receiving less time for vegetative and generative growth with overall negative implications on all quality and agronomic properties of the genotypes used in this study. 25th March (short days) sowing date accumulated more growing degree days due to long periods of sunshine. These gradually received increasing day length photoperiod in comparison to 10th May (long days) sowing date with less sunshine days of 14 h 02 min to 15 h 35 min day light photoperiod with lesser vegetative growth.

All genotypes began to recover from the stress by continuing flowering after returning to 20/15 °C. The maximum seed yield was determined in A2. A2 and AK (122.4 and 114.2 g, respectively) genotype. The differences in the seed yield of genotypes were due to the better performance of genotypes under the existing agro-climatic conditions of Ankara. This difference might be due to well adaptation and genetic potential of Brassica genotypes. Similar results were obtained by Yousaf et al. (2013) in other Brassica genotyppes under hot humid agro climatic conditions of Bahawalpur, Pakistan. The climate of Yenimahalle Ankara lies in the Central Anatolia with hot humid climate. The Bahawalpur lies very close to River Sutlej and Beyas with hot humid climate. The similarity between the two climates could be a potential reason for similarity in the results. Thurling and Das (1980) analyzed the reaction of six cultivars of spring rape (*B. napus*) to varieties in terms of planting dates. They demonstrated that both the span of vegetative phase and time of sowing of seeds was significant determinants in measuring yield of seeds. It was likewise demonstrated that variety in seed yield was fundamentally connected with variations in term of stem lengthening. They likewise found a direct connection between the plant growth rate and the average photoperiod, while the connection between the growth rate during warm days and the average photoperiod was curvilinear at low average photoperiod.

Kayacetin et al. (2019) also determined the adaptation of the Brassica species using planting season (spring and autumn planting) and locations in the terms of growth of the crop (emergence, 50% inflorescence, physiological maturity and total cultivation days), in 8 different locations under spring sowing conditions. They found that required total temperature for germination (118 ºC), flowering (453 ºC) and ripening (872 ºC) was higher during first year of study compared to the second year of study (71, 440, 620 ºC, respectively). They also found that the first year seed yield (1830 kg ha^{-1}) was higher compared to second year seed yield (1585 kg ha^{-1}). They further noted that plants from early spring sowing showed increased seed yield compared to the plants obtained from late spring sowing; because of the tendency of genotypes to mature earlier (short duration) by accumulating less heat units and long day length.

Crude oil content (%): A comparison among genotypes showed that the sowing dates day length affected the maximum crude oil content. Maximum crude oil content (25.0%) was determined on 10th April sowing, while the minimum crude oil content (11.9%) was recorded on 10th May (long days) sowing date. The crude oil content (24.8%) of 25th March (short days) sowing was statistically similar to 10th April sowing (25.0%). This suggest that a sowing date between these dates had no significant negative effect on quality parameters of these genotypes. Contrarily, Tobe et al. (2013) reported that 30th March sown spring canola had significantly higher percentage of oil as compared to 14th April, 19th April and 14th May sown crops. The difference in results could be due to different agro ecological and photoperiod conditions in the two experiments. Delayed sowing date cause a reduction in crude oil content due to precocious ripening under hotter and drier conditions (Pritchard et al., 2000). Comparing sowing dates the maximum crude oil content of 27.7% for B30 genotype while the minimum crude oil content of 23.2% for B13. The differences in the crude oil content could be due to genetic potential of the genotypes as affected by meteorological conditions and photoperiod (Yousaf et al., 2013).

Crude oil yield (g m^{-2}): A comparison among genotypes showed the maximum crude oil yield (25.8 g m^{-2}) was found on 25th March (short days) sowing date; whereas, the minimum crude oil yield (0.7 g m^{-2}) was noted on 10th May (long days) sowing date. Average of sowing dates among genotypes showed that the maximum crude oil yield was found for AK (38.4 g m^{-2}) while the minimum crude oil yield was noted for AC2 (13.4 g m^{-2}) genotype. 25th March (short days) sowing date and the genotypes AK had high seed yield, crude oil content and crude oil yield was maximum.

Conclusions

Following conclusions were drawn

Regardless of the dates of sowing all *B. juncea* genotypes used in this study flowered at the same synchronised time when the appropriate photoperiod was available.

Yield performance of *B. juncea* in regard to sowing date was distinctive and important for each period for its use as profitable alternative crop for hot humid climates. Each sowing date after first sowing; meant subsequent decrease in time of vegetative and generative growth of the next sown plants and ultimately studied agronomic characteristics of *B. juncea* genotypes in close association with their genetic background.

Out of 38 studied genotypes, the AK and A2 proved the best in performance in terms of growth, development and yield performance.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

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Evaluating the effects of integrated nutrient management and insitu rainwater harvesting on maize production in dry regions of Zimbabwe

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Abstract

Moisture stress and inherent soil fertility caused huge loss in crop production. The use of insitu rainwater harvesting and integrated nutrient management can improve soil health and crop production. The Objective of the study was to evaluate the effects of integrated nutrient management and insitu rainwater harvesting on maize productivity in dry regions of Zimbabwe. Experiment was laid out as a factorial with three main factors which include cattle manure, insitu rainwater harvesting and inorganic manure. Data was collected from harvested net plot to obtain maize grain and stover yields for two growing years. Data was analysed based on analysis of variance using IBM SPSS version 25 and means which were significant different were separated using least significant different. The results show that there was significant different (p<0.001) between all treatments combinations. Results recorded higher maize grain yields from100 kg N/ha + 5 t/ha cattle manure treatments for both seasons. High grain yield of 3.41± 0.042 t/ha was recorded from $ZN_{100}C_5$ treatments during the second year and highest maize grain yield of 3.11 t/ha was recorded in first year. There was significant different (p<0.001) on the effects of combination of cattle manure and inorganic fertiliser alone on maize grain yields. Control treatments recorded lowest maize (1.17 ± 0.031 t/ha) and stover yields of 4.36 ± 0.046 t/ha. Results indicated significant different (p<0.001) on the effects of insitu rainwater harvesting, cattle manure and inorganic manure on maize stover yields. The use of integrated nutrient management and insitu rainwater harvesting has the capacity to increase maize yields and reduce food insecurity in dry regions of most sub-Saharan African countries.

Keywords: Cattle manure, Integrated nutrient management, Maize stover, Insitu rainwater harvesting, Dry regions

Introduction

Increased soil moisture stress and inherent soil fertility has been major causes of poor maize production in dry regions of Zimbabwe (Motsi et al., 2019; Nyagumbo et al., 2019). Erratic and low rainfall in arid and semi-arid regions contributed to poor soil moisture and low crop production in dry regions (Mugwe et al., 2019; Shumba et al; 2020; Muchai et al., 2020). Combination of low rainfall and low nutrient status has significantly affected crop production in most dry regions in African countries (Yazar and Ali, 2016; Mugwe et al., 2019; Shumba et al; 2020). Erratic rainfall and frequent dry spells affected soil moisture which lead to poor maize growth due to reduced nutrient absorption. Climatic variability, long mid-season droughts and dry spell during growing season reduced crop production in dry regions (Nyagumbo et al., 2020).

Maize (*Zea mays* L.) is ranked first in Zimbabwe and is used a one of staple food in African countries. It is widely grown in all regions in Zimbabwe. Integrated nutrient management and insitu rainwater harvesting can be one of the best options to increase maize production in dry regions of Zimba-

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bwe. Integrated nutrient management increases nutrient availability to plant root zone (Vanlauwe *et al*., 2010; Vanlauwe et al., 2014; Shumba et al., 2020). The use of insitu rainwater harvesting techniques increases soil moisture content, reduce soil erosion and improve water availability in the plant root zone (Nyamadzawo *et al*., 2013; Mudatenguha *et al*., 2014). The use of integrated nutrient management and insitu rainwater harvesting techniques has been successfully used by smallholder farmers to improve soil fertility and maize productivity (Mugwe, 2007; Mugwe et al., 2019). Integrated nutrient management option increases nutrient availability in the plant root zone (Mugendi *et al*., 2004; Vanlauwe *et al*., 2010) and this improves both plant growth and nutrient absorption. The use of rainwater harvesting techniques such as tied ridges and *zai* pits harvest and store water for later use by crops (Milkias *et al*., 2018) which if amended with organic and inorganic fertiliser leads to increased crop yield. The use of integrated nutrient management has been used by smallholder farmers for many years (Rusinamodzi et al., 2011; Nyamangara et al., 2013; Shumba et al., 2020) in Zimbabwe to improve crop production. The use of insitu rainwater harvesting and integrated nutrient management has the capacity to improve maize production in dry regions across Africa. The objective of study was to evaluate the effects of integrated nutrient management and insitu rainwater harvesting on maize production in dry regions of Zimbabwe.

Materials and Methods

Study area

The study was carried out in ward 4 of Masvingo district which is located between 20º 2´ 43″ S and 30º 40´ 29″ E. The area is characterised by deep sandy loam soils which are moderately fertile soils associated with *Terminalia-Combretum* species with few Mopane trees on the river banks of Shashe River. Farming in this area has immensely contributed to the siltation and pollution of Shashe River which negatively affect Muzhwi Dam. The area receives 450-500 mm of rainfall per annum on average and temperatures ranges from 18 º C minimum and 32 ºC maximum. This area is made up of small scale commercial farms and few smallholder farmers in the adjacent of the ward.

Experimental Design and Treatments

The experiment was laid out as Randomised Complete Block Design with three main factors namely cattle manure, inorganic fertiliser and water harvesting techniques (tied ridges and zai pits). Each treatments was replicated three times. Cattle manure was applied at a rate of 0 and 5 t/ha, inorganic fertiliser at a rate of 0 kg N/ha, 50 kg N/ ha and 100 kg N /ha and with tied ridges and *zai* pits as rain water harvesting technique. Tied ridges were spaced 2 metres in width and ridges were 35 cm in height. Cross ties were placed at 10 m intervals at a height of 25 cm to prevent damages caused by flowing water. Zai pits were dug to a depth of 20 cm and width of 30 cm in plots. Maize plants were spaced at 0.9 m between rows and 0.3 m within the row to achieve a plant population of 37 037 plants/ ha. Plots used were having measurements of 10 m by 7 m and a net plot of 4.5 m by 4.5 m was marked in each experimental plot.

Land preparation

The trials were established at one site during the short rain season (December 2017 to March 2018 and December 2018 to March 2019) and a short season variety SC 403 was used under supplementary irrigation. Land was ploughed using ox-drawn plough to a depth of 36 cm. Ridges were made using the plough and ties were placed using hand hoe. Seeds were spaced at 0.9 m by 0.3 m. Zai pits were done on lad which was not ploughed to make sure their effectiveness can be evaluated. Cattle manure was applied at before planting in opened farrows and zai pits using two rates of 0 and 5 t/ha. Fertilizers were pre-weighed for each plot before going to the field and applied using dollop cups to ensure uniform distribution within the plot. Weeding was done twice using hand hoe weeding.

Table 1. Experimental treatments used

No.	N kg/ha	Cattle manure (t/ha)	Rainwater harvesting	Treatment combinations
1	0	0	Tied Ridge	TN_0C_0
2	0	5	Tied Ridge	TN_0C_5
3	50	0	Tied Ridge	$TN_{50}C_0$
4	50	5	Tied Ridge	$TN_{50}C_5$
5	100	0	Tied Ridge	$TN_{100}C_0$
6	100	5	Tied Ridge	$TN_{100}C_5$
7	0	0	Zai pit	ZN_0C_0
8	0	5	Zai pit	ZN_0C_5
9	50	0	Zai pit	$ZN_{50}C_0$
10	50	5	Zai pit	$ZN_{50}C_5$
11	100	0	Zai pit	$ZN_{100}C_0$
12	100	5	Zai pit	$ZN_{100}C_5$

Data collection

Harvesting was done using hand hoe to cut maize in the net plot and a sharp knife was used to remove husks. All cobs from each plot/ treatment were collected and separately placed in different sacks which were well labelled.

Grain and stover yield

Grain and stover yields were measured from plants harvested net plots 120 days after planting. Ears and stover were sun dried for 7 days to allow drying. Ears were threshed and weighed at 12 % moisture content. Grain yields was then converted from kilogrammes per net area to tonnes per hectare (t/ha).

$$\text{Grain yield (kgha}^{-1}) = \frac{\textit{Yield in the treatment}}{\textit{Harvest area}} \times 10000$$

Where harvest area = 20.25 m^2 and 10000 is equivalent to area of one hectare.

Stover yields was also measured from the net plot after cutting them into small pieces and weigh using a digital scale and convert the mass to kg ha^{-1}.

$$\text{Stover yield (kgha}^{-1}) = \frac{\textit{Yield in the treatment}}{\textit{Harvest area}} \times 10000$$

Where harvest area = 20.25 m^2 and 10000 m^2 is equivalent to area of one hectare.

Data analysis

Data was processed using Microsoft excel and analysed for analysis of variance (ANOVA) using IBM SPSS version 25. Means were separated using least significant different (LSD) at 0.05 to identify means which were significantly different.

Results and discussion

Effects of integrated nutrient management and tied ridges on grain yields

The results show that there was significant different ($p<0.001$) between all treatments combinations. Results recorded higher maize grain yields from100 kg N/ha + 5 t/ha cattle manure treatments for both seasons. High grain yield of 3.41 t/ha was recorded from $ZN_{100}C_5$ treatments during the second year and highest maize grain yield of 3.11 t/ha was recorded in first year. These results were in agreement with results by Muna-Mucheru et al. (2007) who reported that cattle manure releases nutrients slowly and supply these nutrients throughout the season to crops. Milkias et al. (2018) also reported that the use of insitu rainwater harvesting in combination with mineral fertiliser and cattle manure produces higher maize grain yields. Combining cattle and inorganic manure in combination with insitu rainwater harvesting retains a lot of moisture which boost absorption of nutrients making them available to plants for growth. High soil moisture and nutrient availability in the plant root zone promotes high grain yields.

Table 2. Interactive effects of integrated nutrient management and insitu rainwater harvesting on grain yields

Treatment combinations	Mean Grain yield (t/ha)	
	Farming Seasons	
	Firs year (t/ha)	Second year (t/ha)
TN_0C_0	1.20 ± 0.025	1.24 ± 0.025
TN_0C_5	1.62 ± 0.035	1.72 ± 0.03
$TN_{50}C_0$	1.56 ± 0.045	1.65 ± 0.015
$TN_{50}C_5$	2.11 ± 0.032	2.13 ± 0.026
$TN_{100}C_0$	1.92 ± 0.025	1.9 ± 0.02
$TN_{100}C_5$	3.06 ± 0.067	3.11 ± 0.015
ZN_0C_0	1.17 ± 0.031	1.17 ± 0.031
ZN_0C_5	1.78 ± 0.055	1.75 ± 0.042
$ZN_{50}C_0$	1.69 ± 0.025	1.75 ± 0.025
$ZN_{50}C_5$	2.31 ± 0.036	2.42 ± 0.035
$ZN_{100}C_0$	2.03 ± 0.05	2.02 ± 0.068
$ZN_{100}C_5$	3.18 ± 0.06	3.41 ± 0.042
P-value	<0.001	<0.001

Control treatments (TN_0C_0 and ZN_0C_0) recorded the lowest grain yield for both season which were an average of 1.20 ± 0.025 t/ha and 1.24± 0.025 t/ha for tied ridges and 1.17 ± 0.031 for zai pits the two farming seasons respectively. On average treatments without cattle manure had lower maize grain yields compared to those with cattle manure. All treatments with 5 t/ha cattle manure performed above 2.0 t/ha for both seasons with higher yields of 3.41 1.17 ± 0.042 t/ha obtained from a combination of Zai pits + 100 kg N/ha + 5t/ha cattle manure treatments in the second year. The results were 6.7% higher than same treatment in first year and 8.8 % higher than same treatments with tied ridges in 2019. The results coincides with results by Muchai et al. (2020) who reported that zai pits were amended with soil fertility options to improve maize, sorghum and millet in Kenya. The results were similar from findings by Kilasara et al. (2015) who reported improved sorghum yields from combination of insitu rainwater harvesting and integrated nutrient management options in Tanzania. Higher maize yields from treatment with mineral fertiliser and cattle manure concur with results by Shumba et al. (2020) who reported increased maize grain yields in Zimbabwe after combining cattle manure and mineral fertiliser. Treatments with 5 t/ha cattle manure and 50 kg N/ha recorded 2.31 ± 0.036 t/ha and 2.42 ± 0.035 t/ha from Zai pits treatments and were second highest yields for both farming seasons as indicated in Table 2. The results were in agreement with findings by Muna-Mucheru et al. (2007) who indicated that the use of cattle manure increases nutrient availability which promote plant growth and increase grain yields. These results also coincide with results by Motsi et al. (2019) who reported increased maize grain yields after combining cattle manure and Nitrogen fertiliser. The results show that there was significant different ($p<0.001$) for both two farming years and indicated that the effects of Zai pits and tied ridges in combination with integrated nutrient management were significantly different.

Results also show that the interactive effects of cattle manure and inorganic manure significantly affected maize grain yields. There was significant different ($p<0.001$) on the effects of combination of cattle manure and inorganic fertiliser alone on maize grain yields (Table 2). Higher results (3.21 t/ha and 3.26 t/ha) were obtained from combination of 5 t/ha cattle manure and 100 kg N /ha from both seasons respectively. Results show no significant different between combination of 5 t/ha cattle manure + 50 kg N/ha from treatments with 100kg N/ha only for both years (Table 2). These results coincides with findings by Coulibaly (2015) who reported higher pearl millet yields after combining mineral fertiliser and cattle manure. The findings also concur with results by Shumba et al. (2020) who reported higher yields form a combination of mineral fertiliser and cattle manure on maize grain yields. Nyamangara et al. (2005) reported that cattle manure contains both macro and micro-nutrients which improves grain yields and soil properties. This was also affirmed by Shumba et al. (2020) and Kilasara et al. (2015) who all reported that cattle manure improves water retention and releases nutrients slowly in the plant root zone which later improve plant growth and yields.

Table 3. Interactive effects of cattle manure and inorganic manure on maize grain yields

Cattle manure (t/ha)	Inorganic manure (kg N/ha)	First year Mean grain yield (t/ha)	Second year Mean grain yield (t/ha)
0	0	1.19^{f}	1.21^{e}
	50	1.63^{de}	1.7^{d}
	100	1.98^{bc}	1.96^{bc}
5	0	1.7^{d}	1.73^{d}
	50	2.21^{b}	2.27^{b}
	100	3.12^{a}	3.26^{a}
	P-value	0.001	<0.001

Interactive effects of insitu rainwater harvesting and inorganic manure show significant different ($p=0.043$) between treatments of tied ridges and zai pits in combination with inorganic manure in the first year. During the second year, results show significant different ($p<0.001$) effects between all treatments. Highers yields (2.61 t/ha and 2.71 t/ha) were obtained from combination of zai pits and 100 kg N/ha for both years respectively. These results were 4.6 % and 7.7% higher than results from combination of tied ridges and 100 kg N/ha from same years respectively. All treatments with 0 kg N/ha recorded low yield which were not significantly different as indicated in Table 3. The results concur with findings by Coulibaly (2015) who reported high pearl millet yields from a combination of zai pits with inorganic manure compared with tied ridges with inorganic manures. These results also coincide with results by Milkias et al. (2018) who reported that combining insitu rainwater harvesting with mineral fertiliser increases maize grain yields.

Table 4. Interactive effects of insitu rainwater harvesting and inorganic manure on maize grain yields

Insitu rainwater harvesting	Inorganic manure (kg N/ha)	First year Mean grain yield (t/ha)	Second year Mean grain yield (t/ha)
Tied ridges	0	1.41^{e}	1.48^{e}
	50	1.84^{cd}	1.89^{d}
	100	2.49^{ab}	2.5^{b}
Zai pits	0	1.47^{e}	1.46^{e}
	50	2.0^{c}	2.08^{c}
	100	2.61^{a}	2.71^{a}
	P-value	0.043	<0.001

Results from the study indicates that cattle manure significantly affect grain yields. The use of 5 t/ha cattle manure increased maize grain yields by 31 % under tied ridges for both seasons. Maize grain yield increased with 32.6 % and 34.8% under zai pits (Table 4). This show that combining zai pits and cattle manure produces higher yields. These results were similar to results by Mudatenguha et al. (2014) who reported higher maize yields under zai pits and organic manure. This concurred with results by Coulibaly (2015) who reported increased pearl millet yields under zai pits and 2500 kg/ha cattle manure.

Table 5. Interactive effects of insitu rainwater harvesting and cattle manure on maize grain yields

Insitu rainwater harvesting	Cattle manure (t/ha)	First year Mean grain yield (t/ha)	Second year Mean grain yield (t/ha)
Tied ridges	0	1.56^{cd}	1.6^{c}
	5	2.26^{ab}	2.32^{ab}
Zai pits	0	1.63^{c}	1.65^{c}
	5	2.42^{a}	2.53^{a}
	P-value	0.006	<0.001

Sole effects of inorganic manure, cattle manure and rainwater harvesting show that, use of 100 kg N/ha produces higher maize grain yields compared to all levels of cattle manure and inorganic manure. Zai pits alone produces higher yields than the use of tied ridges (Table 5). This supports the results produced under interaction of organic and inorganic manure in combination with zai pits which produces higher results. This was in support with results by Mudatenguha et al. (2014) who reported high yields from zai pits. The results also concur with results by Kilasara et al (2015) and Milkias et al. (2018) who reported higher yields from using inorganic fertiliser in sorghum production and maize production respectively. These results were in support of findings by Shumba et al. (2020) who indicated that mineral fertiliser produces higher maize grains when used alone. Results show no significant different (p=0.204) between the effects inorganic manure and season on maize grain yields. This show that inorganic manure was not affected by seasonal changes. Results also indicate significant different (p=0.005) on the effect of cattle manure and season on maize grain yields.

Table 6. Sole effects of inorganic manure, cattle manure and rainwater harvesting on maize grain yields

Inorganic manure (kg N/ha)	First year Mean grain yield (t/ha)	Second year Mean grain yield (t/ha)
0	1.44	1.47
50	1.92	1.99
100	2.55	2.61
P-value	<0.001	<0.001
Cattle manure (t/ha)		
0	1.6	1.62
5	2.34	2.42
P-value	<0.001	<0.001
Rainwater harvesting		
Tied ridges	1.91	1.96
Zai pits	2.03	2.09
P-value	<0.001	<0.001

Effects of integrated nutrient management and tied ridges on stover yields

The results show positive correlation between grain and stover yields for all treatments used in the experiment. Higher stover yields were recorded from treatment combinations of 100 kg N/ha and 5t/ha cattle manure ($TN_{100}C_5$) which recorded highest stover yields of 5.78 ± 0.11 t/ha and 5.79 ± 0.075 t/ha for the year 1 and 2 respectively. Integrated nutrient management of cattle and inorganic manure increased stover yields. The results concur with findings by Vanlauwe et al. (2010) and Mugwe et al. (2019) who reported that integrated nutrient management improves plant growth and yields. Combining integrated nutrient management with insitu rainwater harvesting boost crop production and yields. The results were in support of findings by Kanonge *et al.,* (2009) who reported significant increase of maize grain and stover yields after using cattle manure and mineral fertiliser. The results were also supporting findings by Bationo *et al*., (2004); Kokerai and Kugedera (2019) and Shumba et al. (2020) who all reported increased maize yields after using integrated nutrient management options of cattle manure and mineral fertiliser. The results were also in support of reports by Kugedera *et al*., (2018) who indicated high sorghum stover yields after using insitu rainwater harvesting, mineral fertiliser and cattle manure.

The results show significant different ($p<0.001$) on the effect of cattle manure, inorganic manure and rainwater harvesting on maize stover yields. The use of cattle manure, inorganic manure and insitu rainwater harvesting improves soil structure, water retention and nutrient availability which increased plant growth and yields. The results concur with findings by Nyamangara et al. (2005) who reported that cattle manure improves soil structure hence more effective to increase crop productivity. This was also affirmed by Tirol-Padre et al. (2007) who indicated significant increase in stover yields after using cattle manure and mineral fertiliser. Mudatenguha et al. (2014) also reported that the use of insitu rainwater harvesting in combination with organic and inorganic fertiliser increase grain and stover yields. Results from this study were also in agreement with results by Kilasara et al. (2015) who reported increased sorghum stover after combining insitu rainwater harvesting with farm yard manure and mineral fertiliser. Findings from this study also support results by Milkias et al. (2018) who reported increased maize stover yields after using insitu rainwater harvesting and integrated nutrient management.

Table 7. Interactive effects of integrated nutrient management and tied ridges on stover yields

Treatment combinations	Mean Stover yield (t/ha)	
	Farming Seasons	
	Firs year (t/ha)	Second year (t/ha)
TN_0C_0	4.39 ± 0.01^{ef}	4.36 ± 0.046^{ef}
TN_0C_5	4.84 ± 0.055^{de}	4.85 ± 0.038^{de}
$TN_{50}C_0$	4.81 ± 0.051^{de}	4.82 ± 0.025^{de}
$TN_{50}C_5$	5.22 ± 0.051^{bc}	5.29 ± 0.021^{bc}
$TN_{100}C_0$	4.99 ± 0.03^{cd}	4.97 ± 0.03^{cd}
$TN_{100}C_5$	5.78 ± 0.11^{a}	5.79 ± 0.075^{a}
ZN_0C_0	4.48 ± 0.026^{e}	4.47 ± 0.055^{e}
ZN_0C_5	4.83 ± 0.04^{cd}	4.89 ± 0.035^{cd}
$ZN_{50}C_0$	4.93 ± 0.04^{cd}	4.99 ± 0.025^{cd}
$ZN_{50}C_5$	5.39 ± 0.026^{bc}	5.45 ± 0.026^{ab}
$ZN_{100}C_0$	5.03 ± 0.053^{c}	5.05 ± 0.055^{c}
$ZN_{100}C_5$	5.5 ± 0.015^{b}	5.54 ± 0.015^{ab}
P-value	0.001	<0.001

Table 8. Sole effects of inorganic manure, cattle manure and rainwater harvesting on maize grain yields

Inorganic manure (kg N/ha): A	First year Mean stover yield (t/ha)	Second year Mean stover yield (t/ha)
0	4.64	4.64
50	5.09	5.13
100	5.33	5.34
P-value	<0.001	<0.001
Cattle manure (t/ha): B		
0	4.77	4.78
5	5.26	5.3
P-value	<0.001	<0.001
Rainwater harvesting: C		
Tied ridges	5.01	5.01
Zai pits	5.03	5.07
P-value	0.178	0.001
Interaction		
AB	<0.001	<0.001
AC	<0.001	<0.001
BC	0.001	<0.001
ABC	0.001	<0.001

The results indicates that cattle manure can significantly cause a positive effect on stover yields because it supplies both macro and micronutrients. Cattle manure also improves soil structure, water retention and regulate soil pH to improve crop growth and nutrient absorption by plants (Tirol-Padre *et al*., 2007; Motsi *et al*., 2019). Generally, year 2 had higher stover yields compared to year 1 and these results were significantly different (p<0.001). The findings also show that TN_0C_0 treatments recorded lower stover yields to all other treatments except the control treatments only for both seasons and the yields were 24% and 24.7 % less than the highest stover yield for the year 1 and year 2 respectively. The results were significantly different as shown in Table 6.

Results also show significant different (p=0.001) on the effect of rainwater harvesting alone on maize stover yields in year 2 and no significant different (p=0.178) in year 1. Combining rainwater harvesting and cattle manure show significant different (p<0.001) for both years on maize stover yields. Results in Table 7 show that combining rainwater harvesting, cattle and inorganic manure significantly increase maize stover yields irregardless of the season. High stover yields were obtained from the use of 100 kg N/ha. This was supporting indications by Shumba et al. (2020) and Nyamangara et al. (2005) who reported that inorganic fertiliser quickly releases nutrients in the soil which will be immediately absorbed by plants to increase plant growth. These results were also in support of findings by Kanonge et al. (2015) who reported increased legume yields after combining cattle manure and mineral fertiliser in smallholder farming systems in Zimbabwe.

Conclusion

The use of integrated nutrient management and insitu rainwater harvesting has the capacity to increase food security in smallholder farming systems and increase maize productivity. Cattle manure decomposes slowly in the soil releases nutrients throughout the growing season and this facilitate high microbial activity which improves soil structure. Insitu rainwater harvesting harvest water to improve soil moisture content, reduce surface runoff and leaching of nutrients. This increase nutrient availability in the plant root zone and retains a lot of moisture which crops can use during dry spell and drought periods. Use of zai pits in combination with integrated nutrient management recorded higher maize yields and this can be adopted by farmers although the method is labour intensive during first year in preparing the pits. Maize grain yields varied with seasons and this can have been caused by management and amount of rainfall received during these two seasons. Farmers can adopt integrated nutrient management and insitu rainwater harvesting and use it on small plots to maximise yields and reduce labour requirements.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Characterization of ethylenediaminetetraacetic acid and acid tolerance of foodborne pathogenic bacteria

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Abstract

Foodborne pathogenic bacteria are subject to different stressful conditions due to process conditions, storage and composition of food. It is crucial to understand the survival characteristics of these bacteria to develop effective measures to limit or eliminate their survival in food. EDTA is a chelating agent and commonly used in food formulations for its function to prevent discoloration or flavor loss in food and to extend shelf life. Due to its common use in food industry, it is important to understand its antimicrobial function for possible interaction with other antimicrobials for elimination of foodborne pathogens. In this study, different foodborne pathogenic bacteria including two Gram-positive (*Listeria monocytogenes* and *Staphylococcus aureus*) and three Gram-negative (*Escherichia coli* O157:H7, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) bacteria were characterized for their survival and growth in the presence of EDTA (0.01 and 0.05%) and under acidic condition (pH 5.0). The presence of EDTA in the growth media caused Gram-positive and Gram-negative bacteria to become more susceptible to subsequent stressful conditions compared to control ($p<0.05$). Gram-negative bacteria were more tolerant to acidic conditions as well as presence of EDTA compared to Gram-positive bacteria ($p<0.05$). This study provides insight on survival characteristics of foodborne pathogenic bacteria against selected stress conditions they are exposed in food and highlights the antimicrobial function of EDTA in food formulations.

Keywords: Stress response, Foodborne pathogens, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, EDTA, Acid tolerance

Introduction

Food safety is a global public health concern and foodborne pathogenic organisms cause significant number of diseases and death worldwide. Food industry and government agencies are focusing on development of new methods or improvement of existing methods to minimize contamination of food with pathogenic microorganisms. The safety of food is ensured by application of different measures including processing, food composition and packaging. Foodborne pathogens are exposed to variety of different stressful conditions through their lifecycle. One key aspect in developing effective food safety measure is the understanding of ecology of foodborne pathogens

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and how they respond to stress conditions. These conditions may lead to selection of strains that develop resistance or tolerance to harsher stress conditions (Wesche et al. 2009).

Key intrinsic characteristics of food that allow control of growth or survival of pathogenic microorganisms include pH, salt content, water activity, etc. (Yeargin and Gibson 2019). Foodborne pathogens show different survival characteristics against these stressful conditions. In negative bacteria demonstrate increased tolerance to external stress factors, including lower pH, increased salt content and presence of detergents. This increased tolerance is mainly due to the differences in cell envelope assembly of Gram-negative and Gram-positive bacteria. Unlike Gram-positive bacteria, Gram-negative bacteria possess an outer membrane providing additional barrier (Jordan et al. 2008). The outer membrane of Gram-negative bacteria consists of lipopolysaccharide molecules making the exterior of bacterial cells hydrophobic and serves as a barrier for entrance of macromolecules and hydrophilic substances. This outer membrane improves the tolerance of Gram-negative bacteria against extracellular stresses. The outer membrane of Gram-negative bacteria could be modified by certain compounds through decomposition of the lipopolysaccharide layer increasing the permeability of the membrane (Vaara, 1992; Alakomi, et al. 2006; McBroom and Kuehn 2007). One such compound is a chelating molecule called Ethylenediaminetetraacetic acid, EDTA. EDTA is commonly used in food formulations for its stabilizer function. It delays or stops the chemical reactions in food that cause discoloration or texture and/or flavor loss. Besides its stabilizer function, it also has antimicrobial characteristics. As a chelator, EDTA, sequesters divalent cations of the outer membrane of Gram-negative bacteria. Divalent cations have a crucial role in enabling the electrostatic interconnections with proteins and lipopolysaccharides serving as the backbone of the stability of the outer membrane in Gram-negative bacteria. Supplementation of benzalkonium chloride with chelating compounds, such as EDTA and polyethylenimine, improved its activity in inhibition of Gram-negative bacteria *Pseudomonas* and *Stenotrophomonas* (Alakomi et al. 2006; Sim et al. 2019; Vale et al. 2019).

Foodborne bacteria are exposed to acid stress in food materials and pH adjustment of food is commonly used as a food protection measure. Some stress response mechanisms that bacteria uses to minimize the lethal impact of acid stress includes membrane composition change, increase in protein efflux, increase in amino acid catabolism, and induction of DNA repair enzymes (Siegumfeldt et al., 2000). These response mechanisms could be initiated by other stress factors or they could be complemented by other response mechanisms to allow bacteria become more tolerant to acidic stress. The impact of stress adaptation of bacteria on increased tolerance to subsequent stressful conditions has been reported in number of were reported in a number of previous studies, Cheng et al. (2003) indicated that acid adaptation of cells increased acid tolerance and this increase was dependent on strain, acid adaptation time, and pH of the acid challenge.

EDTA is commonly used in food industry for its food stabilizer function. Due to its antimicrobial property EDTA could provide additional protection along with other antimicrobial compounds in food or could activate stress response mechanisms of pathogenic bacteria that could increase survival of these bacteria and cause risk in terms of foods safety. Therefore, the impact of EDTA on their survival in acidic conditions was characterized within the scope of this study. Furthermore, the effect of prior adaptation of pathogenic bacteria within different concentrations of EDTA on their growth in specific acidic conditions was studied. These findings would serve as a reference in food product formulation to ensure the safety of the food supply.

Materials and Methods

Bacterial Strains and Growth Conditions

For evaluation of EDTA and acid stress tolerance behavior of bacteria, variety of foodborne pathogens that are most commonly associated with foodborne outbreaks were included in this study. Stress response of two Gram-positive and three Gram-negative foodborne pathogenic bacteria were characterized. Tested Gram-positive bacteria included: a clinical isolate of each of *Listeria monocytogenes* and *Staphylococcus aureus*. Tested Gram-negative bacteria included: a clinical isolate of each of *Escherichia coli* O157:H7, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. Each bacterial isolate were grown on Brain-Hearth Infusion (BHI) agar at 37^{o}C for 24 hours, transferred into BHI broth (BHI-B) (Sigma-Aldrich, St. Louis, MS) and incubated at 37^{o}C for 18 hours before the subsequent stress characterization test.

Adaptation of Bacteria to Presence of EDTA

Prior to evaluation of stress tolerance of each bacteria, the cells were adapted to presence of EDTA in growth media. For this purpose, following overnight growth at 37^{o}C in BHI-B, each culture of bacteria was transferred to BHI-B containing 0.01% or 0.05% EDTA (Sigma-Aldrich, St. Louis, MS). Following inoculation in BHI-EDTA broth, the cultures were incubated at 37^{o}C for 18 hours statically. These cultures were transferred to following stress conditions including acid or EDTA stress.

Screening of Acid Tolerance of EDTA Adapted Bacteria

The growth of selected bacteria was screened in BHI-B adjusted to pH of 5.0. The pH of the growth media is adjusted to desired acidity using HCl. Following the pH adjustment, the media were sterilized before testing. Bacteria grown in 0.01% EDTA containing BHI-B, 0.05% EDTA containing BHI-B or BHI-B were inoculated in BHI-B at pH 5.0. Regular BHI-B without pH adjustment was tested as control. Starting microbial load of each culture was ~10^5 cfu/ml. Growth of each culture was assessed by measurement of optical density at 600nm (OD600). The OD600 were measured every 30 minutes for 24 hours using a plate reader (Tecan, Switzerland). Each condition for each of the tested foodborne bacteria was screened in four independent replicates.

Impact of Presence of EDTA in Acidified Media

Possible effect of presence of EDTA in stress tolerance of bacteria in acidified media was also evaluated. The pH of BHI-B was adjusted to 5.0, and 0.01% of EDTA was supplemented to the media. The growth of each bacteria at 37^{o}C under these conditions were evaluated as described above: measuring OD600 in 30 minutes intervals for 24 hours using a plate reader (Tecan, Switzerland).

Statistical Analyses

Each experiment in this study was replicated four times, and the impact of each condition was statistically assessed by comparison of the growth rates and OD600 value at stationary growth phase using one-way ANOVA. The growth rate in logarithmic growth phase for each test was calculated by following formula: $\ln (OD600_{t2}/OD600_{t1})/(t_2-t_1)$ in which t_1 is the time the bacteria started its logarithmic growth phase and t_2 is a selected later time in its logarithmic growth phase. Statistical significance is defined at $p<0.05$.

Results and Discussion

The composition of food has significant impact on survival and growth of microorganisms in food. It is important to understand how pathogenic bacteria react to the stressful conditions of food due to food's intrinsic properties. EDTA is commonly used as a food stabilizer as it interacts with the chemical reactions within the food to inhibit or stop discoloration or texture and/or flavor losses. Besides its quality improvement function, EDTA has antimicrobial properties to inhibit growth or survival of microorganisms present in food. Although the antimicrobial function of EDTA is known, there are limited studies that focused on its impact against foodborne pathogens and its interaction with other stress conditions within the food. Therefore, in this study the impact of EDTA on acid stress tolerance of five different foodborne pathogenic bacteria associated with majority of foodborne outbreaks was studied.

Impact of EDTA adaptation on growth of Gram-positive and Gram-negative bacteria

Adaptation of *L. monocytogenes* and *S. aureus* in BHI-B with 0.1% or 0.5% EDTA had significant impact on subsequent growth in regular BHI-B ($p<0.05$; Figure 1). Both cells of *L. monocytogenes* and *S. aureus* showed impaired growth in BHI-B following adaptation in the presence of EDTA. The cells adapted to EDTA showed longer lag-phase compared to control cells (Figure 1). This indicates that presence of EDTA caused injury of cells of Gram-positive bacteria and leads to longer lag-phase for cells to recover. On the contrary, the tested Gram-negative bacteria didn't show similar impairment in their growth following adaptation to EDTA. The growth behavior of EDTA adapted and control cells were similar to each other (data not shown). This could be explained due to the chelating function of EDTA against the cell membrane of Gram-positive bacteria. The outer membrane of the Gram-negative bacteria serves as a protectant against the damaging effect of EDTA of cells and prevents injury of the cells (Gill and Holley 2003). Similar enhanced sensitivity of Gram-positive bacteria to other stress conditions was reported in other studies (Guardabassi et al. 2010; Khazandi, et al. 2019; Vale et al. 2019).

Effect of EDTA in acid tolerance of Gram-positive and Gram-negative bacteria

The impact of prior EDTA adaptation in acid tolerance showed similarity between *L. monocytogenes* (Figure 2a) and *S. aureus* ($p>0.05$; data not shown). On the other hand, the impact of EDTA adaptation on their acid tolerance showed significant difference from Gram-negative bacteria tested in this study, *E. coli* O157:H7 (Figure 2b), *S. typhimurium* and *P. aeruginosa* (Figure 2c) ($p<0.05$). This finding highlights the difference between Gram-positive and Gram-negative bacteria in their response to the presence of EDTA and their survival in acidic conditions. Gram-negative bacteria showed more tolerance to the stressful conditions of EDTA compared to the Gram-positive bacteria. This difference in the EDTA and acid stress response could be explained by the differences in the cell wall structure between Gram-positive and Gram-negative bacteria and the presence of outer membrane in Gram-negative bacteria that provides additional protection against the stressful conditions (Alakomi et al. 2006; Khazandi, et al. 2019).

Interestingly, inclusion of EDTA to the acidified media further inhibited the growth of both Gram-positive and Gram-negative bacteria. The cells of *L. monocytogenes* were incapable of survival and growth under the stressful conditions of combination of EDTA and high acidity. Similar impact of presence of EDTA was observed in *S. aureus* (data not shown). This indicates that the cells of tested Gram-positive bacteria were injured in the presence of EDTA and their growth in the presence of EDTA under acidic conditions were completely inhibited (Figure 3a). Although, the cells of *E. coli* O157:H7 showed growth under EDTA and high acid stress, it was significantly less than the cells grown only under acid stress ($p<0.05$; Figure 3b). The presence of EDTA (0.01%) in the growth media that is pH adjusted to 5.0 cause cells to be more susceptible and the growth rate and the cell density at stationary growth phase were significantly lower than the cells grown in BHI-B at pH 5.0 ($p<0.05$; Fig. 3b). *Salmonella typhimurium* showed similar behavior as *E. coli* O157:H7 strain tested in this study (data not shown).

Other tested Gram-negative bacteria, *Pseudomonas aeruginosa,* showed significantly different growth behavior compared to the foodborne pathogenic bacteria tested in this study ($p<0.05$). The EDTA adaptation of *P. aeruginosa* at 0.5% significantly increased the lag-time for the growth of the cells at pH 5.0 compared to the cells adapted to 0.1% EDTA or control cells ($p<0.05$; Figure 2c). On the other hand, interestingly the growth rate and the density of the cells at stationary growth phase were similar to each other regardless of the adaptation or the pH adjustment of the growth media (Fig. 2c & 3c).

This study highlights the differences of EDTA and acid tolerance of different foodborne pathogens and shows the antimicrobial activity of EDTA. The chelating properties of EDTA possibly cause formation of pores in cell membrane of the bacteria and cause leakage of the cell or increase the uptake of the other antimicrobial compounds inside the cell (Alakomi et al. 2006; Sim et al. 2019; Vale et al. 2019). It is worthwhile to note that sublethal levels of EDTA in food could select for more tolerant strains of pathogenic bacteria and could pose threat to food safety. Further phenotypic and genotypic characterization of additional strains of these foodborne pathogens is needed to better understand the stress response mechanisms of these pathogenic microorganisms and develop better control measures to eliminate them from food sources.

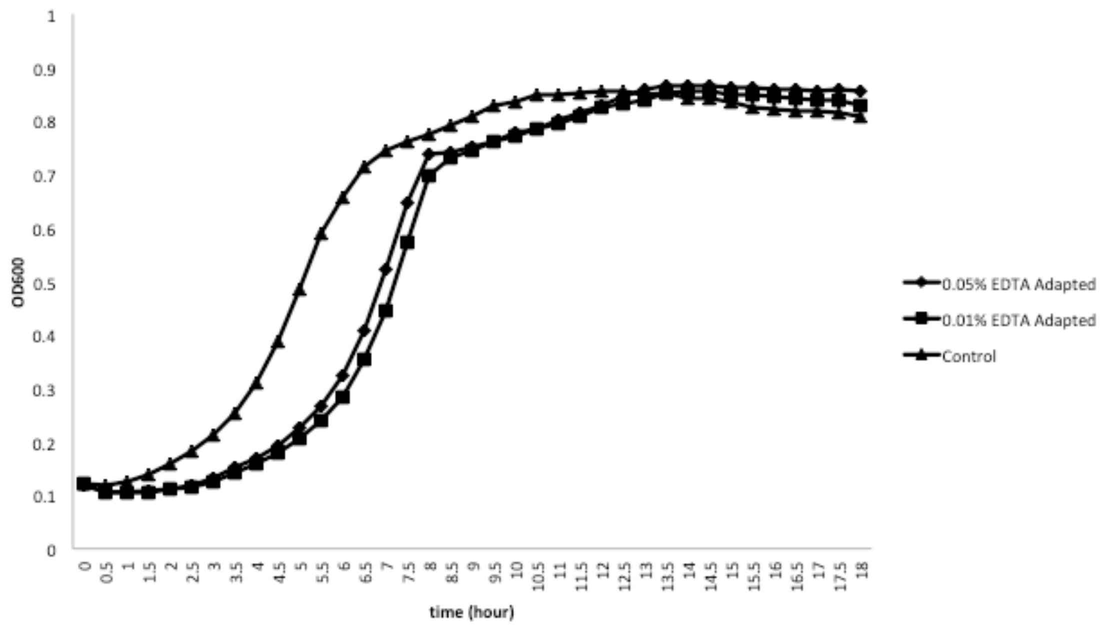

Figure 1- Growth of *L. monocytogenes* in BHI-B following overnight adaptation in BHI-B supplemented with 0.01% EDTA (■) or 0.05% EDTA (◆). Control *L. monocytogenes* only grown in BHI-B is represented by filled triangles (▲).

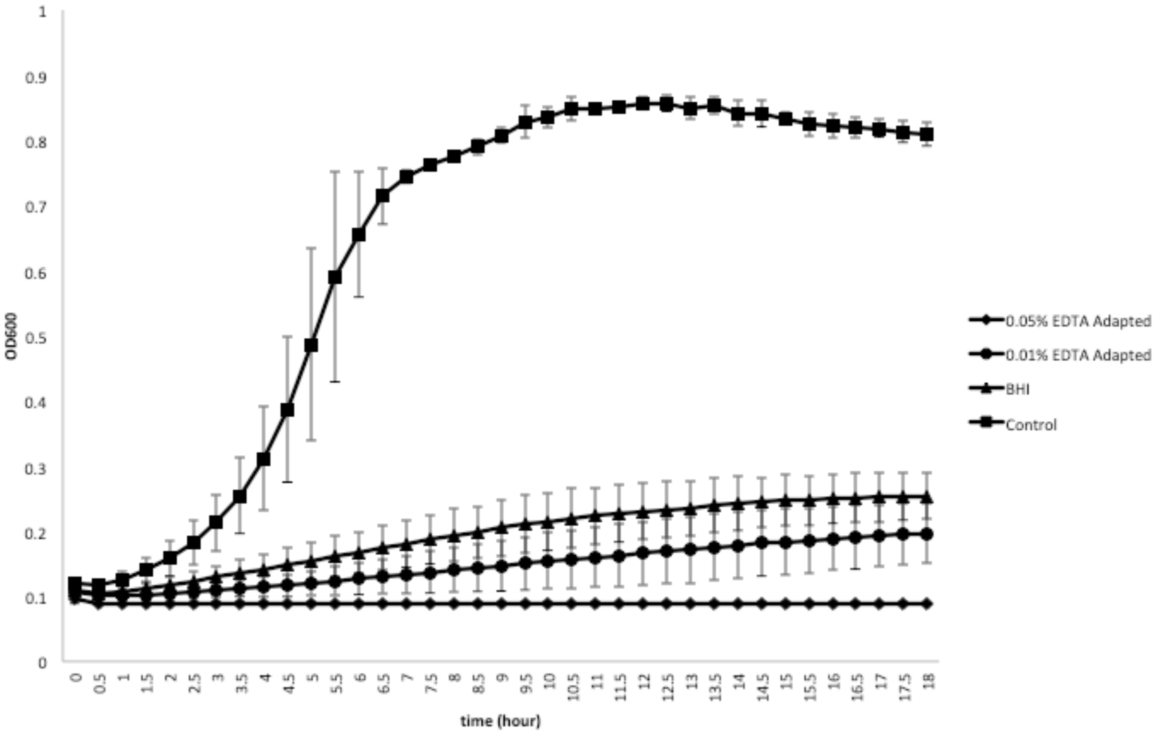

Figure 2a- Growth of *L. monocytogenes* in BHI at pH 5.0 supplemented with different concentrations of EDTA (0.01% and 0.05%) following growth in BHI-B. Control is the cells grown in BHI-B with no EDTA supplementation.

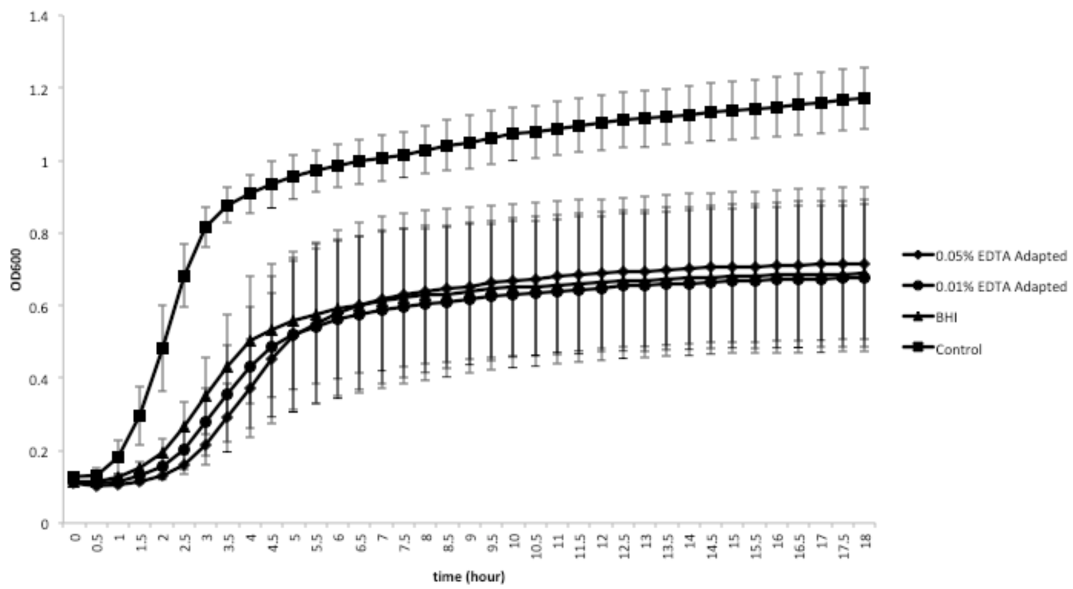

Figure 2b- Growth of *E. coli* O157:H7 in BHI at pH 5.0 supplemented with different concentrations of EDTA (0.01% and 0.05%) following growth in BHI-B. Control is the cells grown in BHI-B with no EDTA supplementation.

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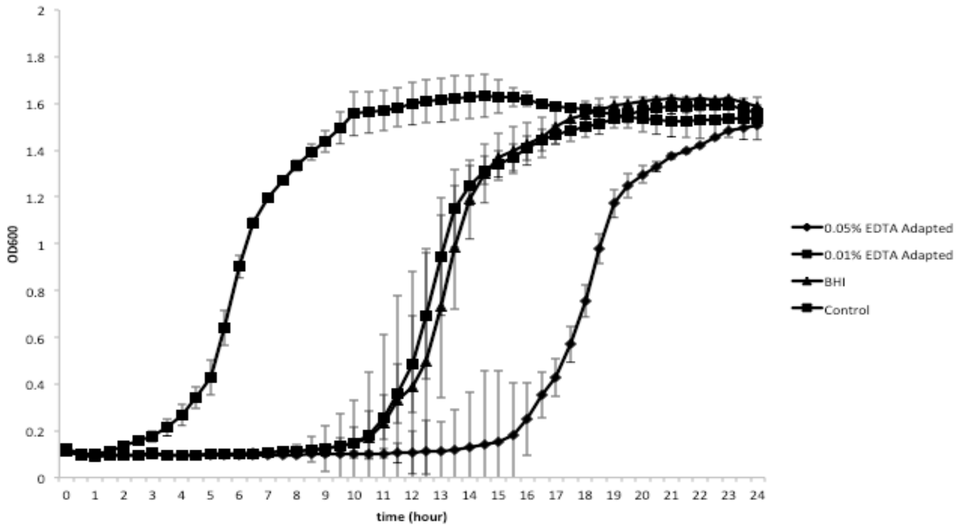

Figure 2c- Growth of *Pseudomonas aeruginosa* in BHI at pH 5.0 supplemented with different concentrations of EDTA (0.01% and 0.05%) following growth in BHI-B. Control is the cells grown in BHI-B with no EDTA supplementation.

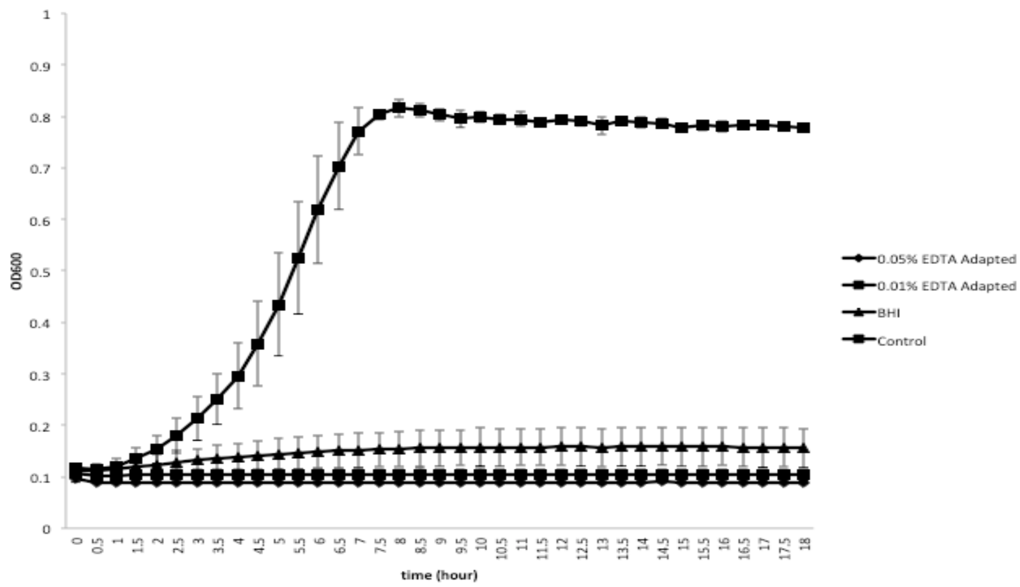

Figure 3a- Growth of *L. monocytogenes* in BHI at pH 5.0 supplemented with different concentrations of EDTA (0.01% and 0.05%) following growth in BHI-B supplemented with 0.01% EDTA. Control is the cells grown in BHI-B with no EDTA supplementation.

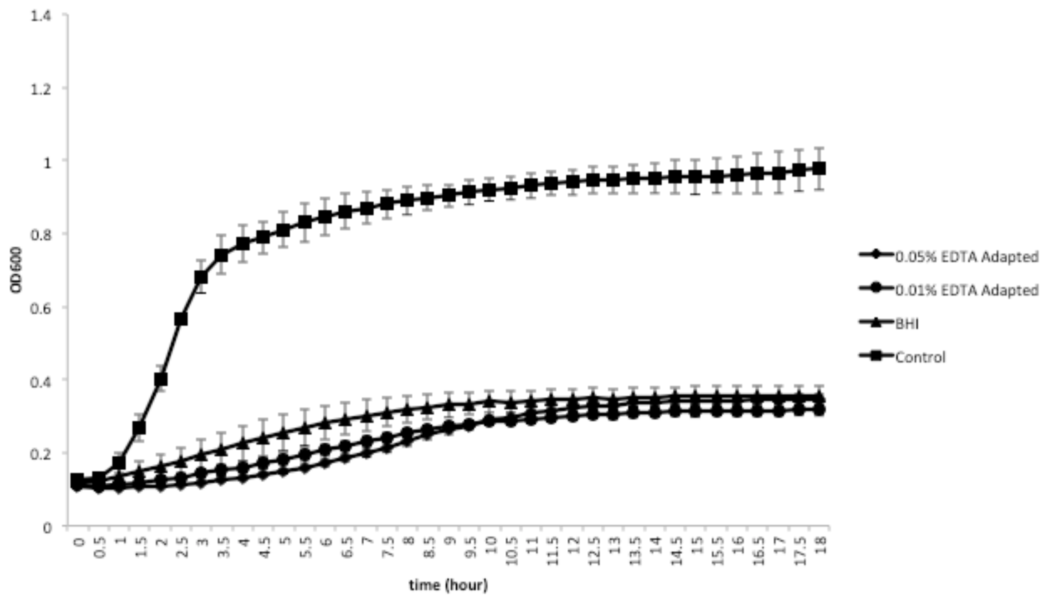

Figure 3b- Growth of *E. coli* O157:H7 in BHI at pH 5.0 supplemented with different concentrations of EDTA (0.01% and 0.05%) following growth in BHI-B supplemented with 0.01% EDTA. Control is the cells grown in BHI-B with no EDTA supplementation.

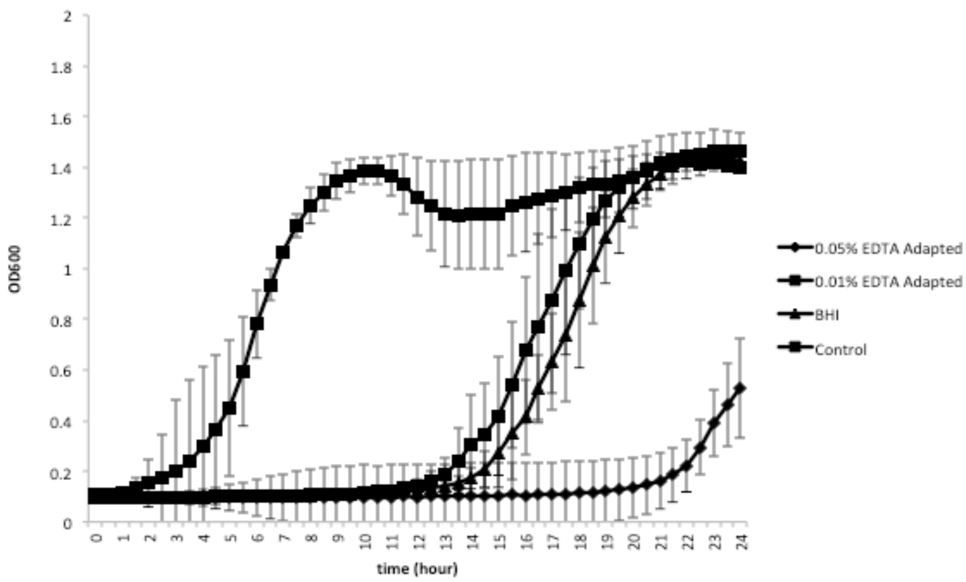

Figure 3c- Growth of *P. aeruginosa* in BHI at pH 5.0 supplemented with different concentrations of EDTA (0.01% and 0.05%) following growth in BHI-B supplemented with 0.01% EDTA. Control is the cells grown in BHI-B with no EDTA supplementation.

Conclusion

Foodborne diseases impact large population around the world causing severe illnesses, therefore it poses a significant public health problem. Foodborne bacteria are usually capable to survive or grow in food and under food storage conditions. In order to develop effective measures to eliminate or limit the occurrence of food-associated illnesses, it is important to understand the ecology of pathogenic microorganisms, and the mechanisms that provide protection for them to survive and grow in food. Therefore, it is crucial to understand how the pathogenic bacteria survive under the stressful conditions of food. In this study, the survival and growth of selected Gram-positive and Gram-negative foodborne bacteria were characterized under the presence of EDTA and acidic conditions. This study showed that the presence of EDTA caused all the tested bacteria become more susceptible to the acidic conditions and possibly to other stress conditions. The differences in EDTA and acid stress response of various foodborne pathogens were also presented. This study highlights the fundamental response of foodborne pathogens against EDTA and acidic conditions. Further phenotypic and genotypic characterization of commonly isolated foodborne pathogenic bacteria would improve our understanding on the stress response mechanisms and allow development of effective methods to eliminate foodborne pathogens from foods.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Variation in the composition of the microbial community in the rhizosphere of potato plants depending on cropping season, cultivar type, and plant development stage

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Abstract

Changes in the structure of the rhizosphere microbiome are influenced by many factors. In the current investigation, the microbial community composition in the rhizosphere of four potato cultivars was monitored using the soil dilution plating technique on specific media. Tested cultivars were grown for two consecutive cropping seasons. Initial soil samples were collected before planting to assess the initial microbial soil species pool. During the growing period, rhizosphere samples were collected at three timing points. For both cropping seasons, the pH and EC of the rhizosphere varied upon sampling periods but not between cultivars. Bacterial and fungal populations at both cropping seasons and that of actinomycetes at the late-season crop were significantly increased by 35-55%, 14-18% and 17-42%, respectively, in the rhizosphere of all grown potato cultivars as compared to the initial soil stage. The relative abundance of *Pseudomonas* spp., actinomycetes, *Aspergillus* spp., and *Fusarium* spp. populations for all potato cultivars combined were 17.4, 26-64, 51-59 and 10-14% higher at the late-season than at the extra-early cropping season, respectively. For both cropping seasons and all sampled soils combined, the highest abundancies of fungal and actinomycetes communities were recorded at plant senescence and 15 days post-harvest. The total culturable bacteria were more relevant at plant emergence and 15 days post-harvest for the late-season crop and at plant senescence for the extra-early crop. The total culturable bacteria were more abundant in the rhizosphere of cvs. Spunta, Elata and El-Mundo at the late-season crop and that of cvs. Spunta and El-Mundo for the extra-early trial. The highest *Pseudomonas* spp. populations were associated to cvs. Cerata, Elata, and El-Mundo for the late-season crop and to Spunta, Elata and El-Mundo for the extra-early crop. The highest fungi counts were noted in the rhizosphere of cv. El-Mundo at the late-season crop and in Spunta for the extra-early trial.

Keywords: Cropping season, Development stage, Potato cultivars, Soil microbial community, Variation

Introduction

The relationship between plants and soil microbial community is very complex leading either to beneficial effects like nitrogen fixation, phosphate solubilization, production of plant growth stimulants, improved water retention, and biosuppression of plant diseases or to negative effects like occurrence of diseases (Ferreira et al., 2008; Berendsen et al., 2012; Singh et al., 2017).

Rhizosphere microbiota are highly dynamic (da Rocha et al., 2009) and they play a key role in plant health and growth and in the preservation of soil fertility (Berendsen et al., 2012). They are also considered as second genome for the plant and

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a key trait in major breeding programs (Marques et al., 2014).

It has been demonstrated that the composition of microbial communities is influenced by the seasonal and the diel changes in temperature (Turpault et al., 2007), the water content (Liddell et al., 2007), pH (Nelson and Mele, 2006), CO_2 concentration, O_2 levels (Jossi et al., 2006) and the biochemical composition of root exudates (Sung et al., 2006).

Different cultivars of the same plant species grown under similar cropping conditions might potentially promote the selection of a distinct microbial community associated to their tissues (Gomes et al., 2003; Ferreira et al., 2008). These shifts in the microbial community may occur due to the direct interaction with plants or as a result of an occurred stress (McDonald et al., 2004; Keswani et al., 2019). According to Sung et al. (2006), plants are capable to increase soil microbial population through their root exudates serving as nutrient sources for associated microorganisms. The chemical composition of root exudates varies between and within plant species (Grayer et al., 2004). The chemical composition of root exudates is the result of different belowground interactions and factors such as the nutritional status, plant age, biotic and abiotic stresses, and environmental conditions; all these factors may affect the microbial community associated to the rhizosphere (Griffiths et al., 1999). Although the effects of plant cultivar on rhizosphere communities has been evidenced on various crops i.e potato, maize, cannabis, and ray-grass (Chiarini et al., 1998; McDonald et al., 2004; Ferreira et al., 2008; Hannula et al., 2010; Winston et al., 2014), these effects are reported to be minimal as compared to the edaphic factors (particularly pH) or the plant development stage (van Overbeek and van Elsas, 2008; Marques et al., 2014; Pfeiffer et al., 2017). The root-associated microbiome variation can be greatly influenced not only by biotic and abiotic stressors, but also by traditional agricultural practices, such as crop rotation (Mardanova et al., 2019).

Potato (*Solanum tuberosum* L.) is an economically important crop worldwide and in Tunisia. Despite its significant role in food security (Devaux et al., 2014), studies about its interactions with microbial communities are scarce (Ferreira et al., 2008). Understanding microbial partnerships with the economically important crop *Solanum tuberosum* L. has the potential to improve plant production and yield, and obviously to maintain soil fertility (Berendsen et al., 2012; Winston et al., 2014). The characterization of soil microbial communities can be part of plant breeding programs and may be useful for studies of environmental risk assessment of selected potato cultivars (Ferreira et al., 2008). Moreover, analysis of the microbial community structure can be used as an indicator of soil quality and bio-fertility (van der Heijden and Wagg, 2013).

In the rhizosphere of potato plants grown under highly different conditions, the root bacterial taxa seem to be tightly linked to cultivars irrespective of the geographical site and the development stage (Pfeiffer et al., 2017). However, Weinert et al. (2011) observed similarities in the relative dominance of the bacterial phyla in potato rhizosphere irrespective of soil type and cultivar. As for the specific effects of the plant developmental stage, İnceŏglu et al. (2013) showed that some bacterial genera are universally present at the plant flowering stage and this based on a 3-year monitoring of the microbial community associated to different potato genotypes grown on the same site.

According to İnceŏglu et al. (2013), potato cultivars are associated with a core microbiome and that the specific patterns of that microbiota are more linked to the local environmental variations. Therefore, to test this hypothesis under Tunisian conditions, the structure of rhizosphere microbial community associated to four potato cultivars grown for two consecutive cropping seasons was monitored at plant emergence, senescence and 15 days post-harvest.

Materials and Methods

Plant Material

Four potato cultivars (cvs. Spunta, Elata, Cerata, and El-Mundo) were used in this study. The morpho-physiological characteristics of tested potato cultivars are listed in Table 1. Seed tubers were gratefully provided by the Regional Research Centre on Horticulture and Organic Agriculture, Tunisia.

Before use, tubers were previously disinfected by dipping for 5 min into 10% sodium hypochlorite solution, rinsed with tap water and air dried. For the initiation of their germination prior to planting, tubers were incubated for two weeks under 15-20°C, 60-80% relative humidity and natural room light.

Table 1. Characteristics of potato cultivars used in the study[aa] Seed tubers were gratefully provided by the Regional Research Centre on Horticulture and Organic Agriculture, Tunisia

Characteristic	Spunta	Elata	Cerata	EL-Mundo
Tuber				
Shape	Elongated oval	Elongated oval	Round	Elongated
Skin color	Yellow	Yellow	Pink	Yellow
Pulp color	Yellow	Yellow	White	Pale yellow
Length/Widgth	1.73	1.74	0.85	1.79
Dry matter content (%)	18.9	19.5	20.7	17.1
Stem				
Number/plant	2.55	3.05	2.75	4.40

[a]Seed tubers were gratefully provided by the Regional Research Centre on Horticulture and Organic Agriculture, Tunisia

Site Description and Experimental Design

Field trial was conducted at the experimental farm of the Regional Research Centre on Horticulture and Organic Agriculture located in Teboulba region (N35°38'38,256"; E10°56'48,458"). This site is under conventional farming system and has a history of potato and other vegetable crops rotations. The soil has a sandy clay texture (Organic matter 76 g/kg at 0-20 cm depth). The precipitation is daily measured and the monthly mean values are given in Table 2 and particularly from September to March corresponding to the experiment duration.

At their pre-germination, potato tubers were planted on October 03, 2017 and November 09, 2017 corresponding to the planting dates of two consecutive cropping seasons (i.e. late season and extra-early). Seeds were arranged into plots consisting of 25 seeds per row and per plot. Seed rows were 0.75 m apart, with four rows per plot, and within-row spacing of 0.33 m.

The trial was carried out according to a completely randomized design with four replications of 100 seed tubers per cultivar. NPK fertilization ratios used for late-season and extra-early cropping seasons are (13:13:24) and (23:9:15), respectively. For both trials, agricultural practices commonly adopted by farmers in the region were used.

Table 2. Average monthly precipitation for the experimental site[a] during the essay (2017/2018 agricultural campaign)

	Precipitation (mm)
September	0
October	26.8
November	11
December	21.5
January	1
February	36
March	27

[a]The experimental farm of the Regional Research Centre on Horticulture and Organic Agriculture in Teboulba region (N35°38'38,256"; E10°56'48,458")

Soil Sampling

For the two crops, composite soil samples were collected from each cultivar and each individual plot at four time points i.e. before planting (soil initial stage), 30 days after planting (plant emergence), 120 days after planting (plant senescence) and 15 days post-harvest.

At each sampling time, 20 soil cores (7 cm in diameter × 15 cm in depth) were collected from the rhizosphere of each sampled plant and were combined to make one composite soil sample per cultivar and per plot. All soil samples were kept in individual plastic bags and were kept under cold conditions during transportation to the laboratory.

Once brought to the laboratory, they were passed through a 2-mm sieve to remove rocks and large organic debris. They were stored at 10°C and processed within 1 to 4 weeks after sampling (Larkin and Honeycutt, 2006). The bulk soil was divided into subparts.

Soil pH and Electrical Conductivity (EC)

Soil samples were air-dried before use. Soil extracts were prepared by suspending soil in distilled water in 1:10 soil/dH_2O ratio. They were filtered through Whatman paper No. 1 and chemically analyzed for the determination of their pH and electrical conductivity (EC) using a glass electrode (VWR sympHony®) and a digital conductivity meter (HANNA®), respectively.

Determination of Soil Microbial Community

The total counts of culturable soil microorganisms focused in this study (bacteria, actinomycetes and fungi) were determined by serial soil dilution and plating on various specific agar media according to Larkin and Honeycutt (2006) with slight modifications. For each soil subsample, 10 g were suspended into 90 ml of sterile distilled water, vigorously stirred for 30 min, serially diluted and a 100 µl sample was spread onto 10% Tryptic Soy Agar (TSA) for total bacterial counts, selective King's B medium (KB) amended with 75 mg/l of penicillin and 75 mg/L of cyclohexamide for *Pseudomonas* spp. counts, Yeast Malt Agar (ISP medium No. 2) amended with 75 mg/L of nalidixic acid and 100 mg/L of cyclohexamide for actinomycete counts, and Potato Dextrose Agar (PDA) amended with 300 mg/L of streptomycin sulphate for total fungal counts. Four replicates of one plate each were used for each soil subsample.

Bacterial and actinomycete plates were incubated at 28°C for 2 and 14 days, respectively, and fungal plates were maintained at 25°C for 7 days. *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. Colonies were identified based on their macro- and micro-morphological traits (Barnett and Hunter, 1987) under light microscope and counted separately.

Statistical Analysis

Data were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software for Windows version 16.0. Data for the microbial community populations counts noted as Colony-forming Unit (CFU) per g of soil were analyzed after a logarithmic transformation to log CFU per g of soil. Data analyses for the pH and EC measurements and the microbial colonies counts were performed according to a completely randomized factorial model with three factors (sampling period, field plot, and cultivars used). Four measures were processed for each soil subsample. The experiment was conducted over two consecutive cropping seasons. Means were separated using LSD or Duncan's Multiple Range tests at $P \leq 0.05$.

Data analyses for the microbial community counts at the initial stage (before planting) were carried out according to a completely randomized design and means were separated using Duncan's Multiple Range test at $P \leq 0.05$. Correlations between soil characteristics (pH and EC), and soil microbial community structure were carried out using bivariate Pearson's test at $P \leq 0.05$.

Results and discussion

Determination of pH and EC of Soil Samples

For both cropping seasons and at each sampling period, no differences in pH values were recorded between potato cultivars (Table 3). ANOVA analysis of the pH values of the soil samples collected from the rhizosphere of the four potato cultivars varied significantly (at $P \leq 0.05$) upon sampling periods and this for both cropping seasons. In fact, for the late-season trial and for all potato cultivars combined, the pH of rhizospheric soils sampled at plant senescence and 15 days post-harvest was 3.9 to 6.9% higher than that removed at plant emergence (Table 3). However, for the extra-early experiment, a significant decrease of about 9.5-10.1%, as compared to the plant emergence, was noted at senescence and at 15 days post-harvest (Table 3). For both cropping seasons and particularly at plant senescence and 15 days post-harvest, no differences were recorded between the pH values of rhizospheres of potato cultivars and that of the initial soil state (Table 3).

The electrical conductivity of the rhizosphere of the four cultivars tested was 50.6-60.6% and 63.9-67.9% higher at plant senescence and 15 days post-harvest for the late-season crop and at 15 days post-harvest for the extra-early trial, respectively (Table 3). For both cropping seasons, no differences were recorded between cultivars at each sampling period (Table 3). At all sampling periods combined, a significant increase in EC values by 18.9-71.1% and 16.8-73.4% was recorded in the rhizosphere of tested cultivars as compared to the initial soil state and this for the late-season and the extra-early crops, respectively (Table 3).

Some works suggest that rhizosphere communities are mostly influenced by edaphic factors (particularly pH) or plant growth stage (van Overbeek and van Elsas, 2008; Winston et al., 2014). Indeed, pH and EC are important determinants of community structure and diversity of soil microbiome. Bacterial communities appeared to differ strongly between the two fields used in Hannula et al. (2012) study, both for bulk soil and rhizosphere.

Soil Microbial Structure in the Rhizosphere of Tested Potato Cultivars

Among the aims of this study is to evaluate the variation of the microbial community in potato rhizosphere according to the environmental changes. For this reason, the field trial was conducted under two cropping seasons namely late-season and extra-early. The composition of microbial communities can fluctuate in response to seasonal and temperature changes (Turpault et al., 2007) and water content (Liddell et al., 2007) among other factors.

Bacterial Population

The total number of bacterial colonies (individual colonies looking like distinct and separate dots) grown on TSA medium varied significantly (at $P \leq 0.05$) depending on sampling periods, field plots and cultivars tested and their interactions (Figure 1). For the two cropping seasons, soil samples removed from the rhizosphere of the four potato cultivars showed 41.-50.2% and 34.6-54.9% significantly higher populations of the total culturable bacterial community (Figure 1) as compared to the initial stage (pre-planting) (1.39-1.47 log CFU/ g of soil) (Table 4). Bacteria are also found to be more abundant in the rhizosphere of rice plants than in bulk soil (Breidenbach et al., 2016).

For the late-season crop, bacterial colonies recovered from the rhizosphere of all potato cultivars at plant emergence (2.64-3.26 log CFU per g of soil) and 15 days post-harvest (2.66-3.05 log CFU per g of soil) 10.3 to 13.2% significantly higher than at plant senescence (2.46-2.66 log CFU per g of soil) (Figures 1A and 2A) and this for all field plots combined. The plant developmental stage is considered as a main factor affecting bacterial communities in the potato rhizosphere (van Overbeek and van Elsas, 2008; Marques et al., 2014).

The distribution of bacteria significantly varied upon field plots whatever sampling times and cultivars tested where the soil samples removed from the second, the third and the first plots had 3.7-7.1% more abundant bacterial community than the last one. Microorganisms are not distributed uniformly in the environment, rather their abundance and activity change along environmental gradients (Nunan et al., 2002).

The total culturable bacteria were significantly more abundant in the rhizosphere of potato cultivars Spunta, Elata and El-Mundo with about 2.76 to 2.82 log CFU per g of soil as compared to 2.63 log CFU per g of soil counted in Cerata rhizosphere (Figure 1A) and this for all sampling periods and field plots combined.

As shown in Figures 1B and 2B, the total bacterial population estimated at the extra-early crop was significantly 4.2-6.4% higher at plant senescence than at plant emergence and 15 days post-harvest. Combined data for all sampling periods and cultivars tested revealed that the total rhizosphere bacterial communities were 1.05 and 1.12 times more abundant in the third and last plots than in the first and the second ones, respectively. For all plots and sampling times combined, the highest population of culturable bacteria, of about 2.67 and 2.62 log CFU per g of soil, was noted in the rhizosphere of potato cultivars Spunta and El-Mundo which was 1.04-1.06 times more than that of Elata, and Cerata (Figure 1B). In the present investigation, shifts noted in the rhizosphere bacterial communities associated to the rhizosphere of the tested cultivars were related to both cultivars and plant developmental stages. These findings are in agreement with those of Inceoğlu et al. (2013) who also demonstrated that the plant growing stage influences the potato rhizosphere microbiota. Also, Chapapro et al. (2014) noted that plant developmental changes affect the rhizosphere microbial community.

As for the composition and the diversity of the bacterial community, the relative abundance of *Pseudomonas* spp. was found to be 17.4% higher in the rhizosphere of potato cultivars grown at the late-season than at the extra-early crop (Figure 3). *Pseudomonas* spp. distribution within potato rhizosphere varied significantly (at $P \leq 0.05$) depending on field plots and cultivars used, and the interactions between the last ones and the sampling periods, and this for the two cropping seasons. As given in Figures 2A and 4A, no significant variation was noted between *Pseudomonas* spp. population estimated at plant emergence, senescence and 15 days post-harvest and this for all field plots and cultivars combined. *Pseudomonas* spp. were found to be more abundant in the fourth and second field plots (1.1-1.3 times higher) than in the remaining plots and this whatever the sampling times and potato cultivars used. Soil systems are particularly heterogeneous, and this heterogeneity arises as a result of the interaction of a hierarchical series of interrelated variables that fluctuate at many different spatial and temporal scales (Ettema and Wardle, 2002). Different subsets of the community are distributed differently across the plot, and this is thought to be due to the variable response of individual populations to the spatial heterogeneity associated with different soil properties (Franklin and Mills, 2003).

As for the variation between tested potato cultivars, the highest *Pseudomonas* spp. counts, of about 2.13 to 2.31 log CFU per g of soil, were noted in soils sampled from Cerata, Elata and El-Mundo rhizosphere followed by those of Spunta, 1.94 log CFU per g of soil, , and this for all plots and sampling periods combined (Figure 4A).

Rhizosphere of all potato cultivars showed an increase in its *Pseudomonas* spp. population at all sampling period of about 1.6 to 2.39 log CFU per g of soil as compared to 1.09 log CFU per g of soil counted at the pre-planting stage (Table 4 and Figure 4A).

Table 3. Soil pH and electrical conductivity (EC) of soil samples[a] removed from the rhizosphere of four potato cultivars grown for two cropping seasons

pH			
Late-season crop			
Initial stage	7.8		
Sampling period	Emergence	Senescence	15 days post-harvest
Spunta	7.3 a B	7.9 a A	7.8 a A
Elata	7.4 a B	8 a A	7.6 a B
Cerata	7.3 a B	7.9 a A	7.8 a A
El-Mundo	7.5 a B	7.9 a A	7.6 a B
Extra-early crop			
Initial stage	7.1		
Sampling period	Emergence	Senescence	15 days post-harvest
Spunta	7.9 a A	7.1 a B	7 a B
Elata	7.9 a A	7.1 a B	7.1 a B
Cerata	7.9 a A	7.1 a B	7 a B
El-Mundo	8 a A	7.1 a B	7.1 a B

EC (dS/m)			
Late-season crop			
Initial stage	0.09		
Sampling period	Emergence	Senescence	15 days post-harvest
Spunta	0.117 a B	0.297 a A	0.259 a A
Elata	0.119 a B	0.267 a A	0.266 a A
Cerata	0.103 a C	0.250 a A	0.184 a B
El-Mundo	0.115 a B	0.297 a A	0.230 a A
Extra-early crop			
Initial stage	0.121		
Sampling period	Emergence	Senescence	15 days post-harvest
Spunta	0.087 a B	0.135 a B	0.449 a A
Elata	0.241 a B	0.135 a B	0.460 a A
Cerata	0.172 a B	0.156 a B	0.449 a A
El-Mundo	0.155 a B	0.135 a B	0.448 a A

[a] Soil sampled at the initial stage (Pre-planting), the emergence stage (30 days post-planting), the senescence stage (120 days post-planting), and at 15 days post-harvest.Values within each column, followed by the same lower letter are not significantly different according to Duncan Multiple Range test at $P \leq 0.05$. Values within each line, followed by the same up letter are not significantly different according to Duncan Multiple Range test at $P \leq 0.05$.

Table 4. Soil culturable microbiome estimated at the initial stage (pre-planting)

Culturable microbiome (log CFU/g soil)			
Late-season crop			
Bacteria	*Pseudomonas* spp.	Actinomycetes	Fungi
1.47	1.09	0.68	1.91
Extra-early crop			
Bacteria	*Pseudomonas* spp.	Actinomycetes	Fungi
1.39	0.54	0.68	1.07

Pseudomonas population monitored for the extra-early crop varied significantly (at $P \leq 0.05$) upon sampling times, field plots and cultivars used, and their interactions. In fact, as shown in Figure 4B, the highest abundance of *Pseudomonas* spp. colonies, for all field plots and cultivars combined, was noted at plant emergence, with 2.23 log CFU per g of soil, as compared to plant senescence and 15 days post-harvest with 1.52 log and 0.29 log CFU per g of soil, respectively. Based on Ferreira et al. (2008) study, differences among rhizosphere bacterial communities are reported to be clearest at the earliest plant developmental stages and tend to decrease in later stages like senescence or after harvest. Whatever the sampling times and the cultivar grown, *Pseudomonas* spp. community was found to be more abundant in the first, the second and the third plot (1.3 to 1.5 times higher) than in the last one. For all sampling times and field plots combined, culturable *Pseudomonas* spp. were more abundant in the rhizosphere of cvs. Spunta, Elata and El-Mundo, 1.46-1.33 log CFU per g of soil, as compared to Cerata with 1.24 log CFU per g of soil. Weinert et al. (2011) reported that the composition of bacterial communities in the rhizosphere of potato plants is dynamic and is strongly influenced by the plant cultivar and the geographical site. Different microbial communities are found as defined by cultivar. According to Inceoğlu et al. (2012) soil and cultivar type shaped the potato root-associated bacterial communities that are responsive to some of the substrates in phenotype arrays.

Rhizospheric soils removed around the four potato cultivars sampled at plant senescence and emergence showed a significant increase (of about 5.2 and 7.6 times higher, respectively) in their average *Pseudomonas* community as compared to the initial soil stage (Table 4 and Figure 4B).

Actinomycetes Population

Soil samples removed from the rhizosphere of the four potato cultivars grown as a late-season crop showed an increment in the average actinomycetes populations of about 1.2 to 1.5 times higher than the initial soil (0.68 log CFU per g of soil) (Table 4 and Figure 5A).

The relative abundance of actinomycetes populations from all potato cultivars was more abundant at the late-season crop, of about 0.58-1.17 log CFU per g of soil, than at the extra-early crop where 0.21-0.86 log CFU per g of soil were noted (Figure 3). For both cropping seasons, no differences in actinomycetes populations were recorded between potato cultivars.

The culturable actinomycetes community monitored for the late-season crop varied significantly (at $P \leq 0.05$) depending on sampling periods × cultivars and field plots × cultivars interactions (Figure 5A). The highest average actinomycetes colonies, in rhizospheric soils of all tested potato cultivars grown in all field plots combined, was estimated at 0.84-1.04 log CFU per g of soil at 15 days post-harvest and plant senescence as compared to 0.72 log CFU per g of soil noted at plant emergence (Figures 2A and 5A).

In the extra-early crop, actinomycetes community varied significantly (at $P \leq 0.05$) depending on the interaction between sampling periods, field plots (within-field plots) and cultivars tested (Figure 5B). The actinomycetes population estimated in the rhizospheric soils associated to all tested potato cultivars, for all field plots combined, were 1.2-1.5 times more abundant at 15 days post-harvest and plant senescence than at emergence (0.45 log CFU per g of soil) (Figures 2A and 5B). Our results confirmed those of Broekling et al. (2008) who found that microbial community structures are abundant at the senescence stage and 15 days-post harvest.

Fungal Population

Soil samples removed from the rhizosphere of the four tested potato cultivars grown as late-season and extra-early crops showed a significant increase in their average fungal populations for all sampling periods which was estimated at 1.91-2.23 and 1.31-2.14 log CFU per g of soil, as compared to 1.91 and 1.07 log CFU per g of soil noted at the initial soil stage (pre-planting), respectively (Table 4 and Figure 5A). These results confirmed previous findings reporting that fungal composition and abundance is strongly influenced by the presence of potato roots (i.e. a strong rhizosphere effect) (Hannula et al., 2010). Mardanova et al. (2019) reported that bacterial and fungal microbiomes in the rhizosphere and rhizoplane of potato plants is remarkably diverse and is dependent on the host plant.

For both cropping seasons, the total culturable fungal community varied significantly (at $P \leq 0.05$) depending on sampling periods, field plots, cultivars grown and their interactions (Figure 6). The highest abundance of the fungal population was noted at 15 days post-harvest, with 2.09 log CFU per g of soil, as compared to 2.02 log and 1.92 log CFU per g of soil estimated at plant senescence and emergence, respectively and this for all field plots and cultivars combined (Figures 2A and 6A). Whatever the sampling times and the cultivars grown, the fungal population was more abundant in the first, the second and the third field plots (1.04- 1.06 times higher) than in the last one. As shown in Figure 5A, for the late-season crop and for all plots combined, the highest fungal population, estimated at 2.09 log CFU per g of soil, was noted in the rhizosphere of cv. El-Mundo compared to 1.97-1.98 log CFU per g of soil noted in cvs. Cerata, Elata and Spunta rhizospheres.

For the extra-early cropping season, the total fungal population was 5.4 and 28.4% significantly higher at 15 days post-harvest than at plant senescence and emergence, respectively (Figures 2B and 6B). Hannula et al. (2012) indicate that at the senescence stage, plants harbor the most diverse, active and abundant fungal communities. The presence of the highest fungal biomass and diversity at senescence is more expected as decomposable plant material (dead roots and leaves) is already available while root exudation still continues thereby broadening the spectrum of substrate availability (Broeckling et al., 2008).

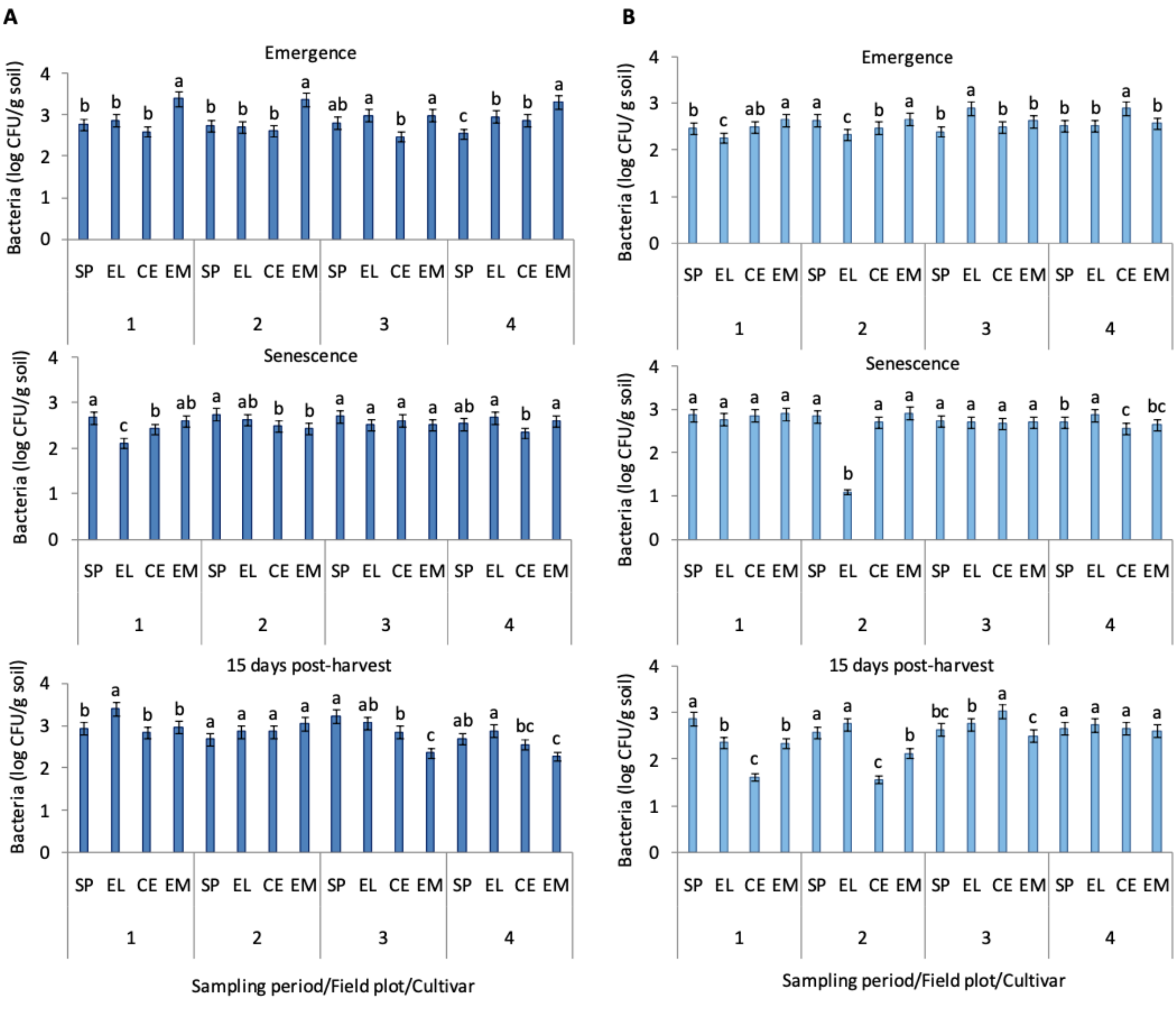

Figure 1. Variation in the total culturable bacterial population in the rhizosphere of four potato cultivars[a] depending on cropping seasons[b], sampling periods[c] and field plots[c]

[a] SP: Spunta; EL: Elata; CE: Cerata; EM: El-Mundo.

[b] (A) Late-season crop; (B) Extra-early crop.

[c] Sampling periods: Emergence (15 days post-planting), Senescence (120 days post-planting), and 15 days post-harvest.

[d] 100 plants per cultivar were grown per field plot.

Results are presented as mean ± SE (n = 8, $P \leq 0.05$).

For each sampling period and each field plot, bars (cultivars) sharing the same letter are not significantly different according to Duncan Multiple Range test at $P \leq 0.05$.

Soil dilution was made from a concentration of 10% (w/v).

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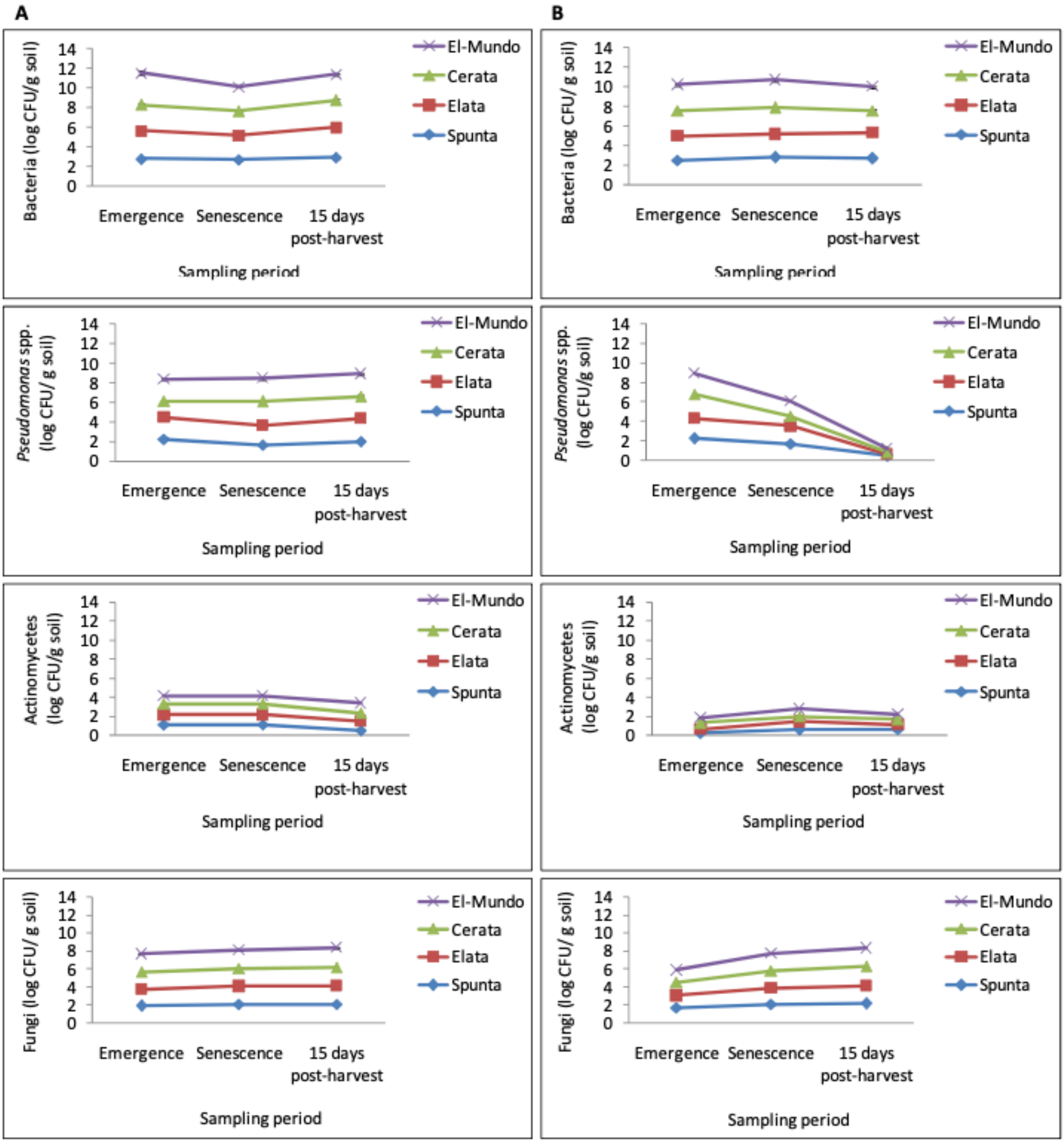

Figure 2. Variation in the total culturable bacterial population in the rhizosphere of four potato cultivars depending on cropping seasons[a] and sampling periods[b]

[a] (A) Late-season crop; (B) Extra-early crop.

[c] Sampling periods: Emergence (15 days post-planting), Senescence (120 days post-planting), and 15 days post-harvest. Results are presented as mean ± SE (n = 8, $P \leq 0.05$). Soil dilution was made from a concentration of 10% (w/v).

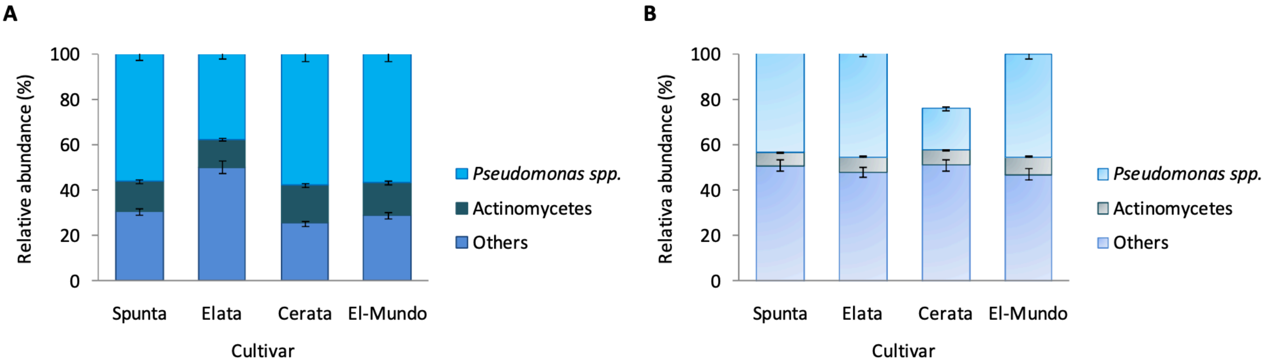

Figure 3. Variation in the bacterial community structure in the rhizosphere of four potato cultivars depending on cropping seasons. Results are presented as mean ± SE (n = 8, $P \leq 0.05$). The relative abundance was estimated per the total bacteria counted in each sampled soil. Soil dilution was made from a concentration of 10% (w/v). (A) Late-season crop (B) Extra-early crop.

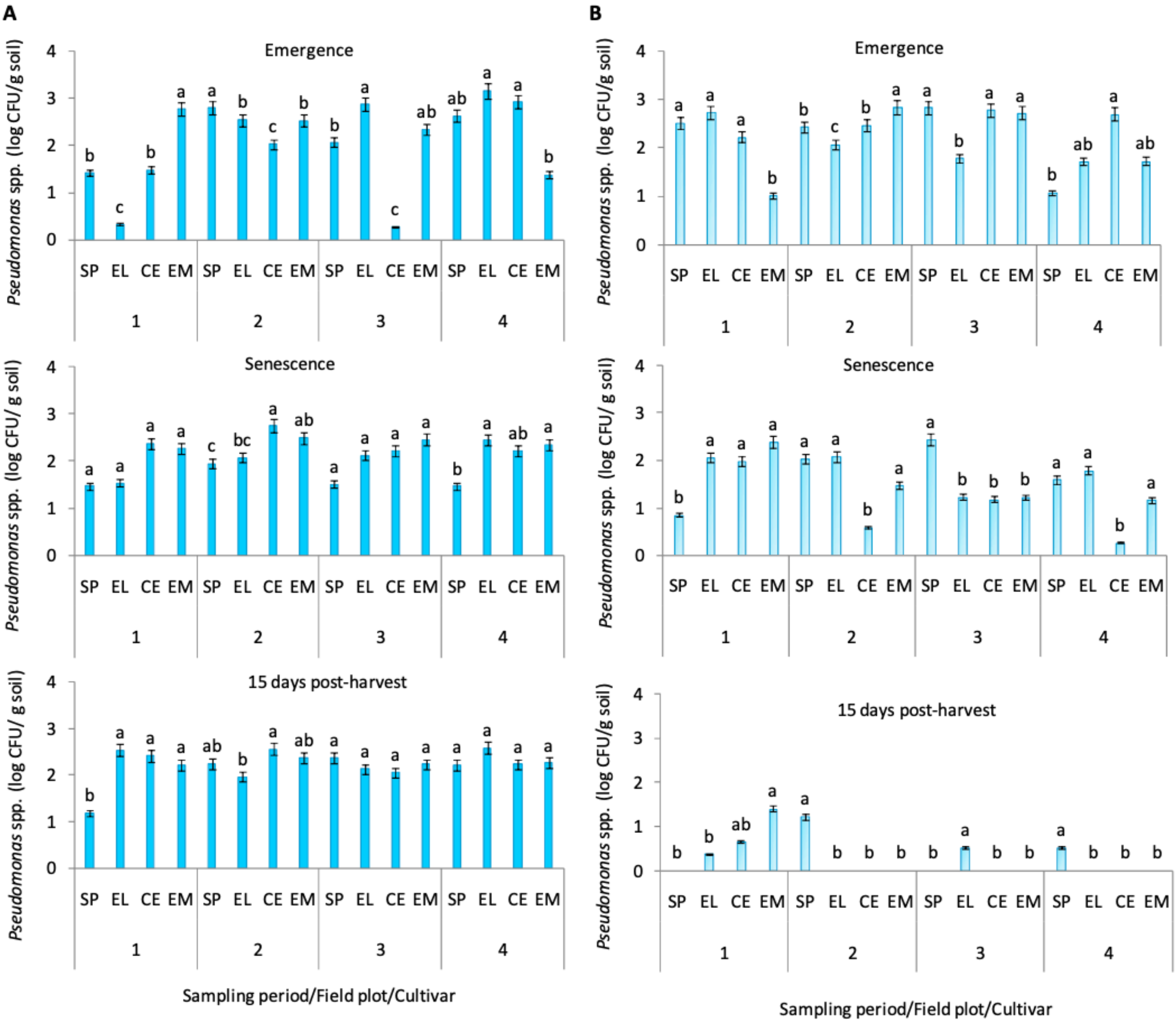

Figure 4. Variation in *Pseudomonas* spp. community in the rhizosphere of four potato cultivars[a] depending on cropping seasons[b], sampling periods[c] and field plots[d]

[a] SP: Spunta; EL Elata; CE: Cerata; EM: El-Mundo. [b] (**A**) Late-season crop; (B) Extra-early crop [c] Sampling periods: Emergence (15 days post-planting), Senescence (120 days post-planting), and 15 days post-harvest. [d] 100 plants per cultivar were grown per field plot. Results are presented as mean ± SE (n = 8, $P \leq 0.05$). For each sampling period and each field plot, bars (cultivars) sharing the same letter are not significantly different according to Duncan Multiple Range test at $P \leq 0.05$. Soil dilution was made from a concentration of 10% (w/v).

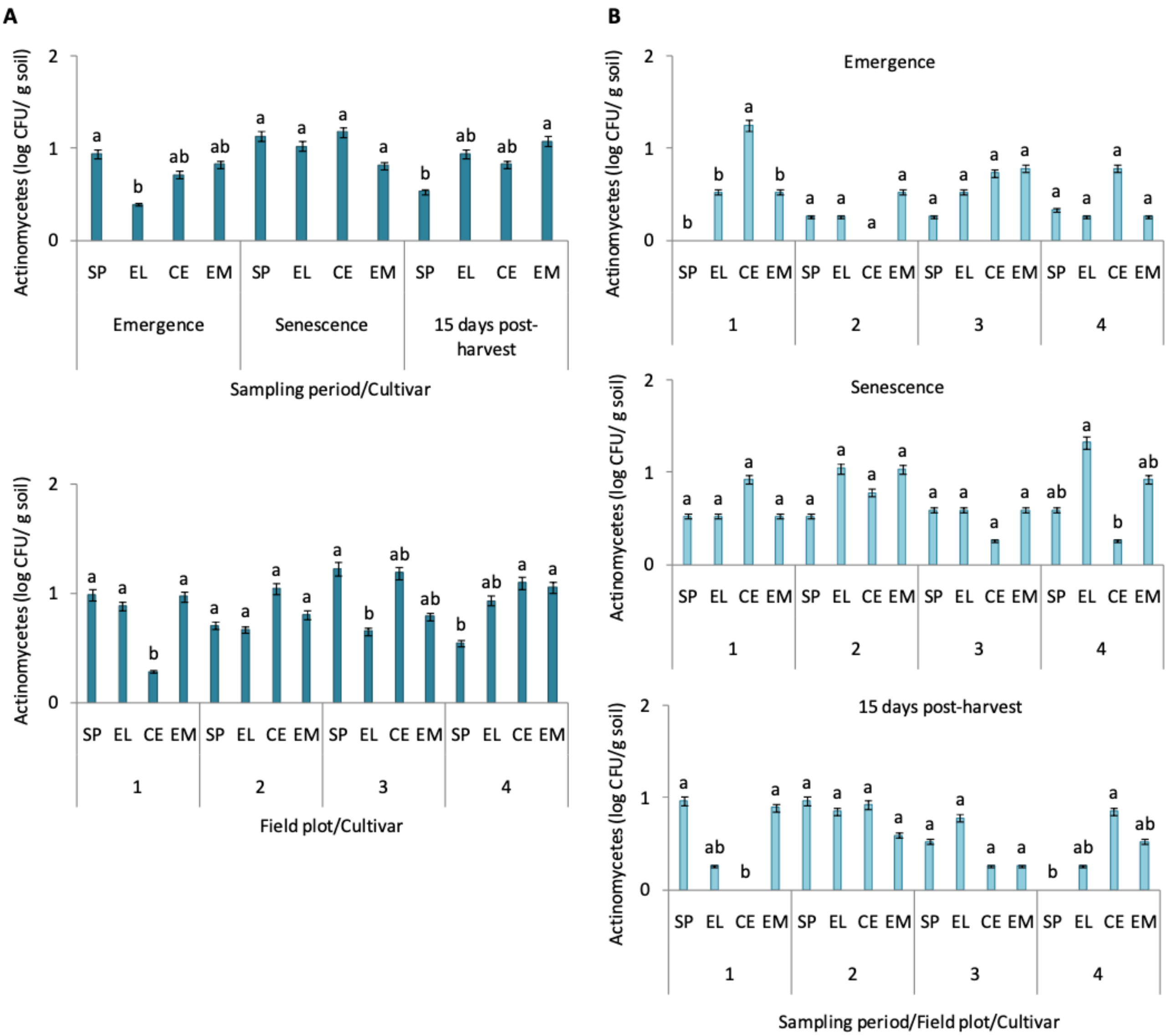

Figure 5. Variation in the Actinomycetes population in the rhizosphere of four potato cultivars[a] depending on cropping seasons[b], sampling periods[c] and field plots[d]

[a] SP: Spunta; EL: Elata; CE Cerata; EM: El-Mundo.

[b] (A) Late-season crop; (B) Extra-early crop

[c] Sampling periods: Emergence (15 days post-planting), Senescence (120 days post-planting), and 15 days post-harvest.

[d] 100 plants per cultivar were grown per field plot.

Results are presented as mean ± SE ($n = 8$, $P \leq 0.05$).

For each sampling period and each field plot, bars (cultivars) sharing the same letter are not significantly different according to Duncan Multiple Range test at $P \leq 0.05$.

Soil dilution was made from a concentration of 10% (w/v).

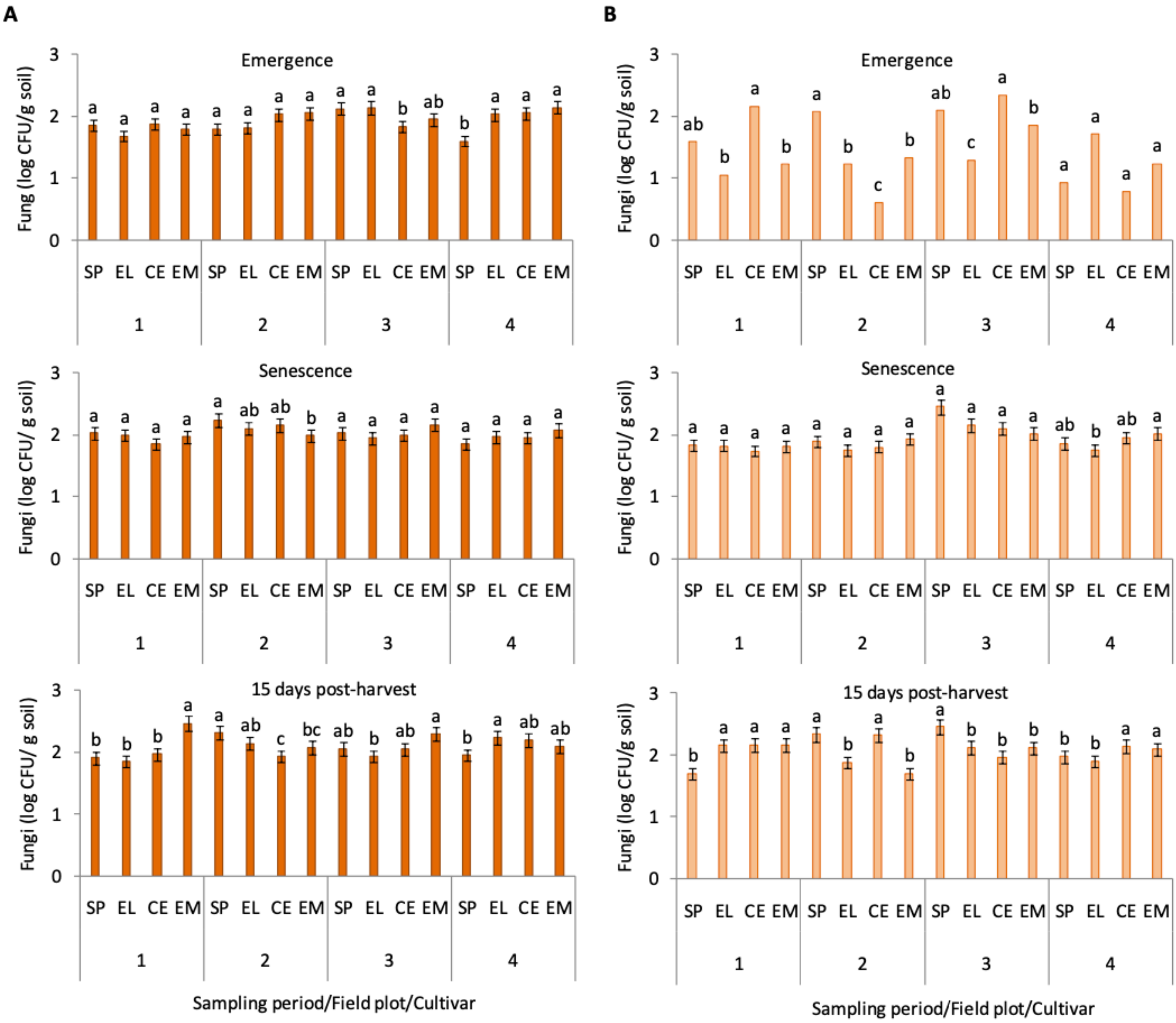

Figure 6. Variation in the total culturable fungal community in the rhizosphere of four potato cultivars[b] depending on cropping seasons[b], sampling periods[c] and field plots[d]

[a] SP: Spunta; EL: Elata; CE: Cerata; EM: El-Mundo.

[b] (A) Late-season crop; (B) Extra-early crop

[c] Sampling periods: Emergence (15 days post-planting), Senescence (120 days post-planting), and 15 days post-harvest.

[d] 100 plants per cultivar were grown per field plot.

Results are presented as mean ± SE (n = 8, $P \leq 0.05$).

For each sampling period and each field plot, bars (cultivars) sharing the same letter are not significantly different according to Duncan Multiple Range test at $P \leq 0.05$.

Soil dilution was made from a concentration of 10% (w/v).

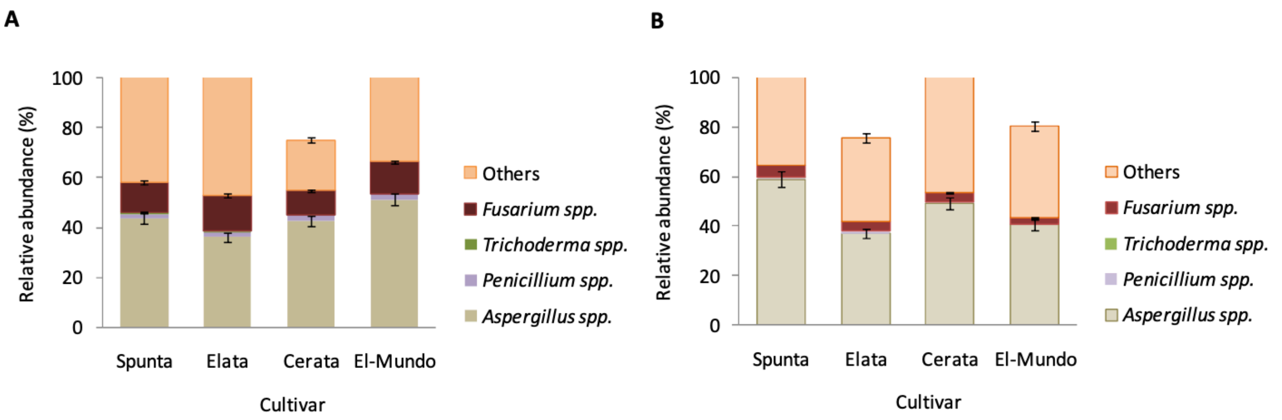

Figure 7. Variation in the fungal community structure in the rhizosphere of four potato cultivars depending on cropping seasons Results are presented as mean ± SE (n = 8, $P \leq 0.05$). The relative abundance was estimated per the total fungi counted in each sampled soil. Soil dilution was made from a concentration of 10% (w/v).
(A) Late-season crop (B): Extra-early crop.

Combined data for all sampling periods and cultivars tested revealed that the total fungal community was 1.2 times more abundant in the third field plot than in the remaining ones.

For all sampling periods and field plots combined (Figure 5B), the rhizospheric soil associated to cv. Spunta showed 1.05 and 1.08-1.11 times more abundant fungal population than those of cvs. Cerata, and El-Mundo and Elata.

It is well known that the rhizosphere microbiome or plant-associated microbial communities are generally influenced by root exudates and thereby change throughout plant development (Chaparro et al., 2014). The chemical compositions of root exudates vary not only among different plant species but also among cultivars of the same plant species (Kerdchoechuen, 2005). Furthermore, the chemical composition of root exudates is a result of the interactions of different factors such as the nutritional status, plant age, stress, diseases, and environmental factors that subsequently influence the microbial community associated to the rhizosphere (Griffiths et al., 1999).

As for composition and the diversity of the fungal community, *Aspergillus* spp. colonies were found to be more abundant in all sampled soils where their populations represented 36.2-51.2% and 37-58.9% of the total culturable fungi isolated from the late-season and the extra-early crops, respectively (Figure 7). For all sampling periods and field plots combined, the highest abundance of *Aspergillus* spp. colonies was 51.2 and 58.9% higher in El-Mundo and Spunta rhizospheres grown as late-season (Figure 7A) and extra-early crop (Figure 7B), respectively. *Fusarium* spp. population in the rhizosphere of all potato cultivars tested represented was estiamted at 9.6-14.2% in the late-season crop (Figure 7A) and at 2.5-5% in the extra-early season (Figure 7B). The variation in the microbial communities depending on cropping seasons can be explained by two possible mechanisms (Wang et al., 2009). The first one is related to temporal changes in abiotic conditions such as soil moisture, precipitation and temperature. The second mechanism may be attributed to the changes in quality and quantity of root exudates and rhizodeposits which are influenced by environmental and edaphic factors and/or root morphology (Marschner et al., 2003). The largest explaining factor for the variation of composition and function of fungal communities in the rhizosphere was the plant phonological growth stage, followed by the year and the soil type (Hannula et al., 2012).

Correlations Between Soil Characteristics (pH and EC), and Soil Microbial Community Structure

Pearson's correlation analysis indicated that, for all sampling periods, field plots and cultivars combined, the pH values were significantly and positively ($r = 0.332$, $P = 0.021$) correlated to the *Pseudomonas* spp. population in late-season crop and negatively ($r = -0.307$, $P = 0.034$) correlated to *Pseudomonas* spp. community at the extra-early crop . EC values were significantly and positively linked to *Pseudomonas* spp. ($r = 0.351$, $P = 0.015$) and fungal populations ($r = 0.306$, $P = 0.034$) in the analyzed soil samples of potato cultivars grown at the late-season crop. For the extra-early crop, EC significantly and positively correlated to fungal community ($r = 0.321$, $P = 0.026$) and this for all cultivars r, field plots and sampling periods combined. Our findings confirm in part those of Kim et al. (2016) who demonstrated a significant positive correlation between soil chemical parameters such as soil pH, EC, and exchangeable cations and bacterial community. Rousk et al. (2010) reported that both the relative abundance and diversity of bacteria are positively related to pH whereas the relative abundance of fungi is not influenced by pH and the fungal diversity is weakly related with pH. Although the bacterial community composition is clearly influenced by soil pH and EC values, the specific relationships between each bacterial phylum and pH and/or EC can vary (Kim et al., 2016). Besides, *Bacteroidetes*, *Spirochaetes*, and *Tenericutes* are positively correlated with the EC values, but *Acidobacteria* had a negative correlation (Kim et al., 2016). Many studies have shown that soil microbial communities are influenced by various environmental factors, including soil pH (Xu et al., 2014), electrical conductivity (Ma et al., 2016; Min et al., 2016), soil texture (Lauber et al., 2008), soil parental material (Sun et al., 2015), and salinity (Lozupone and Knight, 2007). Pfeiffer et al. (2017) observed that the taxonomic composition of bacteria repeatedly occurring at particular stages of plant development was almost unaffected by highly diverse environmental conditions.

Conclusions

Four potato cultivars were evaluated for searching the eventual relationships between associated rhizosphere mirobiomes and grown cultivars, plant age and/or cropping seasons. The obtained results clearly demonstrated that the distribution of bacterial and fungal populations varied significantly upon cultivars. For both cropping seasons, fungi were more associated to EL-Mundo and Spunta cultivars, while bacteria were more abundant in cvs. Spunta, Elata and El Mundo rhizospheres. Indeed, *Pseudomonas* spp. were associated to the four tested potato cultivars, but *Aspergillus* spp. were more abundant in the rhizosphere of El-Mundo and Spunta cultivars. In the present study, the highest abundance of the total culturable bacteria was noted at plant emergence and 15 days post-harvest for the late season and at senescence for the extra-early crop. The actinomycetes and fungal populations were more prevalent at plant senescence and 15 days post-harvest and this for both cropping seasons. The relative abundance of *Pseudomonas* spp., actinomycetes and *Fusarium* spp. populations was found to be relatively important in the rhizosphere of all potato cultivars grown for a late-season than for an extra-early crop. Furthermore, the distribution of bacteria, and fungal communities significantly varied upon field plots whatever the sampling time and the cultivar tested and this for both cropping seasons.

Variations in the rhizosphere microbial community composition were noted at different developmental stages for four potato cultivars grown for two cropping seasons. Besides, the microbial community structure significantly correlated with pH and EC of soils sampled from potato rhizopshere. This will help researchers to explore specific microbes that are required for the improvement of potato growth and/or health under different growing conditions and during different developmental stages. In addition, this study can be valorized in order to incorporate associated microbiome in future strategies for plant breeding programs.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

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Not applicable.

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The growth curve of body weight in Kacang goats managed by smallholders at Tambang District of Indonesia

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Abstract

Kacang goat (*Capra hircus*) is one of Indonesian germ plasm and kept by smallholders for meat production. This research was carried out to obtain the growth curve of body weight from 1 to 24 months of age in Kacang goats managed by smallholders at Tambang District of Indonesia. Total of 100 goats (51 buck and 49 does) were used in this study to obtain Logistic and Gompertz growth curves. Research showed that the Logistic and Gompertz growth curve have similar of coefficient of determination value (R^2= 0.98). However, the standard error (SE) value in Gompertz was lower than Logistic curves. The inflection weight (IW) was reached of 13.16 to 15.30 kg (Logistic) and 12.76 to 14.41 kg (Gompertz). The inflection age (IA) was reached of 12.95 to 15.57 months (Logistic) and 12.71 to14.41 months (Gompertz). The growth rate (GR) were reached of 1.06 to 1.23 kg/month (Logistic) and 1.02 to 1.10 kg/month (Gompertz). It was concluded that Gompertz growth curve was the best predictor to predict body weight in Kacang goats.

Keywords: Body weight, Growth curve, Kacang goats, Smallholders, Inflection

Introduction

Kacang goat (*Capra hircus*) are one of Indonesian native that kept by smallholders as meat production. Kacang goat was decided as the one of Indonesian native cattle through decision of Ministry of Agriculture No: 2840/Kpts/LB.430/8/2012. The average of body kg weight and carcass weight in Kacang bucks were 10.50 - 14.75 kg (Yurmiaty, 2006) and 5.63 kg (Sumardianto et al., 2013) respectively. Morever, Nuriadin et al. (2017) reported that the litter size, kidding interval, mortality rate at preweaning age and kid crop of Kacang does were 1.59±0.06; 8.05±0.38 months; 18.62±3.31% and 208.84±20.96% respectively.

Selection in the livestock can be performed based on growth traits. The growth of a livestock is a collection of the growth of parts of each component that can be seen from the physical appearance and weight of their life (Lawrence and Fowler, 2002). The growth of livestock can be evaluated based on their growth curve (Cak et al., 2017). Growth curve is a reflection of the ability of an individual or population to actualize themselves as a measure of the development of body parts to the maximum size (adult) in the existing environmental conditions. In general, growth in the form of sigmoid or "S". The "S" curve represents a form of acceleration and deceleration that is limited by turning points or inflection points. The inflection point is the maximum point of growth in body weight (Lawrence and Fowler, 2002).

Two growth curve of Logistic and Gompertz models were widely used to predict growth curve of body weight in many

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goat breeds with highly accuracy. Previous studies used these model to predict the body weight in Katjang (Kacang), Angora, Alpine, Beetal, Damascus, Repartida, Saanen, Raeini Cashmere, Markhoz, Sirohi and Boer goats (Tsukahara et al., 2008; Özdemir and Dellal, 2009; Kume and Hajno, 2010; Waheed et al., 2011; Gaddour et al., 2012; Regadasfilho et al., 2014; Pires et al., 2017; Ghiasi et al., 2018; Kheirabadi and Rashidi, 2018; Waiz et al., 2019; Garcia-Muniz et al., 2019).

This study aimed to obtain the growth curve of body weight in Kacang goats managed by smallholders at Tambang District of Indonesia using Logistic and Gompertz models. The results of this study can be used as the early information to develop breeding program of Kacang goats at villager breeding center (VBC) in the future.

Materials and Methods

The records data of body weight (BW) in 100 Kacang goats (51 bucks and 49 does) at Tambang District, Kampar Regency of Indonesia were used in this study. All goats in this study were born with single birth status. The research site located between latitude 01°00'40" N - 00°27'00 S and longitude 100°28'30" - 101°14'30" E. The average of maximum air temperature about 21 - 35 °C with rainfall intensity about 2,846 mm/year and 26-100 m above sea level. The BW of goats were obtained from 1 to 2 animals per ages group with animal weighing scale. All goats were managed by smallholders with extensive traditional system.

Two growth curve models of Logistic and Gompertz were performed in this study using CurveExpert 1.4. software. The Logistic and Gompertz growth equations were presented in Table 1.

Table 1. The mathematics equation in the growth curve model of Logistic and Gompertz

Model	General formula				
Logistic	$Y_t = \frac{a}{1+be^{-ct}}$	$IW = 1/2(a)$	$IA = (\ln.b)/c$	$GR = c(IW)\left(1 - \frac{IW}{a}\right)$	$AR = GR / a$
Gompertz	$Y_t = ae^{-e^{b-ct}}$	$IW = a / e$	$IA = a\exp(-b e^{-c})$	$GR = c(IW)\ln.\left(\frac{IW}{a}\right)^{-1}$	$AR = GR / a$

Where: Y_t is the predicted body weight at t^{th} age; a is the asymptotic; b is the scale parameter; c is the the average rate of body growth until the animal reaches body maturity; e is the logarithm base (2.72); t is the animals age (months); IW is the inflection weight (kg) IA: is the inflection age (months); GR is the maximum growth rate (kg/month) and AR is the adult rate (kg/month).*Amrullah (2016).

Results and Discussion

The average of BW in Kacang goats in this study was presented in Table 2. Abadi et al. (2015) reported that the average of BW in Kacang does at 18-30 months old (3PPI) in Grobogan Regency was 21.13 kg and similar to Kacang does in this study at 21 months old. Waheed et al. (2011) reported that the average of BW at 12 months old in Beetal goat was 22.10±1.90 kg (male) and 20.70±1.54 kg (female) and close to the animals study at 21 months old. Özdemir and Dellal (2009) the average of BW in Angora goat at 12 months old was 22.70±0.70 kg (male) and 16.40±0.70 kg (female) and higher than animals study at the same age. Thus, Waiz et al. (2019) reported that the average of BW in Sirohi goat at 12 months old was 26.58±6.16 kg (male) and 23.65±3.13 kg (female) and close to the animals study at 24 months old.

The growth curve of Logistic and Gompertz models had similar of coefficient of determination value ($R^2 = 0.98$).

Table 2. The average of body weight in Kacang goats

Age (months)	$\bar{X}_{BW}$ (kg)		Age (months)	$\bar{X}_{BW}$ (kg)	
	Male (N)	Female (N)		Male (N)	Female (N)
1	1.50 (3)	1.43 (3)	13	12.75 (2)	14.30 (2)
2	2.50 (3)	2.45 (2)	14	13.25 (2)	14.65 (2)
3	3.20 (2)	3.35 (2)	15	13.95 (2)	15.80 (2)
4	3.40 (2)	4.45 (2)	16	15.75 (2)	16.40 (2)
5	4.45 (2)	5.75 (2)	17	16.60 (2)	14.30 (2)
6	5.35 (2)	7.55 (2)	18	18.00 (2)	18.45 (2)
7	6.40 (2)	8.70 (2)	19	18.15 (2)	18.80 (2)
8	7.05 (2)	9.25 (2)	20	19.50 (2)	19.35 (2)
9	8.00 (2)	10.00 (2)	21	21.10 (2)	20.50 (2)
10	10.05 (2)	11.00 (2)	22	22.55 (2)	21.40 (2)
11	10.75 (2)	11.90 (2)	23	23.85 (2)	23.00 (2)
12	12.10 (2)	12.65 (2)	24	25.23 (3)	24.30 (2)

$\bar{X}_{BW}$: the average of body weight; N: number of animal

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Table 3. The statistical analysis in growth curve of body weight in Kacang goats

Model / Sex	a	b	c	R^2	SE	IW (kg)	IA (months)	GR (kg/month)	AR (kg/month)
Logistic									
Male	30.60	12.08	0.16	0.98	0.71	15.30	15.57	1.23	0.04
Female	26.31	7.94	0.16	0.98	1.16	13.16	12.95	1.06	0.04
Gompertz									
Male	42.90	1.17	0.07	0.98	0.53	14.59	14.41	1.10	0.03
Female	31.12	0.98	0.09	0.98	0.99	12.76	12.71	1.02	0.04

a is the asymptotic; b is the scale parameter; c is the the average rate of body growth until the animal reaches body maturity; R^2: coefficient of determination; SE: standard error; IW is the inflection weight; IA is the inflection age; GR is the maximum growth rate and AR is the adult rate

In sheep, the R^2 value of 0.97 to 0.99 in the Logistic and Gompertz growth curves were reported in Morkaraman, Awassi, Akkaraman, Dorper cross, Norduz, Mengali, Hemsin, Madras red, Thalli, Mehraban and Mecheri breeds (Topal et al., 2004; Kucuk and Eyduran, 2009; Malhado et al., 2009; Kum et al., 2010; Tariq et al., 2013; Kopuzlu et al., 2014; Ganesan et al., 2015; Waheed et al., 2016; Hojjati and Hossein-Zadeh, 2017; Balan et al., 2017). Previous studies obtained the R^2 = 0.96 (Logistic) and 0.97 (Gompertz) in Hair, Markhoz and Sirohi goats (Tatar et al., 2009; Kheirabadi and Rashidi, 2019; Waiz et al., 2019). Meanwhile, the R^2 value in both models for Angora and Repartida goats were 0.91 and 0.96 respectively (Özdemir and Dallal, 2009; Pires et al., 2017).

The standard error (SE) value in Gompertz was lower than Logistic models. In Gompertz model, the growth curve in males was higher than females. Meanwhile, the growth curve of males in both models was lower than females (Figure 1). However, previous study reported that the growth curve of BW in bucks was higher than does (Waheed et al., 2011; Waiz et al., 2019; Kheirabadi and Rashidi, 2019). Despite, previous study reported that the growth curve of BW in rams was higher than ewes (McManus et al., 2003; Keskin et al., 2009; Gautam et al., 2018). Soeparno (2005) stated that the mechanism sex hormonal (androgen) was caused the growth rate in male animal was faster than female animal. The IW and IA in Gompertz model were lower than Logistic model.

According to the Gompertz model, the mature weight in Kacang does of this study was reached at 12.71 months old and lower than Kacang does at Malaysia (18 months old) as reported by Tsukahara et al. (2008). Malhado et al. (2009) obtained the IW values in Dorper × Morada Nova (DMN); Dorper × Rabo Largo (DRL) and Dorper × Santa Ines (DSI) sheeps based on Gompertz model were 11,96 kg; 10.50 kg and 11.03 kg respectively with IA around 58.50 days (DMN), 67.10 days (DRL) and 75.70 days (DSI). Thus, Garcia-Muniz et al. (2019) reported IW and IA in Boer goat based on Logistic model were 24.8 kg and 115.1 days respectively. Hence, the IW and IA in Boer goat based on Gompertz model were 20 kg and 92 days respectively. Morever, Najari et al. (2007) obtained IW of 5.93 kg and IA of 39 days in native Tunisian goats recorded from birth until 8 months old.

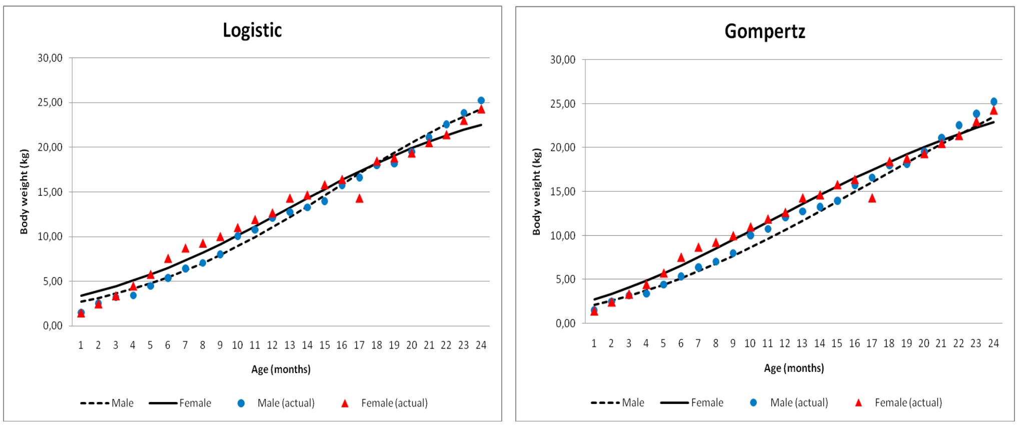

Figure 1. The growth curve of body weight in Kacang goats adjusted by Logistic and Gompertz models

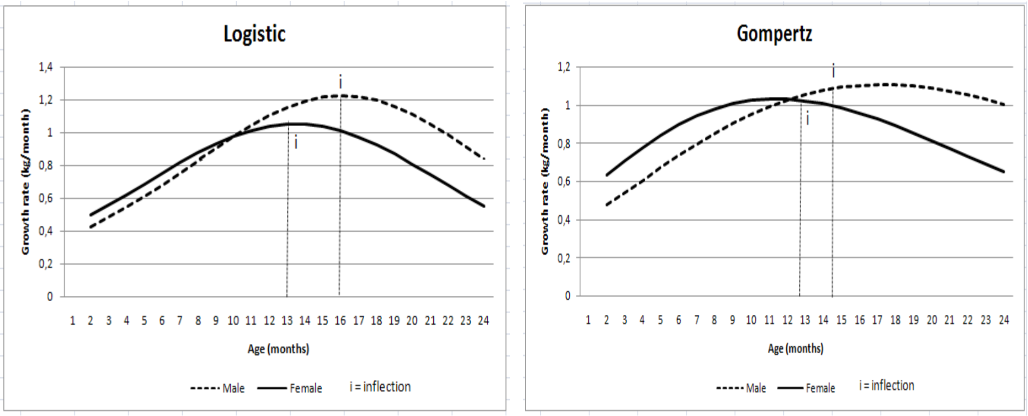

Figure 2. The absolute growth rate of body weight in Kacang goats estimated by Logistic and Gompertz models

The IW and IA values in animals studied with Gompertz were lower than Logistic models and similar to Boer goat (Garcia-Muniz et al., 2019). In this study, the mature weight of Kacang bucks was about 15 kg and reached at about 14-16 months old. Hence, the mature weight in Kacang does was about 13 kg and reached at about 13 months old. The IA value in animals studied based on Logistic and Gompertz models were presented in Figure 2. In addition, the GR for Kacang bucks in this study was 1.23 kg/month (Logistic) and 1.10 kg/month (Gompertz). Hence, the GR for Kacang does in this study was 1.06 kg/month (Logistic) and 1.02 kg/month (Gompertz) as presented in Figure 2. Morever, the AR in animals studied was 0.03-0.04 kg/month. Amrullah (2016) obtained the AR value in Brahman cows of 0.03 kg/month in Logistic or Gompertz models and close to the present study.

Conclusion

The growth curve of BW with Logistic and Gompertz models in animals studied had R^2=0.98. However, the Gompertz model had lower of SE value rather than Logistic model. Despite, selection of Kacang goat can be performed based on the BW at 14-16 months old (male) and 13 months old (female).

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Factors affecting the use of artificial insemination of farmers in dairy farming

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Abstract

The study was carried out in the districts of Harmacık, Keles, Orhaneli and Büyükorhan known as the mountain region of Bursa province, Turkey with the purpose of determining factors affecting farmers' use of artificial insemination in dairy farming. The number of the farms was determined using a stratified random sampling method. The data were collected from 252 dairy cattle farms between April and December 2018. The study employed the logit model to evaluate the data. The results revealed that the educational level of the farmers, dairy farming experience, the number of dairy cattle, livestock diseases, off-farm income, artificial insemination support and access to veterinary services variables had a positive effect on farmers' use of artificial insemination; whereas age and household size variables had a negative impact. As a result, the use of artificial insemination in dairy farm can be increased with farmers who are open to innovations regarding livestock activity and knowledgeable about artificial insemination.

Keywords: Artificial insemination, Dairy cattle, Income, Logit model

Introduction

Livestock sector has socio-economical contributions such as providing employment for households living in rural area, supplying raw materials to different sectors, and increasing national income (Aksoy et al., 2011). Thus, it is critically important for the sustainability of livestock activities that farms keep up with the recent changes by following innovations and developments. The methods and techniques used in livestock activities vary in time depending on the developments in the sector. However, to keep up with these changes, farmers need to give up old methods used in livestock and adopt the innovations in a short time (Sezgin et al., 2010). Furthermore, it is rather important in this sector to have fertile breeds, and to increase the number of the healthy animal. Therefore, it can be said that artificial insemination (AI) is one of the key practices in livestock sector. It is a technique aimed at improving all breeds of animals. Particularly used as a reproduction method in dairy farming, AI provides significant economic contributions to milk production and to farmers by genetically improving animals (Howley et al., 2012). In Turkey, AI efforts began in 1926, and was used for domestic cattle and sheep breeds from 1926 to1936. Both private sectors and public institutions were entitled to use AI as per the 1985 regulations. All these developments accelerated the studies related to this technique and it made widespread (Gençdal et al., 2015). In Turkey, it is estimated that the number of AI implemented in 2018 was about 2.8 million. Bursa province is the 4th biggest city of Turkey, located in the northwest of Turkey and the southeast of the Marmara Sea. The province has a great potential in terms of livestock and animal production due to its economic structure and geographical features. In Bursa province, there has been significant increases in the culture breeds in animal population, especially in the recent years. Bursa province ranks 28th in Turkey in terms of the number of bovine animals. In 2018, this

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ratio was 72.4% in Bursa province while the share of culture breeds (dairy cattle) was 48.9% in Turkey. In the same year, the number of AI was 81,146 in Bursa province (TURKSTAT, 2018). With its significant location for dairy farming, Bursa province includes the following districts, which are known as the mountain region: Harmancık, Keles, Orhaneli and Büyükorhan. The majority of the households in these districts live on agriculture and livestock (Karahan et al., 2015). Bovine animals in these four districts respectively constitute about 1.1%, 1.9%, 5.2% and 4.3% of the bovine animals in total in Bursa province. Therefore, four districts in the mountain region make significant contributions to livestock farming. Although there are many studies on AI in the literature, there is a limited number of studies aimed at determining of the factors affecting farmers' use of AI and their decision-making trends regarding this technique (Kaaya et al., 2005; Sezgin et al., 2010; Howley et al., 2012; Gençdal et al., 2015). Besides, there is no comprehensive study conducted in these four districts in relation to this subject. For this reason, it is important to evaluate the factors that have an impact on farmers' decision-making trends on the use of AI for improving dairy farming activities in the research area. Therefore, the purpose of the present study is to determine the factors that impact on farmers' use of AI in these four districts known as the mountain region. To elaborate on the issues that serve the purpose of the study, this study tests the following hypotheses:

H_0=Institutional services, socio-economical and farm characteristics have no significant effect on farmers' use of AI

H_1= Institutional services, socio-economical and farm characteristics have a significant effect on farmers' use of AI

The precautions to increase milk yield in dairy cattle farms, which constitute an important portion of the livestock sector, is essential for the sustainability of farms. Therefore, it is necessary to increase the number of healthy and fertile animals in farms. This study, which offers some significant insights into the factors affecting the farmers' use of AI, is of great importance for this research field. The results of this study will potentially contribute to livestock activities in Bursa province and its districts, and to other studies in the relevant literature.

Material and Methods

The Study Area and Sample Size

This study was based on the data obtained from dairy cattle farms in the districts of Harmancık, Keles, Orhaneli and Büyükorhan of province of Bursa in Turkey, which are known as the mountain region, through survey method between April and December, 2018. The data were collected by face-to-face interviews with farmers. A total of 3,505 farms involved in dairy farming activities were the target population of the research. The number of animals in farms was considered in determining the sample size. The farms were selected by the stratified random sampling method. The sample size was calculated using the Neyman method (Sezgin et al., 2010; Yamane, 1967). This method is as follow;

$$n=\frac{(\sum N_h\ S_h)^2}{N^2D^2+\sum N_h S_h^2} \quad , \qquad D^2=d^2/z^2 \qquad [1]$$

where, n is the sample size (252 farms), N is the number of dairy cattle farms in the districts (3,505 farms), N_h is the number of dairy cattle farms in the h stratum; S_h is the standard deviation for the h stratum, S_h^2 is the variance for h stratum, d is desired absolute precision, z is desired confidence level (1.96 for 95%), D is acceptable error limit in population mean. Farms were divided into three groups as 2 to ≤11 cattle (67 farms), 12 to <21 cattle (53 farms) and equal 22 and >22 cattle (132 farms). The total sample size was calculated as 252 dairy farms. However, all farms were included in the evaluation because there were not important differences between the farms in the strata.

Econometric Model

This study determined the socio-economical characteristics of the farmers through descriptive statistics. The logit model was also used in the identification of the factors affecting farmers' decision-making trends for the use of AI. Logistic regression is non-linear a model which can be linearization with designed transformations for the binary dependent variable, and dependent variable represents the occurrence or non-occurrence of an event (coded as 1 or 0). The logit model is one of methods used to estimate of the binary dependent variable models (Yohannes, 2014). The dependent variable that indicates the farmers' use of AI was divided into two response categories as the farmers who use AI (coded 1= event) and those who do not use AI (coded 0= no event) according to the logit model. The STATA statistical analysis program was utilized in the analysis of the data (StataCorp, 2005). This model is defined as follows:

$$P_i=\frac{1}{1+e^{-(\beta_0+\beta_i X_i)}}=\frac{1}{1+e^{-(Z_i)}} \qquad [2]$$

where, is dependent variable, P_i is the probability of use AI for the i[th] farmers and it ranges from 0-1, is constant term, =parameter to be estimated, X_i=independent variables. Based on the natural log of this equation (2), the following equation can be written:

$$L_i=\ln\left[\frac{P_i}{1-P_i}\right]=Z_i=\beta_0+\beta_1 X_1+\ldots+\beta_k X_k \qquad [3]$$

McFadden's pseudo R-squared value and the likelihood ratio (LR) were calculated to the goodness of fit of the model and its explanatory power. McFadden's pseudo R-squared value can be as low as 0 but can never be equal to 1, and parameter values between 0.2 and 0.4 contain a reference to an appropriate model fit (Karahan et al., 2015). The likelihood ratio chi-square (LR chi^2) is defined as -2 (L_0- L_1), where L_0 describes the log likelihood for the constant only model and L_1 is the log likelihood for the full model with constant and predictors (Yohannes, 2014). When the LR statistic value of the model is greater than the value of chi-square, the null hypothesis (H_0) is rejected.

Definition of Variables

The dependent variable of the logit model has two response categories. To determine the dependent variable, the farmers were asked if they use AI or not. Accordingly, these results were combined into a dummy variable; if the farmer do not use AI, this is given the value of 0 (0=no event) and the value of 1 is given when the farmer uses AI (1=event). The independent variables included in the model were selected from some studies in the literature (Tefera et al., 2014; Gençdal et al., 2015). Age is one of the important demographic characteristics affecting farmers' behaviors. Because, farmers' preferences may change depending on their age. Old farmers are more likely to refuse innovations on agriculture and livestock compared to younger or middle-aged farmers to avoid the risks of new technologies (Yohannes, 2014**)**. Therefore, the increasing age is expected to negatively affect the farmers' use of AI. Education enhances the ability of farmers to use their knowledge (Paulos et al., 2004). Since the more educated farmers are, the more successful they are in adapting to innovations and developing skills. Therefore, it is expected that educational level of the farmers has a positive effect on the farmers' use of AI. Experience is an important factor for farmers. Because, the farmers with knowledge and experience in dairy farming activities may find it easier to adapt to new technologies (Yohannes, 2014**)**. Thus, it is expected that the farmers with more experiences are more likely to use AI and thus, experience in dairy farming may have a positive impact on the farmers' use of AI. The likelihood to use AI also depends on the increase in the number of animals and the income level of the farmers. Hence, the use of AI may decrease depending on the increase in the total number of dairy cattle, particularly in the farms with a low income. So, the higher the number of animals in these farms is, the less likely the farmers use AI. Yet, the farmers with a middle or high income and an additional income may prefer to use AI to maintain the health of their animals and to ensure herd management (Yohannes, 2014**)**. Thus, the higher the number of animals in these farms is, the more likely the farmers use AI. It is expected that the number of dairy cattle may have a positive or negative impact on the farmers' use of AI. Household size plays an important role in the decision-making process of the farmers on the use of any technological innovations or practices in their farms. Since the increase or decrease in the number of family members in a household can affect the tendencies of the farmers towards the adoption of new technologies (Bamire et al., 2002). Particularly small family farms are by nature subsistence farms. Therefore, when the number of family member in a household increases, the farmers use their income to meet the needs of these family members rather than buying new technologies. Hence, it is expected that the number of the farmers using AI decreases depending on the increase in the number of family members in the household. Livestock diseases are substantially important for dairy cattle farms because farmers often face with various animal diseases in their farms. Farmers encounter problems such as loss of income and production due to animal deaths caused by diseases. Therefore, it is expected that the farmers use AI more often to maintain herd management and to prevent animal diseases in their farms. Thus, in this model, the variable of livestock diseases takes values as 1 for the farmer faced with epidemic diseases and as 0 for those who do not. Farmers need an off-farm income in order to boost their income level. Additional income can enhance the financial strength of farmers and the opportunities to use new technologies (Yohannes, 2014**)**. Therefore, farmers may follow innovations on livestock depending on the increase in income in their farms. Hence, the likelihood of the farmers with an off-farm income to use AI is expected to be high. Thus, in this model, the variable of off-farm income takes values as 1 for the farmers with an off-farm income and as 0 for those who do not. It can be said that farmers benefit from various livestock supports. AI is one of these supports that benefit by farmers. This support is key in ensuring the sustainability of livestock activities and protecting the farmers. Because, it can significantly increase the animal production in farms. Thus, the farmers who benefit from this support are expected to have an increasing tendency to use AI. Therefore, in this model, the variable of artificial insemination support takes values as 1 for the farmers who benefit from this support and as 0 for those who do not. Having access to veterinary services is an important for a healthy and productive livestock farming (Oladele et al., 2013). Since these services can reduce the productivity losses and economic losses in production. For this reason, farmers need to easily access to veterinary services. Thus, it is assumed that the farmers' use of AI may increase with the provision of easy access to veterinary services. Therefore, in this model, the variable of access to veterinary services takes values as 1 for the farmers who have easy access and as 0 for those who do not.

Results and Discussion

In this study, the ratio of the farmers who use AI was 85.3% and those who do not use AI was 14.7%. Similarly, the ratio of the farmers using AI was determined as 49%, 50.3%, 54.5%, 75.2% and 56%, respectively, in some studies (Howard and Cranfield, 1995; Aksoy et al., 2011; Özyürek et al., 2014; Gençdal et al., 2015). According to these results, it can be said that the ratio of AI use in the four districts known as the mountain region is higher than the ratio found in previous studies. This result shows that the tendencies of the farmers to use AI are high and they hold positive opinions regarding this technique. The socio-economical characteristics of the farmers were explained by descriptive statistics (Table 1). It was found that 38.1% of the farmers were aged between 26-36 and the average age was 40.8. In studies conducted in province of Van in Turkey and Uganda, the average age of the farmers was reported to be 45.0 and 44.5, respectively (Kaaya et al., 2005; Gençdal et al., 2015). Thus, it can be said that the results of this study are congruent with the findings of previous studies. Educational level is one of the most important demographic features affecting the farmers' behaviors, and their preferences may change depending on their educational level. It was found that the majority of the farmers (72.2%) attended primary education and the average year of schooling was 6.2 in this study. In the studies conducted by Kaaya et al. (2005) and Gençdal et al. (2015), it is reported that the average year of schooling of the farmers was 10.3 years and 4.5 years, respectively. Therefore, the average year of schooling of the farmers in this study is lower than the value reported by Kaaya et al. (2005) and higher than the value explained by Gençdal et al. (2015). Also, 55.6% of the farmers had a household size of 3-4 persons and the average household size was 3.1 persons. Thus, it can be said that this value is below the average household size (3.5 persons) at the national level. It was found that 58.7% of the farmers had a dairy farming experience of 11-20 years and the average experience was 15.1 years. In studies conducted by Howard and Cranfield (1995), Kaaya et al. (2005) and Gençdal et al. (2015) found that the dairy farming experience of the farmers was 16.5 years, 19 years and 25 years, respectively. Thus, the results of this study on the dairy farming experience are lower than those obtained from previous studies. Furthermore, it was found that 15.1% of the farmers had the highest income (≥€5512).

Table 1. Socio-economical, farm characteristics and institutional services (n=252)

Characteristics	Frequency	%	Mean	**SD
Farmers' age (year)				
26-36	96	38.1	40.78	9.85
37-47	86	34.1		
48-58	60	23.8		
≥59	10	4.0		
Education level (year)				
Primary school graduate	182	72.2	6.25	2.29
Secondary school graduate	42	16.7		
High-school graduate	23	9.1		
University graduate	5	2.0		
Household size (person)				
≤2	80	31.7	3.10	1.05
3-4	140	55.6		
≥4	32	12.7		
Farmers' dairy farming experience (year)				
≤10	59	23.4	15.10	5.59
11-20	148	58.7		
≥20	45	17.9		
*Household income (€ $year^{-1}$)				
≤€2718.5	66	26.2	€3639.8	1036
€2719-€5511.8	148	58.7		
≥€5512	38	15.1		
Number of dairy cattle (head)				
2≤11	67	26.6	21.38	14.70
12-21	53	21.0		
≥21	132	52.4		
Livestock diseases				
Yes	181	71.8	0.72	0.45
No	71	28.2		
Off-farm income				
Yes	204	81.0	0.81	0.39
No	48	19.0		
Artificial insemination support				
Yes	229	90.9	0.91	0.29
No	23	9.1		
Access to veterinary services				
Yes	224	88.9	0.89	0.31
No	28	11.1		

*1 Euro=5.31 TRY (Turkish lira) in June 2018, **SD=Standart deviation

In this study, the McFadden's pseudo R-squared value and likelihood ratio (LR) were calculated to test the goodness of fit of the established model and its explanatory power. The LR and chi-square statistic (chi^2) values were calculated as 72.15 and 16.92, respectively. The null hypothesis at 5% significance was rejected because the LR value was found to be greater than chi^2 value. The McFadden's pseudo R-squared value was calculated as 0.34. These results revealed that the model is statistically significant and fits for the study (Table 2).

Table 2 presents the parameter estimates of the logit model employed to identify the factors affecting the farmers' use of AI. According to these results, it was found that age had a negative effect on farmers who use AI at 1% level of significance. Hence, it can be said that institutional services, farm and socio-economical and farm characteristics have a significant effect on the farmers' use of AI. Thus, a one-year increase in the age of the farmer would decrease the likelihood that the farmer uses AI by 9.92%. The relevant results revealed that old farmers are more likely to refuse innovations on livestock compared to their young or middle-aged counterparts. In this regard, the majority of the farmers using AI were young and middle-aged, and the variable of age was effective in the preferences of farmers on new practices. Thus, these results are congruent with the findings from the studies of Sezgin et al. (2010) and Howley et al. (2012), but are not with those from Howard and Cranfield (1995), Tambi et al. (1999) and Kaaya et al. (2005), which indicated that there was a positive relationship between the farmers' age and the use of AI. Therefore, it is essential that old farmers in the research area are informed on AI to ensure herd management and to enhance their yields from dairy cows and their tendencies to use such techniques are increased.

Table 2. Factors affecting farmers' use of artificial insemination

Variables	Coefficient	Standart Error	z-statistic	p-value>|z| (probability)	Marjinal Effects
Age of farmers	-0.2159	0.0386	-5.59	0.000**	-0.0992
Educational level	0.4710	0.1355	3.48	0.001**	0.0216
Dairy farming experience of farmers	0.1282	0.0514	2.50	0.013*	0.0059
Number of dairy cattle	0.0514	0.0191	2.70	0.007**	0.0024
Household size	-0.8953	0.2541	-3.52	0.000**	-0.0411
Livestock diseases	1.3958	0.5255	2.66	0.008**	0.0884
Off-farm income	1.1030	0.5210	2.12	0.034*	0.0707
Artificial insemination support	2.1729	0.6525	3.33	0.001**	0.2277
Access to veterinary services	1.5044	0.6486	2.32	0.020*	0.1208
Constant	3.3771	1.3780	2.45	0.014*	

McFadden's pseudo R-squared =0.34	log likelihood (L_0)= -105.12499
log likelihood (L_1)= -69.05208	likelihood ratio (LR)=72.15
Prob>chi square (chi^2)=0.000(Probability)	$LR>chi^2(9)_{(0.05)}$ = 72.15>16.92

The levels of significance: $^{*}p<0.05$; $^{**}p<0.01$

Educational level had a positive influence on the farmers using AI at 1% level of significance. Thus, a one-year increase in the school education of farmers would increase the probability of the use of AI by 2.16%. These results show that the number of the farmers using AI may increase depending on increase in their education and knowledge level. These findings are consistent with the findings of the study conducted by Gençdal et al. (2015), which concluded that there was a positive relationship between the farmers' educational level and the use of AI, but are not congruent with those of Tefera et al. (2014). Thus, AI practices are preferred by the farmers with a high educational level more than others in the farms in the research area.

The dairy farming experience had a positive influence on the farmers using AI at 5% level of significance. Thus, a one-year increase in dairy farming experience would increase the probability of the use of AI by 0.59%. The preferences of the farmers on AI may change due to the increase in their knowledge and experiences depending on their dairy farming experiences. Similar findings were obtained from study conducted in Kenya (Tambi et al., 1999). The findings of this study are not congruent with the results of the studies conducted in Uganda and in Canada, which reported that there was a negative relationship between the dairy farming experiences of the farmers and the use of AI (Howard and Cranfield, 1995; Kaaya et al., 2005). Thus, the probability of the use of AI may increase with the improved knowledge and skills due to increased dairy farming experiences in the farms.

The number of dairy cattle had a significant positive influence on the farmers who use AI at 1% level of significance. Thus, a one-unit increase in the number of dairy cattle would increase the probability of the use of AI by 0.24%. Therefore, it can be said that AI is preferred more by the farmers with a middle and high income, as well as an additional income, compared to other farmers. Although these results are not congruent with the findings of Tefera et al. (2014), which indicated that there was a negative relationship between the number of dairy cattle that the farmers had and the use of AI, the present results are similar with the findings of the study conducted in Ireland (Howley et al., 2012). Thus, it is expected that an increase in the number of the farmers using AI to increase milk yield and to protect the health of animals in the farms depends on the increase in the number of dairy cattle.

Household size had a significant negative effect on the farmers using AI at 1% level of significance. Thus, an increase in the household of the farmers by one person would decrease the probability of the use of AI by 4.11%. Thus, the larger the household is, the less likely the farmers use AI. When the number of family member in a household increases, the farmers use their income to meet the needs of these family members rather than buying new technologies. Therefore, household size is significant for the farms. Similar results were obtained by Asfaw et al. (2011). The results of the present study are not congruent with the results of Aksoy et al. (2011), which indicated that there was a positive relationship between the number of persons in the family and the use of AI. Thus, the negative effects of household size on the adoption of innovations by the farmers may decrease with the precautions taken to increase the income level and milk yield of the farmers in the farms.

Livestock diseases had a significant positive effect on the farmers who use AI at 1% level of significance. Thus, the probability of the use of AI increases by 8.84% for the farmers who face with epidemic diseases compared to others. Because, it can be said that farmers take various measures to protect the herd health in their farms. AI, which is one of these measures, may be more preferable by farmers who have encountered with epidemic diseases, and so the probability of the use of AI is higher in the farms that have faced with epidemic diseases. Because, losses of production and income negatively affect the income of farmers.

Off-farm income had a significant positive effect on farmers the using AI at 5% level of significance. The probability of the use of AI increases by 7.07% for the farmers with an additional income compared to others. According to these results, off-farm income had a significant positive effect on the farmers using AI. In other words, the farmers who have an off-farm income can increase likely to use AI. Because, these income can increase the tendencies of the farmers to use new technologies (Yohannes, 2014). Therefore, farmers need off-farm income. These results are in line with the findings of Mal et al. (2012) and Beshir et al. (2012). Thus, most of the farmers in the research area have an off-farm income besides an on-farm income.

Artificial insemination support had a significant positive effect on the farmers who use AI at 1% level of significance. The probability of use of AI increases by 22.77% for the farmers who receive such support compared to others. According to these results, artificial insemination support had a significant positive effect on the farmers who use AI. In other words, the farmers who benefit from this support are expected to have an increasing tendency to use AI. Furthermore, AI is critical to protect consumers and ensure sustainable livestock activities. One of the most important purposes of this support is to increase profitability. For this reason, AI is required to convert the existing breeds into high-yielding breeds (Demir and Yavuz, 2010). Thus, benefiting from this support increases the number of the farmers using AI.

Access to veterinary services had a significant positive effect on the farmers using AI at 5% level of significance. Thus, the probability of the use of AI increases by 12.08% for the farmers who have easy access to these services compared to others. According to these results, access to veterinary services had a significant positive effect on the farmers using AI. Veterinary services prevent diseases from spreading to animals, and from animals to humans (Oladele et al., 2013). Therefore, it can be argued that it is key for farmers to benefit from these services. Gaining an easy access to veterinary services means that farmers an also easily benefit from AI service. The results of the present study are not congruent with the findings of Gençdal et al. (2015), which indicated that there was a negative relationship between the distance from farm to the district and the possibility of AI use. Therefore, providing an easy access to veterinary services can increase the number of the farmers using AI.

Conclusion

In conclusion, it is essential to make AI more popular in the districts of Bursa province that old farmers are informed on AI and its importance, that financial capacities are reinforced by increasing the income obtained from off-farm activities, and that farmers are provided with the access to veterinary services in the shortest time possible and the supports on AI are improved. In other words, the farmers who are knowledgeable on AI, open to innovations on livestock activities, and aware of the fact that AI is key for a profitable farming can reduce the ratio of low-yielding breeds and increase the use of AI in farms.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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How does the environmental knowledge of Turkish households affect their environmentally responsible food choices? The mediating effects of environmental concerns

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Abstract

The main objective of the present study is to examine whether Turkish households' environmental knowledge effects environmentally responsible food choices through the mediating effect of households' environmental concerns. A face-to-face survey was conducted, resulting in 450 responses from households that have recently chosen environmentally responsible foods in Erzurum, Turkey. The hypotheses were tested using the partial least squares-based structural equation modelling (PLS-SEM) technique to detect how the mediating role of environmental concerns plays in the relationships between environmental knowledge and households' environmentally responsible food choices. The findings confirm that environmental knowledge and environmental concerns positively and significantly enhance households' environmentally responsible food choices. It is also the first study to examine the mediational effect of environmental concerns on environmental knowledge and households' environmentally responsible food choice relations. The findings of the study and its implications are expected to benefit the development of environmentally responsible foods in the Turkish food industry.

Keywords: Environmental knowledge, Environmental concerns, Environmentally responsible food choices

Introduction

In recent years, households' interest in environmentally responsible foods has increased significantly. For example, organic food consumption on a global scale reached $97 billion in 2017 (Willer and Lernoud, 2019). It is estimated that this figure will increase to approximately $323.56 billion by 2024 (Chauke and Duh, 2019). Ninety per cent of environmentally responsible foods are demanded by developed countries (Chauke and Duh, 2019). Environmentally responsible food awareness and demand for these products are also increasing in developing countries (Asif et al., 2018). For example, many stores have recently opened in Turkey which sell environmentally responsible foods like organic and eco-labeled. This can be considered as an important indicator of the increase in households' interest in environmentally responsible foods.

On the other hand, according to the European Commission (2005) "food consumption is one of the most important areas where environmental sustainability can be improved as it is responsible for one third of a household's total environmental impact" (cited in Eldesouky et al., 2020, p. 65). There are many studies in the literature on environmental information, environmental concerns and environmentally responsible food choices (e.g. Haron, Paim and Yahaya, 2005; Peschel et al., 2016; Suki, 2016; Zareie and Navimipour, 2016; Lin and Niu, 2018). However, most of these studies have focused on developed countries as the demand for environmentally friendly food is in developed countries. However, the widespread use of environmentally responsible foods in developing countries

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increases the research need on this subject in developing countries. In addition, studies on households' environmentally responsible food choices in Turkey, which is an important food manufacturer, are expected to provide important information.

The current study aims to fill a gap in the literature about the relationship between environmental information, environmental concerns and households' environmentally responsible food choices in Turkey. Previous studies have examined direct and indirect relationships between environmental knowledge and sustainable choices (e.g. Haron et al., 2005; Pescel et al., 2016), environmental knowledge and pollution concerns (e.g. Tong et al., 2020), and environmental concerns and purchase intentions (e.g. Shamsudin et al., 2018). The current study provides the first empirical evidence of the direct relationship between environmental knowledge and environmentally responsible food choice. In addition, the role of environmental concerns as a mediator variable was examined for the first time in the relationship between environmental knowledge and environmentally responsible food choice.

Construct definition and research hypotheses

Households' environmental knowledge is believed to be a determinant of their environmentally responsible food choices. Furthermore, households' environmental concerns may enhance the impacts of environmental knowledge on their environmentally responsible food choices. Therefore, environmental concerns should be considered as a mediator variable in the research framework (see Fig. 1). Environmental concerns are thus the third variable that represents the reproductive mechanism through which the centric independent variable (i.e. environmental knowledge) is able to affect the dependent variable (i.e. environmentally responsible food choices) in this hypothetical relation.

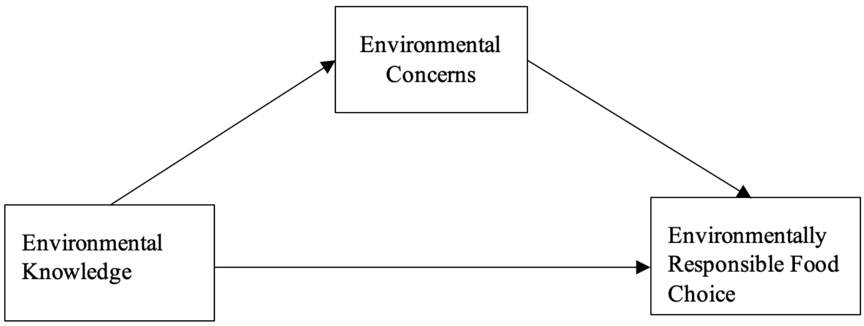

Figure 1. The research framework

Environmentally responsible food choice directly affects households' quality of life. In this respect, it can differentiate from other sustainable consumption dimensions (i.e. waste generation and recycling, personal transport choice, residential energy demand, residential water use) (Başar, 2018). For example, reducing household energy demand may not directly affect individuals' quality of life, or even consuming less energy can be perceived as a factor that decreases the quality of life. However, environmentally responsible food choice improves the quality of life by helping households to protect their own health (Kareklas, 2014). In this regard, it can be said that environmentally responsible food choice has emerged as a result of individuals' concerns about health. When individuals do not choose environmentally responsible foods they are primarily affected by the consequences of this (Saxe, 2014).

Previous research on environmentally responsible behavior has assessed various factors that influence such behavior, such as environmental knowledge. The importance of knowledge in consumers' environmentally responsible behavior has been examined in numerous studies. For instance, Thøgersen et al. (2010) have examined the effect of consumer knowledge relative to consumers' environmentally responsible behavior. Especially, consumers who had at one time purchased environmentally responsible products have been observed to show a greater likelihood of choosing products with a lower carbon footprint. According to Safari et al. (2018), consumer knowledge about an issue impacts significantly upon decision making. In particular, people dislike, and hence tend to abstain from situations where they do not have enough information to guide their behavior and where there is a high probability of confusion. This explains why some people may prefer not to accept sustainable consumption activities such as involvement in reducing residential energy demand because they feel they do not know enough about reduction (Haron et al., 2005). On the other hand, Diamantopoulos et al. (2003) argue that consumers having high-level environmental knowledge may not necessarily lead to the development of positive environmentally friendly behaviors. It has also been discussed, moreover, that there was not a significant correlation between environmental knowledge and environmentally responsible behavior (Oğuz et al., 2010).

As seen, there is no consensus in the literature as to how environmental knowledge affects environmentally responsible behavior. Therefore, more empirical evidence is needed on this issue.

Against this background, this study aims to assess the impact of environmental knowledge on environmentally responsible food choices via one hypothesis:

H1: Households' environmental knowledge directly and positively effects their environmentally responsible food choices.

Environmental concerns

Another important motivational factor towards households' environmentally responsible food choices is environmental concerns. According to Hines et al. (1987), the basis of environmental researches is the individual's concerns for the environment (cited in Kim and Choi, 2005, p. 593). Environmental concerns, referring to an individual's general orientation toward environmental issues, have been found to be a useful predictor of environmentally responsible behavior (Chen, 2019; Saleh and Danmaliki, 2020; Sanchez-Sabate and Sabaté, 2019; Shamsudin et al., 2018). Ling (2013), in his paper, has analyzed the intentions of young consumer groups to buy eco-friendly food products. The findings showed that young consumers demonstrated more altruistic motivations and more concern towards eco-friendly foods. Environmental concerns should be included as a mediator that intervenes with the independent variable (i.e. environmental knowledge levels) and the dependent variable (i.e. environmentally responsible food selection). The basic reasoning behind this is that though households may have high-level environmental knowledge, environmentally responsible food may not be easily chosen if households cannot objectively or will not subjectively undertake environmental concerns. Some people may have high-level environmental knowledge but are not willing to consume environmentally responsible foods due to the higher price of these foods (Başar, 2018; Chen, 2009). In other words, although environmental knowledge and environmental concerns are correlated, some people still follow an environmentally unconcerned lifestyle even if they have high-level environmental knowledge (Chen, 2009). This means that the positive relationship between environmental knowledge and environmentally responsible food choices will be enhanced if households have environmental concerns.

Based on the above, the following hypotheses are proposed:

H2: Households' environmental knowledge directly and positively effects their environmental concerns.

H3: The positive relationship between households' environmental knowledge and environmentally responsible food choices is mediated by households' environmental concerns.

Research method

Questionnaire design

The survey instruments for each of the constructs were designed to gather exhaustive details and were adapted from the literature, including environmental knowledge - 14 items (Haron et al., 2005); environmental concerns -five items (Kim and Choi, 2005); and environmentally responsible food choice - seven items (Başar, 2016). The seven-point Likert-type scale was used to measure the responses.

Survey administration

This research was based on a face-to-face survey. The survey was conducted with 450 randomly chosen households of Erzurum, Turkey. To qualify for this survey, respondents had to buy environmentally responsible foods such as organic and eco-labeled food products and/or dairy products from local producers in recent months (≤12 months) and had to be at least 18 years old.

Data analysis

Partial least squares-structural equation modelling (PLS-SEM) was used to determine the causal relationships for theory confirmation in this study. In the analysis of the data, the SmartPLS 3 and SPSS 25 software was used.

Results

The sample consisted of 230 males and 220 females. The sample's average age was 41 (standard deviation = 9.78). A large percentage of the samples were married (90%). The average monthly income of samples was 6000 TL (standard deviation = 10.65). Most households (76%) had undergraduate degree qualifications.

The Cronbach's alpha (CA) value is more than 0.75, indicating good internal consistency for each of the constructs. Composite reliability values for all the constructs were satisfactory (>0.65). Both test results confirmed the internal consistency and reliability of the measures.

By examining the outer loadings of the items in each of the constructs, convergent validity was ensured.

According to Hair et al. (2014), an outer loading <0.40 should be eliminated and values >0.70 are acceptable. For all that, all item loadings were statistically significant (t-value >1.96; Fig. 2).

Examining the Fornell-Larcker criterion, the heterotrait-monotrait (HTMT) ratio and cross-loadings of items were used to ensure discriminant validity. First, the Fornell-Larcker criterion was used. Accordingly, the square root of the AVEs of all constructs should be greater than the highest correlation value for other constructs. Table 1 shows convergent and discriminant validity test results (Hair et al., 2014).

As seen in Table 1, the square root of the AVEs of all constructs should be greater than the highest correlation value for other constructs (Sultan et al., 2020). Thus, discriminant validity has been established.

Second, the heterotrait-monotrait ratio of correlations (HTMT) was also used in assessing the discriminant validity of reflective constructs.

As seen in Table 2, none of the HTMT values of the constructs exceeded 0.90 (Henseler et al., 2015). Hence, discriminant validity has been established between reflective constructs.

Third, the "cross-loadings of items (Appendix 1), where item loadings for their own constructs are relatively higher than loadings for the other constructs, confirming the discriminant validity of the items" (Sultan et al., 2020: 5).

Fig. 2 shows that environmental concern explains 64% of the variance of environmental knowledge and that environmentally responsible food choice explains 13% of the variance of the independent constructs. Both constructs are statistically significant ($p < 0.05$). The normalized fit index is 0.75 (value closer to 1). The standardized root mean square residual value is 0.06 (value <0.08). The goodness-of-fit value, the square root of the product of average AVE and average R^2, which is 0.498 (value >0.36), is considered satisfactory for model fit indices, confirming the predictive validity (Hair et al., 2014).

The relationships among the three main variables must be tested and satisfy the following four conditions: (a) environmental knowledge (the independent variable) significantly influences environmentally responsible food choice (the dependent variable); (b) environmental knowledge significantly influences environmental concerns (mediator); (c) environmental concerns (the mediator variable) significantly influence environmentally responsible food choice (the dependent variable); and (d) the impact of the independent variable on the dependent variable must be reduced or must become statistically insignificant after controlling for the effect of the mediator. If the first three conditions are significant and the relationship between environmental knowledge and environmentally responsible food choice is still significant but reduced, this is called "partially" mediated. But if the relationship between environmental knowledge and environmentally responsible food choice is not significant, the effect of environmental concerns is called "fully" mediated.

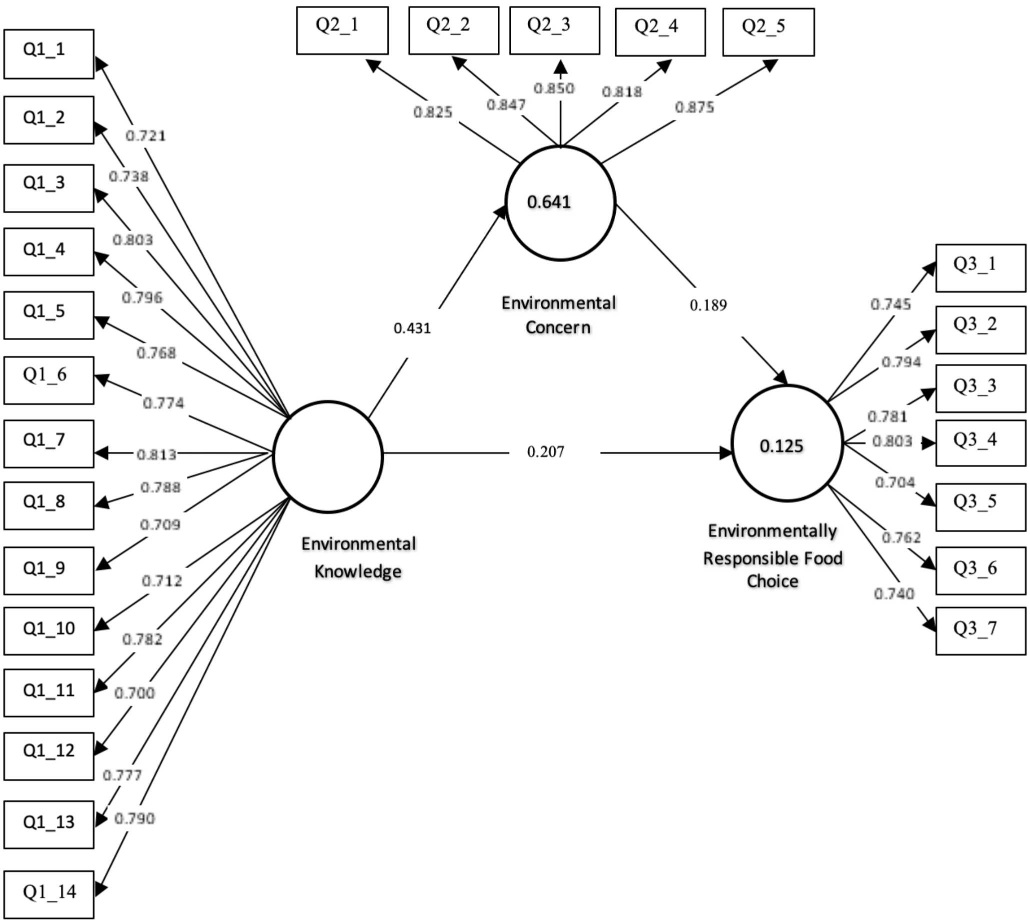

Figure 2. The structural model with outer loadings and path coefficients

Table 1. Convergent and discriminant validity test results

	CA	CR	AVE	01	02	03
Environmental Knowledge	0.852	0.965	0.601	0.775		
Environmental Concern	0.870	0.959	0.693	0.768	0.832	
Environmentally Responsible Food Choice	0.789	0.823	0.647	0.691	0.375	0.804

CA = Cronbach's alpha; CR = Composite reliability; AVE = Average variance extracted

Table 2. Heterotrait-monotrait ratio of correlations (HTMT)

	Environmental Knowledge	Environmental Concern	Environmentally Responsible Food Choice
Environmental Knowledge	-		
Environmental Concern	0.469	-	
Environmentally Responsible Food Choice	0.680	0.672	-

The results show that environmental knowledge positively effects environmentally responsible food choice (β = 0.21, p value <0.001), implying that H1 is supported. It is also found that environmental knowledge positively effects environmental concern (β = 0.43, p value <0.001), hence supporting H2. Also, environmental knowledge–environmental concern is stronger than other path coefficients in the model. All path coefficients are statistically significant, using all data set bootstrap samples in the 95% confidence interval (casual hypotheses are confirmed; Table 3).

Regarding the mediating effects of environmental concern on the association between environmental knowledge and environmentally responsible food choice, the indirect results show a significant indirect effect (β = 0.200, p value <0.05). This finding, along with the fact that the direct effect between environmental knowledge and environmentally responsible food choice is still significant but decreased, as in the basic model, suggests that environmental knowledge directly and indirectly affects environmentally responsible food choice via environmental concern. These results offer empirical support for H3, suggesting that the relationship between environmental knowledge and environmentally responsible food choice is partially mediated by environmental concern. Furthermore, Table 3 shows the mediating effect of environmental concern in the model.

Table 3. Hypotheses and mediation test results

Causal hypotheses		β	t	p	Results
H1		0.207	3.421	0.000	Accepted
H2		0.431	10.879	0.000	Accepted
Mediation test					
H3	Direct effect	0.200	3.402	0.001	Partial mediation effect
	Indirect effect	0.053	2.461	0.012	

Discussion, Limitations and Future Directions

The aim of the present study is to examine the mediating effect of households' environmental concerns on environmental knowledge and environmentally responsible food choice relationships. Thus, the current study proposed two causal and one mediating hypothesis. This study validated the research framework and found statistically significant results for all hypotheses, using the PLS-SEM technique. In summary, the results show positive and significant effects of environmental knowledge (β = 0.207, p <0.01) and environmental concern (β = 0.431, p <0.01) on environmentally responsible food choice, supporting H1 and H2. These results offer empirical support for the findings of related research (e.g., Bazoche et al., 2013; Bezawada and Pauwels, 2013; Scalvedi, 2018; Heo and Muralidharan, 2019; Tong et al., 2020), suggesting that households having environmental knowledge and environmental concern tend to choose environmentally responsible foods that in turn can lead to sustainable consumption.

The mediation test results show that environmental concern has a partial-mediation effect between environmental knowledge and environmentally responsible food choice relationships, confirming H3. This result offers novel findings for several reasons. Environmental knowledge itself is found to be sufficient in raising households' environmentally responsible food choices. However, the mediation test results clearly point to the importance of the indirect effect role, including that for environmental knowledge which raises households' environmentally responsible food choices through environmental concerns. Previous studies attempting to explain households' environmentally responsible food choices (e.g. Yu et al., 2014, Zhu et al., 2013) have been addressed in single-equation models and led to largely biased predictions. The PLS-SEM application used in the current study contributes to the literature methodologically by revealing two direct and one indirect pathway in which observed and latent variables interact in explaining households' environmentally responsible food choices (Tong et al., 2020). A possible explanation of the partial mediation may be due to the role of other factors, such as income, regulations, and/or best practices, in encouraging households to strengthen their environmentally responsible food choices.

This research supplies new evidences on the roles that households' environmental knowledge, and environmental concerns play in Turkish households' environmental responsible food choices. However, the sample size of the work is

Appendix 1. Cross-loadings of items

Items	Environmental Knowledge	Environmental Concern	Environmentally Responsible Food Choice
Q1_1 All living things play an important role in maintaining balance in the ecology	0.721	0.512	0.603
Q1_2 Natural resources should be conserved for future generations	0.738	0.500	0.614
Q1_3 The condition of our environment can affect our health	0.803	0.534	0.623
Q1_4 Destruction of forests will cause biological imbalances	0.796	0.525	0.701
Q1_5 There is an abundance of natural resources that can never be depleted	0.768	0.565	0.687
Q1_6 The main cause of air pollution in our country is fumes from vehicles	0.774	0.545	0.694
Q1_7 Most rivers in our country are polluted	0.813	0.516	0.642
Q1_8 Our country is faced with serious solid waste (garbage) and landfill problems	0.788	0.601	0.709
Q1_9 Alternative energy, e.g. solar energy can be utilized in place of electricity	0.709	0.574	0.638
Q1_10 The natural environment should be sacrificed in the name of development	0.712	0.541	0.687
Q1_11 Usage of disposable goods should be encouraged as it provides convenience to consumers	0.782	0.597	0.691
Q1_12 Unleaded petrol is better than leaded petrol as it is less harmful to the environment	0.700	0.571	0.672
Q1_13 Using public transport can help alleviate air pollution	0.777	0.634	0.688
Q1_14 Vehicles improperly maintained will cause pollution	0.790	0.593	0.675
Q2_1 I am extremely worried about the state of the world's environment and what it will mean for my future	0.698	0.825	0.532
Q2_2 Mankind is severely abusing the environment	0.691	0.847	0.593
Q2_3 When humans interfere with nature it often produces disastrous consequences	0.703	0.850	0.631
Q2_4 The balance of nature is very delicate and easily upset	0.661	0.818	0.633
Q2_5 Humans must live in harmony with nature in order to survive	0.671	0.875	0.644
Q3_1 I can pay more for organically grown food	0.602	0.645	0.745
Q3_2 I avoid consuming food with GMO (genetically modified organism)	0.590	0.632	0.794
Q3_3 I prefer to consume eco-label food	0.596	0.665	0.781
Q3_4 I am careful not to consume too much meat	0.613	0.687	0.803
Q3_5 I prefer to buy dairy products from local producers	0.601	0.674	0.704
Q3_6 I avoid consuming imported food such as a variety of exotic fruits	0.552	0.641	0.762
Q3_7 I avoid consuming canned "ready-made" food	0.568	0.662	0.740

limited and hence not representative of all Turkish households. Larger sample sizes could give more evidence in the generalizability of results. Furthermore, it would be interesting to apply the study with the same constructs to other countries or cultures. It could also be worthwhile to assess data using PLS-SEM, to determine further impacts, including the potential effects of demographic variables.

Our findings also shed light on one of the mechanisms through which households' characteristics may affect environmentally responsible food choice. Future research can build upon this relationship by examining other aspects of households' environmentally responsible food choice in addition to environmental knowledge. Moreover, the partial mediation of environmental concern suggests that there may be other behaviors that households endorse to improve environmentally responsible food choice. Hence, researchers may investigate other mechanisms, such as purchase intention and environmental education, to explain the environmental knowledge–environmentally responsible food choice relationship.

Conclusions

The analysis in this paper shows that the effect of households' environmental knowledge on environmentally responsible food choice is enhanced via the mediator variable (i.e. environmental concern). The application of PLS-SEM in this study provides a great depth of insight into the effect pathway that shows how households' environmental concern and environmental knowledge affect their environmentally responsible food choices. It is found that households' environmental knowledge is essential for forming environmentally responsible food choices, while their environmental concerns are direct and indirect additional drivers of a growing environmentally responsible food choice.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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PCA analysis on postharvest quality characterization of fenugreek depending on seed weight

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Abstract

In this study, the changes of quality characters of fenugreek according to thousand-seed weight (TSW) was examined and compared with Principal Component Analysis (PCA). Fenugreek seeds were separated into 5 different TSW ranges (W1, W2, W3, W4, W5). Different TSW had significant effects on crude protein content, palmitic, stearic, linoleic and linolenic acid contents and K, Ca and Mn contents. Linoleic acid had the highest content among fatty acid components. Increasing K and Mn contents and decreasing Ca contents were observed with increasing TSW. The highest values were obtained from W1 range in terms of crude ash and crude oil content, palmitic and linolenic acid, Ca, Mg, P, Na and S. At the end of the study, the W1 (10.00-13.00 g) range, which stands out in many quality features, can be recommended in fenugreek cultivation for high quality.

Keywords: Thousand-seed weight, Fenugreek, Fatty acids, Linoleic acid, Mineral composition

Introduction

Fenugreek is an annual medicinal and aromatic plant of *Fabaceae* family (Kaviarasan et al., 2007; Hassanzadeh et al., 2011). It has long been used as a traditional foodstuff (Pandey and Awasthi, 2015). Fenugreek plants have a gene center including South Europe, West Asia and Mediterranean basin (Tunçtürk and Çiftçi, 2011). Plant pods are about 15 cm long and include about 10-20 seeds in golden yellow color (Petropoulos, 2002; Pandey and Awasthi, 2015; Venkata et al., 2017). Plant seeds are used as spice or used in folk medicine (Gürbüz et al., 2000).

Protein, ash and oil contents are significant quality parameters of fenugreek plants. Fenugreek seeds contain about 27% protein, 3-4% ash, 8% crude oil and trace amounts of mucilage, trigonelline, diosgenin, choline, phytin, coumarin (Tunçtürk and Çiftçi, 2011). Besides high protein content, fenugreek seeds are also rich in calcium, iron and minerals (Pandey and Awasthi, 2015). Plant seeds constitute a good source of protein both in human and animal nutrition (Gökçe and Efe, 2016). Seeds are also rich in saturated and unsaturated fatty acids. Sulieman et al. (2008) reported that fenugreek plants contained around 43.2% linoleic acid, 22.0% linolenic acid and 16.7% oleic acid.

It was indicated in previous studies that several morphological characteristics (plant height, number of branches, number of pods, number of seeds per pod, pod length, seed weight) of fenugreek plants were closely related to seed yield (Sade et al., 1996; McCormick et al., 2009). A yield parameter, TSW is among the most significant ones of these characteristics. Since fenugreek plants are generally grown for their seeds, high seed yields are desired in fenugreek plants. Previous studies put forth direct and positive impacts of TSW on yield (Ayanoğlu et al., 2004; Fikreselassie et al., 2012; Gurjar et al., 2016).

Starting from the positive effect of TSW on seed yield, besides the treatments to increase TSW in field studies, laboratory studies are also needed to elucidate the effects of TSW on

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quality attributes. Therefore, in this study, after harvesting, the changes of quality characters of fenugreek according to TSW was examined and compared with PCA analysis.

Materials and Methods

Materials

Seeds of Gürarslan fenugreek cultivar were used in this study. Seeds were collected from the cultivar field in July 2018 and preserved in sacks until separation of them into different seed weight.

Determination of Thousand Seed Weight Ranges

Following the harvest, the seeds preserved in sacks were separated into five different TSW (g) ranges (W1, W2, W3, W4 and W5). Each range was formed in three replicates. For TSW, 1000 seeds were counted, weighed in a precise scale and expressed on dry matter in g. Resultant ranges are provided in Table 1 and average of replicates of ranges are provided in Table 2.

Table 1. Thousand-seed weight (g) ranges formed from fenugreek seeds

Thousand-seed weight ranges	
10.00-13.00	W1
13.01-16.00	W2
16.01-19.00	W3
19.01-22.00	W4
22.01-25.00	W5

For chemical composition, crude protein, ash and oil contents of the seed groups were determined by AOAC (1990). Crude protein content was determined from 0.2 g ground fenugreek seeds with the aid of Kjeldahl method. Resultant nitrogen content was multiplied by 6.25 to get crude protein content. Crude ash content of fenugreek seeks was determined from 1 g ground samples through ashing the samples in an ash oven at 550 ºC for 4 hours. For crude oil quantities, 3 g ground samples of each group were used. Samples were analyzed with petroleum ether in a device. Crude oil content was calculated over dry matter. Chemical composition analysis were replicated three times for each group and average of resultant measurements were expressed in % (Table 2).

Fatty Acid Composition

Fatty acid methyl esters for samples were prepared using 1-step extraction-transesterification process (Sukhija and Palmquist, 1988). In the study, the analysis was performed on a GC device (Schimadzu, GC 2010 plus) equipped with a FID (flame ionization detector) detector and column (a 100 m fused silica capillary column - i.d. 0.25 mm). Helium was used as the carrier gas. Fatty acid methyl esters were separated using a temperature gradient program (Chilliard et al., 2013) and the peaks were identified based on comparison of retention times with authentic standard (Supelco #37, Sigma, Supelco Inc., Bellefonte, PA, USA). Fatty acid composition analysis were repeated three times for each group and average of resultant values was expressed in % (Table 3).

Mineral Composition

Mineral composition of fenugreek samples was determined in an ICP OES spectrophotometer (Inductively Couple Plasma spectrophotometer) (Perkin-Elmer, Optima 4300 DV, ICP/OES, Shelton, CT 06484-4794, USA). 10 ml of a mixture of nitric + perchloric acid was added to 0.5 g of ground fenugreek seed and subjected to wet digestion until it had 1 ml of sample. After the digestion process, distilled water was added to the obtained solutions and readings were made on the ICP OES spectrophotometer to determine the mineral contents (Mertens, 2005). Mineral composition analysis were repeated three times for each range and resultant values were expressed in % (Table 4).

Statistical Analysis

All analysis were performed in three replicates. SPSS Statistics (version 17.0) for Windows was used for statistical analysis of the experimental data. Data were subjected to one-way analysis of variance (ANOVA) and significant means were compared with the aid of Duncan's multiple range test ($p < 0.05$). PCA analysis was performed using PAST (version 3.20) (Hammer et al., 2001).

Results and Discussion

TSW average of each range is provided in Table 2. Variance analysis for TSW revealed that the experimental ranges were significant and fenugreek seeds were separated into 5 different weight ranges. TSW varied between 11.96 - 23.01 g (Table 2). In previous studies, Tunçtürk et al. (2011) reported TSW as 18.5 g in the first year and as 18.0 g in the second year; Ayanoğlu and Mert (1999) reported TSW as between 12.23 - 18.58 g. Present findings on TSW thus comply with those earlier ones.

Chemical Composition

Results for chemical composition of fenugreek seeds are provided in Table 2. Variance analysis on investigated traits revealed that crude ash and oil contents of the ranges were not significantly different ($p>0.05$). Crude protein content of the ranges was found to be significant ($p<0.05$).

Crude protein contents of the TSW ranges varied between 20.43-24.91%, crude ash content varied between 3.20 - 3.50% and crude oil content varied between 2.81 - 3.88%. The greatest crude protein content was observed in W3 range and the greatest crude ash content and crude oil content were observed in W1 range (Table 2). The lowest crude protein and crude oil contents were observed in W4 range and the lowest crude ash content was observed in W5 range (Table 2).

In previous studies about chemical composition of fenugreek plants, Ali et al. (2012) reported protein content as 19.8% and ash content as 4%; Al-Jasass and Al-Jasser (2012) reported protein content as 12.91%, ash content as 4.23% and crude oil content as 4.51%; Sulieman et al. (2008) reported protein content as 25.0%, ash content as 3.0% and crude oil content as 8.4%; Acharya et al. (2006) reported protein contents as between 26.0 - 31.6%; Tunçtürk et al. (2011) reported protein content as 23.3% in the first year and as 23.2% in the second year. Niknam et al. (2004) reported protein content as 29.93% and Beyzi (2020) reported crude oil content as between 4.01-4.95%.

Table 2. Thousand-seed weight averages and chemical composition of experimental ranges

Characters	Ranges										p
	W1		W2		W3		W4		W5		
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	
TSWA (g)	11.96[e]	0.21	14.83[d]	0.86	17.68[c]	0.57	20.22[b]	0.35	23.01[a]	1.28	*<0.05*
CP (%)	24.18[a]	0.17	24.10[a]	0.79	24.91[a]	0.50	20.43[c]	0.25	21.86[b]	0.70	*<0.05*
CA (%)	3.50	0.55	3.43	0.43	3.23	0.28	3.35	0.40	3.20	0.18	*NS*
CO (%)	3.88	0.50	3.14	0.62	3.14	0.02	2.81	0.78	3.60	0.49	*NS*

TSWA: thousand-seed weights averages of ranges; CP: crude protein content; CA: crude ash content; CO: crude oil content; SD: standard deviation; a-b: different letters indicate differences in same raw at $p<0.05$; NS: not significant

Fatty Acid Composition

Results for fatty acid composition of fenugreek seeds are provided in Table 3. According to variance analysis on fatty acid composition, while the differences in linoleic, linolenic, palmitic, stearic acids and UFA/SFA content of the experimental ranges were significant ($p<0.05$), the differences in the other components were not found to be significant ($p>0.05$).

In fatty acid composition analysis, 8 components were identified. Linoleic acid had the highest content among these components. This component (linoleic acid) was followed by linolenic acid and oleic acid, respectively. Linoleic acid of TSW ranges varied between 43.85 - 46.02%, palmitic acid varied between 8.43 - 8.95%, stearic acid varied between 4.24 - 4.67% and linolenic acid varied between 26.47 – 28.04%. The greatest linoleic acid was observed in W4 range. The greatest palmitic acid was observed in W1 range and a regular decrease was observed in palmitic acid with increasing TSW. Contrary to palmitic acid, the greatest stearic acid content was observed in W5 range and a regular increase was in stearic acid with increasing TSW.

Among the insignificant fatty acids, the greatest oleic acid was observed in W2 range, arachidic acid in W5 range, eicosenoic acid in W3 range and behenic acid in W2 range. The UFA/SFA contents of experimental ranges varied between 5.62 – 5.86% with the greatest value in W4 range and the lowest value in W1 range (Table 3).

In previous studies about fatty acid composition of fenugreek plants, Bieńkowski et al. (2017) reported linoleic acid as 37.9%, α-linolenic acid as 28.2%, oleic acid as 13.3%, palmitic acid as 13.1%, stearic acid as 3.8%, arachidic acid as 1.4%. Ciftci et al. (2011) reported linoleic acid as between 45.1 – 47.5%, α-linolenic acid as between 18.3 – 22.8%, oleic acid as between 12.4 – 17.0%, palmitic acid as between 9.8 –11.2% and stearic acid as between 3.8 – 4.2%; Ali et al. (2012) reported palmitic acid as 10.5%, stearic acid as 6.5%, oleic acid as 20%, linoleic acid as 42.5%, linolenic acid as 18%, arachidic acid as 2% and behenic acid as 0.5%; Sulieman et al. (2008) reported average palmitic acid as 11.0%, stearic acid as 4.5%, oleic acid as 16.7%, linoleic acid as 43.2% and linolenic acid as 22.0%. Dinu et al. (2013) reported average palmitic acid as 8.77%, stearic acid as 3.71% and linoleic acid as 43.15%. Beyzi (2020) reported linoleic acid as between 39.62-43.68%, linolenic acid as between 26.11-29.89%, oleic acid as between 11.60-14.10%, palmitic acid as between 8.94-9.50% and stearic acid as between 4.67-5.55%.

Mineral Composition

Results for mineral composition of different weight ranges are provided in Table 4. Of the macro elements, the differences in K and Ca contents of the experimental ranges were found to be significant ($p<0.05$), but the differences in Mg, P, S and Na contents were not found to be significant ($p>0.05$). For micro elements, Mn was found to be significant ($p<0.05$), but Cu, Fe, Zn and B were not found to be significant ($p>0.05$).

K contents of the TSW ranges varied between 8908 - 10356 mg/kg. Increasing K contents were observed with increasing TSW and the greatest value was observed in W5 range. Ca contents varied between 1353 - 1897 mg/kg. Ca contents decreased with increasing TSW and the greatest value was observed in W1 range. Mn contents varied between 6.31 - 12.15 mg/kg. Mn contents increased with increasing TSW and the greatest value was observed in W5 range (Table 4). For insignificant minerals, the greatest Mg, P, S and Na contents were observed in W1 range, the greatest Cu content was observed in W5 range, the greatest Fe and Zn contents were observed in W4 range and the greatest B content was observed in W2 range.

In previous studies about mineral composition of fenugreek plants, Bouhenni et al. (2019) reported average Ca, K, Mg, P, S, B, Cu, Fe and Zn contents respectively as 1445, 10605, 1229, 5143, 2648, 11.8, 9.9, 91 and 30.9 mg/kg, Ali et al. (2012) reported average Ca, Cu, Fe, Zn, K, Mg, P, Na and Mn contents respectively as 226, 5.4, 11.6, 4.4, 1080, 78.4, 200, 290 and 1.6 mg/100 g; Al-Jasass and Al-Jasser (2012) reported K, Mg, Ca, Zn, Mn, Cu and Fe contents respectively as 603, 42, 75, 2.4, 0.9, 0.9, 25.8 mg/100 g.

Table 3. Fatty acid composition of the thousand-seed weight ranges

Fatty acid composition (%)		Ranges										
		W1		W2		W3		W4		W5		
		Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	p
C16:0	Palmitic acid	8.95[a]	0.14	8.75[a]	0.08	8.72[a]	0.24	8.44[b]	0.03	8.43[b]	0.12	<0.05
C18:0	Stearic acid	4.24[b]	0.05	4.25[b]	0.07	4.35[b]	0.09	4.36[b]	0.10	4.67[a]	0.15	<0.05
C18:1	Oleic acid	12.47	0.18	12.63	0.54	12.28	0.30	12.48	0.54	12.56	0.05	NS
C18:2	Linoleic acid	43.85[c]	0.15	44.28[c]	0.40	45.35[ab]	0.25	46.02[a]	0.06	45.07[b]	0.71	<0.05
C18:3	Linolenic acid	28.04[a]	0.24	27.65[ab]	0.36	27.16[bc]	0.29	26.47[d]	0.36	26.94[cd]	0.49	<0.05
C20:0	Arachidic acid	1.26	0.02	1.25	0.01	1.08	0.18	1.19	0.07	1.31	0.05	NS
C20:1	Eicosenoic acid	0.53	0.02	0.52	0.01	0.62	0.21	0.46	0.02	0.56	0.01	NS
C22:0	Behenic acid	0.66	0.04	0.67	0.10	0.44	0.21	0.59	0.08	0.46	0.01	NS
SFA		15.11		14.92		14.59		14.57		14.87		NS
UFA		84.89		85.08		85.41		85.43		85.13		NS
UFA/SFA		5.62[b]		5.70[ab]		5.85[a]		5.86[a]		5.73[ab]		<0.05

SD: standard deviation; [a-b]: different letters indicate differences in same raw at $p<0.05$; NS: not significant; SFA: sum of saturated fatty acids; UFA: sum of unsaturated fatty acids

Table 4. Mineral composition of the thousand-seed weight ranges

Mineral composition (mg/kg)	Ranges										
	W1		W2		W3		W4		W5		
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	P
Macro minerals											
K	9779[ab]	363.67	8908[b]	1007.83	9943[a]	137.96	10166[a]	194.69	10356[a]	339.00	<0.05
Ca	1897[a]	40.43	1668[b]	259.31	1512[bc]	18.21	1431[c]	46.42	1353[c]	68.13	<0.05
Mg	992	26.94	862	106.69	904	55.79	904	34.63	880	8.16	NS
P	5605	116.56	5007	498.80	5526	453.99	5143	211.78	5216	221.04	NS
S	2080	245.57	1823	139.12	2075	133.13	2068	182.38	1917	242.79	NS
Na	166.15	10.17	148.29	23.60	149.17	9.71	155.71	4.75	147.33	12.30	NS
Micro minerals											
Cu	17.88	0.60	19.12	1.10	18.25	1.49	18.50	0.97	20.35	0.37	NS
Fe	62.91	4.53	72.81	15.24	73.48	4.17	78.88	13.51	60.23	9.56	NS
Zn	24.77	7.05	22.19	2.53	24.73	5.04	30.83	14.08	22.00	2.96	NS
B	1.29	0.13	1.48	0.25	1.15	0.13	1.21	0.04	1.17	0.25	NS
Mn	6.31[c]	0.77	9.23[b]	2.44	9.67[b]	0.38	10.33[ab]	0.88	12.15[a]	0.83	<0.05

SD: standard deviation; [a-b]: different letters indicate differences in same raw at $P < 0.05$; NS: not significant

Principal Component Analysis (PCA)

The eigenvalue and variability values resulting from the PCA analysis were given in Table 5. According to PCA analysis, the first two PCs explained 67.52% of the total variance. PC1 explained 42.50%, PC2 25.02%, PC3 19.26% and PC4 13.22% of the total variance. In Eigenvalue values, PC1 was 9.775, PC2 5.754, PC3 4.430 and PC4 3.041 (Table 5). It can be said that W1 range was separated from other TSW ranges since it located in a separate location (Fig. 1). The highest values were obtained from W1 range in terms of crude ash and crude oil content, palmitic and linolenic acid, Ca, Mg, P, Na and S (Table 2,3,4). For this reason, W1 range can be recommended in fenugreek cultivation for high quality.

Table 5. Eigenvalue and variance values formed as a result of PCA analysis

	Eigenvalue	Variability (%)
PC1	9.775	42.50
PC2	5.754	25.02
PC3	4.430	19.26
PC4	3.041	13.22

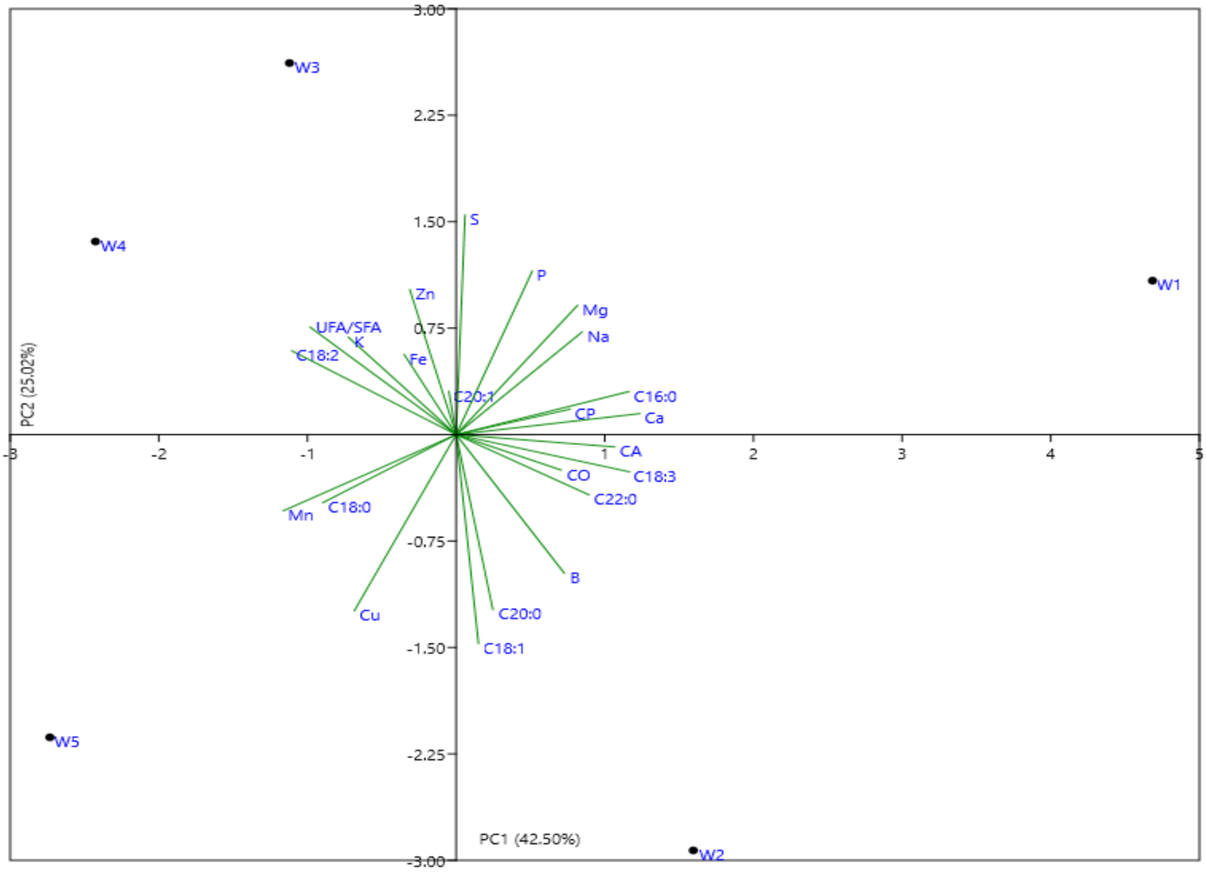

Figure 1. PCA graph of quality characteristics of fenugreek depending on thousand-seed weight; UFA: sum of unsaturated fatty acids; SFA: sum of saturated fatty acids; CP: crude protein content; CA: crude ash content; CO: crude oil content

Conclusion

In this study, the changes of postharvest quality characters of fenugreek according to TSW was examined and compared with PCA analysis. Different TSW had significant effects on crude protein content, palmitic, stearic, linoleic and linolenic acid contents and K, Ca and Mn contents. In this study, it has been determined that PCA analysis can be used for classification according to the quality characteristics of fenugreek depending on TSW. At the end of the study, the W1 (10.00-13.00 g) range, which stands out in many quality features, can be recommended in fenugreek cultivation for high quality.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Use of Water Quality Index to evaluate the groundwater characteristics of villages located in Edirne Province

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Abstract

In this research, water quality of groundwater of some villages located in Edirne Province of Turkey were evaluated by using Weighted Arithmetic Water Quality Index (WAWQI). Groundwater samples were collected from ten villages in winter season of 2019. Thirteen parameters including total dissolved solids – TDS, oxygen saturation – OS, salinity, pH, dissolved oxygen – DO, turbidity, nitrate – NO_3, electrical conductivity – EC, nitrite – NO_2, sulphate – SO_4, oxidation – reduction potential – ORP, phosphate – PO_4 and chemical oxygen demand – COD were measured in groundwater samples. According to detected data, groundwater of the region has 1. Class quality for dissolved oxygen, pH, nitrite, sulphate and COD parameters in general and has 2. Class quality for oxygen saturation, EC, nitrate and phosphate parameters in general. It was also determined that any investigated water quality parameter has not exceeded the drinking water standards. As a result of WAWQI, the values of overall WAWQI were recorded within the permissible limits (<100) and the groundwater quality of the region was found as "A grade" water quality characteristic.

Keywords: Edirne Province, Groundwater quality, Water Quality Index

Introduction

Water quality assessment has become an important mandatory on all over the world, because of increasing population and need of freshwater. One of the main points on an effective management of freshwater resources is monitoring quality of aquatic habitats (Solak et al., 2007; Çiçek et al., 2013; Tokatlı et al., 2014; 2016; Köse et al., 2014; 2016; Ustaoğlu et al., 2017; Atıcı et al., 2018; Onur and Tokatlı, 2020).

Water quality assessment indices are known as an effective tool to determine and evaluate the quality of water ecosystems. Weighted Arithmetic Water Quality Index (WAWQI), which is one of the most commonly used drinking water quality indices, is calculated from the perspective of suitability of drinking water for human consumption (Tyagi et al., 2013; Akter et al., 2016; Mukatea et al., 2019; Tokatlı, 2019; Ustaoğlu and Tepe, 2019; Ustaoğlu et al., 2020).

Edirne Province of Turkey is one of the most productive land because of nice soil structure and rich freshwater resources. But as in many aquatic ecosystems, surface and groundwater of the region is being adversely affected from agricultural and domestic discharges. The aim of the present investigation was to determine the groundwater quality of the villages located in the Edirne Province by determining some water quality parameters and evaluate the detected data according to Turkish Regulations Water Quality Classes and apply the WAWQI in order to assess the water quality in terms of drinking purposes.

Material and Method

Sample collection

In this research, groundwater samples were taken from ten villages located in the Edirne Province in winter season of 2019. The coordinate information of the stations is given in Table 1 and the map of study area and selected stations are given in Figure 1 and.

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Table 1. Coordinate information of villages

Villages	North	East
Büyükdöllük	41.759	26.601
Menekşesofular	41.763	26.640
Küçükdöllük	41.756	26.668
Kayapa	41.775	26.689
Korucu	41.791	26.655
Sinanköy	41.814	26.697
Çömlekakpınar	41.839	26.645
Hanlıyenice	41.870	26.695
Lalapaşa	41.839	26.736
Hıdırağa	41.738	26.661

Physical – chemical and statistical analysis

DO, OS, pH, EC, TDS, ORP and salinity variables were determined by using a multiparameter device (Hach Lange – HQ40D) during the field studies; turbidity variable was determined by using a portable turbidimeter device (Hach Lange – 2100Q) during the field studies; NO_3, NO_2, PO_4, SO_4 and COD variables were determined by using a colorimeter device (Hach Lange – DR890) and a spectrophotometer device (Hach Lange – DR3900) during the laboratory studies.

Cluster Analysis (according to Bray Curtis) was used in the present research by using the "PAST" statistical package program in order to classify the investigated locations according to similar water quality characteristics.

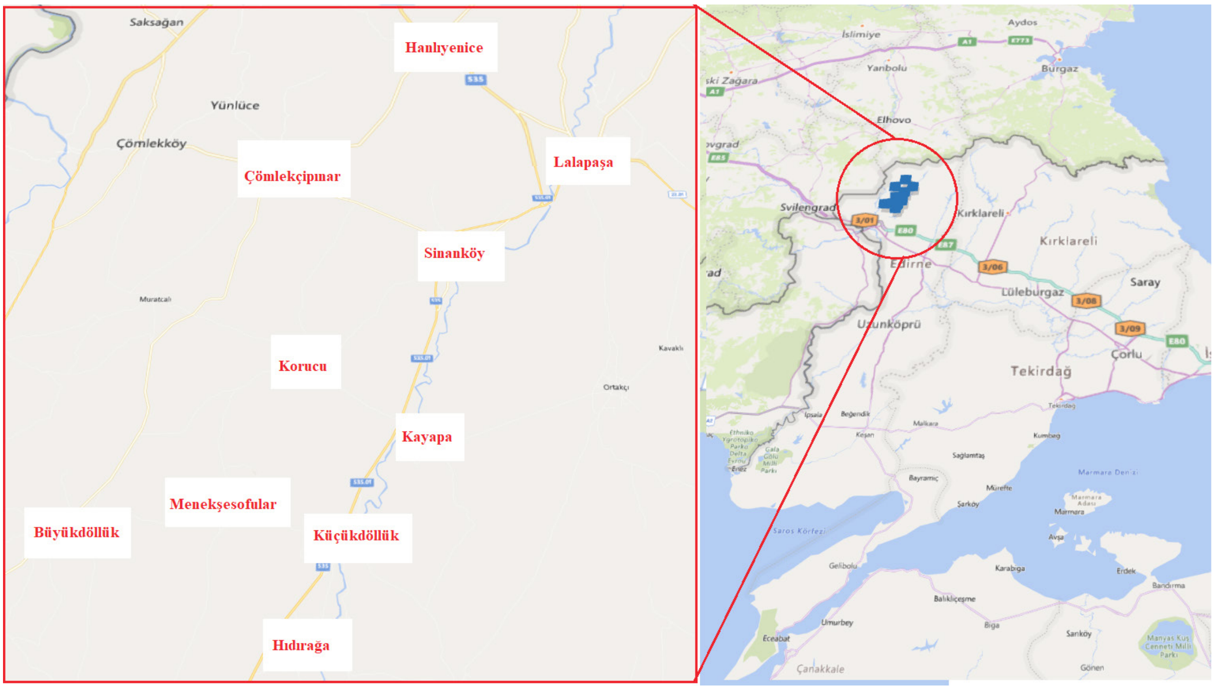

Figure 1. Study area and selected stations

Calculation of Weighted Arithmetic Water Quality Index

WAWQI calculation was made by using the following formula:

$$\text{WAWQI} = \sum QiWi / \sum Wi$$

The following formula was used to calculation of Quality rating scale (Qi):

$$Qi = 100[(Vi - Vo)/(Si - Vo)]$$

"Vi" is estimated accumulation of i^{th} variable, "Vo" is the best value of this variable, "Si" is recommended value of i^{th} variable.

The following formula was used to calculation of "Wi":

$$Wi = K/Si$$

The following formula was used to calculation of "K":

$$K = \frac{1}{\sum(1/Si)}$$

The quality classes of WAWQI is given in Table 2 (Brown et al., 1972).

Table 2. Water quality rating for WAWQI

WAWQI Value	Rating of Water Quality	Usage Possibilities	Grading
0 – 25	Excellent	Drinking, irrigation, industrial	A
25 – 50	Good	Drinking, irrigation, industrial	B
50 – 75	Poor	Irrigation, industrial	C
75 – 100	Very Poor	Irrigation	D
> 100	Unsuitable	Proper treatment is required	E

Results and Discussion

Results of detected physical – chemical parameters in villages located in the Edirne Province are given in Table 3. According to the Turkish Regulations (2004; 2015), groundwater of the region has 1. Class quality for dissolved oxygen, pH, nitrite, sulphate and COD parameters, in general and has 2. Class quality for oxygen saturation, EC, nitrate and phosphate, in general (Uslu and Türkman, 1987). Küçükdöllük village has 3. Class quality for nitrate parameter and Büyükdöllük village has 3. Class quality for nitrite parameter. It was also determined that any investigated station did not exceed the drinking water standards in terms of these parameters (TS266, 2005; EC, 2007; WHO, 2011).

The nitrate in water is caused by the oxidation of ammonia, which occurs as a result of the decomposition of proteins contained in animal and vegetable wastes, and especially nitrate fertilizers used in agricultural areas. A small amount of nitrate in clean waters is the most common form of nitrogen in streams (Wetzel, 2001; Manahan, 2011). Nitrite is an intermediate in biological oxidation from ammonium to nitrate, and it may have oxidized to nitrate or reduced to ammonia. It is mostly low in natural waters. Nitrite can reach high densities in low oxygenated waters with organic pollution and suggests sewage contamination if it is found in high amounts. The most important sources of nitrite in soils and waters are organic substances, nitrogenous fertilizers and some minerals (Wetzel, 2001; Manahan, 2011). The reason of the quite high nitrate and nitrite values detected in drinking water of some villages may be applied intensive agricultural fertilizers in the basin.

Table 3. Results of detected parameters

Villages	Parameters*												
	DO ppm	O_2Sat %	pH	ORP mV	EC mS/cm	TDS ppm	Sal ‰	Tur NTU	NO_3 ppm	NO_2 ppm	PO_4 ppm	SO_4 ppm	COD ppm
Büyükdöllük	9.33	81.6	7.47	206.3	568	404	0.41	0.42	7.90	0.081	0.059	22.0	1.00
	1. Class	2. Class	1. Class	-	2. Class	-	-	-	2. Class	**3. Class**	2. Class	1. Class	
Menekşesofular	9.52	84.3	7.66	202.3	598	416	0.42	1.50	3.99	0.003	0.047	21.7	0.03
	1. Class	2. Class	1. Class	-	2. Class	-	-	-	1. Class	1. Class	2. Class	1. Class	
Küçükdöllük	8.64	79.4	7.32	201.8	853	579	0.58	0.48	11.50	0.004	0.094	123.0	0.69
	1. Class	2. Class	1. Class	-	2. Class	-	-	-	**3. Class**	1. Class	2. Class	1. Class	1. Class
Kayapa	9.85	89.1	7.82	210.3	389	263	0.26	0.70	6.09	0.003	0.058	21.9	1.61
	1. Class	2. Class	1. Class	-	1. Class	-	-	-	2. Class	1. Class	2. Class	1. Class	1. Class
Korucu	9.71	87.5	7.47	192.6	448	305	0.31	0.41	2.29	0.005	0.036	30.4	0.59
	1. Class	2. Class	1. Class	-	2. Class	-	-	-	1. Class	1. Class	2. Class	1. Class	1. Class
Sinanköy	8.59	77.4	7.32	189.3	454	311	0.31	0.67	2.34	0.002	0.030	34.7	0.24
	1. Class	2. Class	1. Class	-	2. Class	-	-	-	1. Class	1. Class	2. Class	1. Class	1. Class
Çömlekakpınar	10.44	92.9	7.82	194.2	331	227	0.23	0.78	5.44	0.006	0.038	15.6	2.11
	1. Class	1. Class	1. Class	-	1. Class	-	-	-	2. Class	1. Class	2. Class	1. Class	1. Class
Hanlıyenice	9.26	82.4	7.58	168.4	711	498	0.50	3.29	0.90	0.008	0.066	127.0	2.03
	1. Class	2. Class	1. Class	-	2. Class	-	-	-	1. Class	1. Class	2. Class	1. Class	1. Class
Lalapaşa	9.83	86.6	7.84	187.3	374	261	0.26	0.48	7.83	0.006	0.035	26.9	3.64
	1. Class	2. Class	1. Class	-	1. Class	-	-	-	2. Class	1. Class	2. Class	1. Class	1. Class
Hıdırağa	10.47	92.8	7.96	176.5	582	405	0.41	0.65	7.18	0.001	0.104	38.6	2.27
	1. Class	1. Class	1. Class	-	2. Class	-	-	-	2. Class	1. Class	2. Class	1. Class	1. Class
min	8.59	77.4	7.32	168.4	331.0	227.0	0.23	0.41	0.90	0.001	0.030	15.6	0.03
mak	10.47	92.9	7.96	210.3	853.0	579.0	0.58	3.29	11.50	0.081	0.104	127.0	3.64
ort	9.56	85.4	7.62	192.9	530.8	366.9	0.36	0.93	5.54	0.012	0.057	46.1	1.42
SD	0.64	5.3	0.22	13.1	164.0	113.5	0.11	0.88	3.23	0.024	0.025	42.1	1.11

DO: Dissolved oxygen; O2sat: Oxygen saturation; Sal: Salinity; Tur: Turbidity
*3. – 4. Class water qualities are given in bold

Cluster Analysis (CA) was applied to detected data to determine the similar groups among the investigated villages according to water quality characteristics. The diagram of CA is given in Figure 3. According to the results of CA, 2 clusters were identified, which were named as "moderate contaminated zones" and "less contaminated zones". The moderate polluted cluster (C1) was formed by the locations of Hıdırağa, Mnekşesofular, Büyükdöllük, Küçükdöllük and Hanlıyenice; and the lower polluted cluster (C2) was formed by the locations of Kayapa, Lalapaşa, Çömlekakpınar, Korucu and Sinanköy.

Monomial and multinomial risks of electrical conductivity, turbidity, nitrate and nitrite parameters in groundwater of the region were determined by using WAWQI. The quality rating scale values (Qi), which means the results of monomial WAWQI, calculated unit weights (Wi) of investigated parameters and the data of overall WAWQI, which means the results of multinomial WAWQI are given in Table 2.

According to the results of WAWQI, the values of overall WAWQI were within the permissible limits (<100), and the investigated villages located in the Edirne Province were found as “A grade” water quality characteristic. It was also determined that the risk sequence of the investigated parameters in groundwater of the region used in the Weighted Arithmetic Water Quality Index as follows; electrical conductivity > turbidity > nitrate > nitrite in general.

In a study performed in the same region, groundwater quality of Ergene River Basin was evaluated by using WAWQI. According to the results of this research, the majority of investigated element concentrations in groundwater of the basin have been found to be in the range of human consumption standards (Tokatlı, 2019). In another study performed in the city of Pogradec (Albania), drinking water quality assessment was made by using the water quality index. According to the results of this research, the drinking water quality in the city of Pogradec was found as “good” level and as similar of the results of the present investigation, turbidity parameters was found as one of the main problem on drinking water quality (Damo and Icka, 2013).

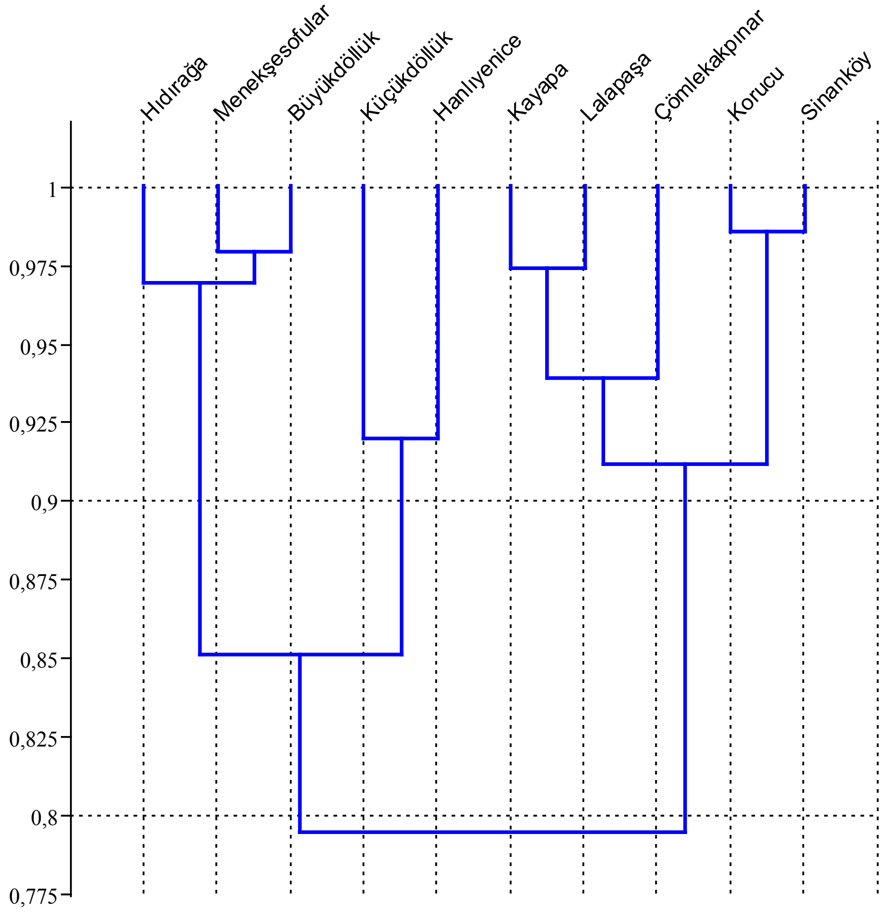

Figure 3. CA diagram of villages

Monomial and multinomial risks of electrical conductivity, turbidity, nitrate and nitrite parameters in groundwater of the region were determined by using WAWQI. The quality rating scale values (Qi), which means the results of monomial WAWQI, calculated unit weights (Wi) of investigated parameters and the data of overall WAWQI, which means the results of multinomial WAWQI are given in Table 2.

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Table 2. Quality rating scale values (Qi), unit weights (Wi) and overall WAWQI

Stations	Parameters used in WAWQI				Overall WAWQI
	EC	Turbidity	NO_3	NO_2	
	Wi				
	0.00007664	0.03832	0.0038	0.958	
	Qi				
Büyükdöllük	22.72	8.4	15.8	40.5	21.864369
Menekşesofular	23.92	30	7.98	1.5	15.856795
Küçükdöllük	34.12	9.6	23	2	17.187365
Kayapa	15.56	14	12.18	1.5	10.814634
Korucu	17.92	8.2	4.58	2.5	8.3035581
Sinanköy	18.16	13.4	4.68	1	9.3139911
Çömlekakpınar	13.24	15.6	10.88	3	10.684578
Hanlıyenice	28.44	65.8	1.814	4	25.024223
Lalapaşa	14.96	9.6	15.66	3	10.809632
Hıdırağa	23.28	13	14.36	0.5	12.790481
min	**13.24**	**8.2**	**1.814**	**0.5**	**8.3035581**
max	**34.12**	**65.8**	**23**	**40.5**	**25.024223**
mean	**21.232**	**18.76**	**11.093**	**5.95**	**14.264963**
SD	**22.72**	**8.4**	**15.8**	**40.5**	**21.864369**

Conclusion

In the present research, groundwater qualities of 10 villages in Edirne Province were assessed by using Weighted Arithmetic Water Quality Index (WAWQI). As a result of this study, the quality of groundwater in the region was found as 1. - 2. Class, in general and any investigated variable has not exceeded the drinking water standards. It was also determined that the values of overall WAWQI were recorded within the permissible limits (<100) and the groundwater quality of the region was found as "A grade" water quality characteristic.

In conclusion, although levels of some of the investigated parameters in some villages of the region were determined as quite high levels, the majority of investigated variables in drinking water of the region have been found to be in the range of human consumption standards.

The detected data reveals that agricultural runoff is the main risk factor for the groundwater of the region and if such contamination persists in especially around the Büyükdöllük and Küçükdöllük Villages, concentrations of nitrogen compounds in drinking water may reach the critical levels and may adversely affect the human health in the near future.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Tolerance of forage pea cultivars to salinity and drought stress during germination and seedling growth

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Abstract

The germination and seedling characteristics of six forage pea cultivars were investigated under different levels of salinity (0, 5, 10, 15, and 20 dS/m) and drought (0, -2, and -4 bar) stresses. All characteristics of germination and seedling growth varied by cultivar, salinity and drought levels, and their interactions. With high seedling lengths overall, Özkaynak, Ulubatlı, and Töre cultivars demonstrated low reduction rates in seedling length when salinity reached 15 dS/m, whereas the Taşkent cultivar's reduction rate increased considerably at that level of salinity, as did Ürünlü and Gölyazı cultivars. Meanwhile, the Gölyazı cultivar had high fresh and dry weights, despite high reduction rates in fresh weight, similar to the Taşkent cultivar. Though the Ulubatlı cultivar had the shortest seedlings, they exhibited a low reduction rate in seedling length at -2 bar of drought stress, as did Töre and Özkaynak cultivars. At that level of drought stress, those cultivars also indicated low reduction rates in fresh weight. Altogether, the Töre cultivar best tolerated salinity and drought conditions, the Özkaynak cultivar showed promise as well, whereas Taşkent and Gölyazı cultivars were the most sensitive to the conditions.

Keywords: Forage legume, Seedling length, Reduction rate, PEG, NaCl

Introduction

Worldwide, 20% of agricultural lands experience salt stress, and that rate is expected to rise to 50% by 2050 (Kang et al., 2010; Tiryaki, 2018). Salinity in the soil mostly occurs in arid and semi-arid regions with low rates of precipitation and high temperatures, where it causes severe losses in yield (Munns and Termaat, 1986; Umezawa et al., 2001). In studies on salinity, germination and seedling development are typically examined in determining how crop genotypes respond to salt (Ghoulam and Fares, 2001; Kara and Uysal, 2010). The top reason why seeds in overly salty soils face adversity during germination is the stunted intake of water through the seed coat (Coons et al., 1990; Mansour, 1994). Beyond that, deterioration in the ion balance in plants grown in saline soils damages their physiological functions, including their capacity for photosynthesis and respiration (Levitt, 1980; Aydınşakir et al., 2012).

Aside from salinity, drought stress, observed in 26% of agricultural lands worldwide, causes a range of physiological, biochemical, and molecular events in plants (Blum and Jordan, 1985). During drought, plants reduce their cell growth as a means to protect against water deficiency (Taiz and Zeiger, 2015), after which turgor pressure drops, and the water balance between plant tissues becomes disturbed (Levitt, 1980). On top of that, drought not only causes damage to pigments for photosynthesis and decreases chlorophyll content (Saeidi and Abdoli, 2015) but also causes the failure of major organs and root elongation by increasing the amount of absicic acid, which decreases the amount of cytokinin and gibberellic acid (Özel et al., 2016).

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In Turkey, barren lands represent 5.48% of all cultivated lands, and 74% of those barren lands are salty; 25.5% have saline–alkaline soils, while 0.5% have alkaline soils (Karaoğlu and Yalçın, 2018). Although the average annual rainfall in Turkey is approximately 640 mm, many regions experience water shortage and drought due to the irregular distribution of precipitation. It has been hypothesized that Turkey ranks among countries at risk to the possible effects of global warming, and in the future, Turkey's Mediterranean and Inner Anatolian regions will be especially more affected by climate change (Kapluhan, 2013). Considering the quality roughage deficit in Turkey, it is critical to identify forage crops resistant to salt and drought stress with high yield potential that can guarantee their use as fodder for livestock in the future.

Among likely candidates, forage peas [(*Pisum sativum* var. *arvense* (L.) Poiret)] produced for fresh forage, hay, and silage have not only shown high yield potential but also been adapted to nearly all of Turkey's regions, regardless of their different climatic and soil conditions (Uzun et al., 2012). Rich in protein, the hay and seed of forage peas are both nutritious and attractive food sources for livestock (Açıkgöz et al., 1985). They also leave clean stubble as well as significant nitrogen and organic matter in the soil for the benefit of subsequent crops (Parr et al., 2011).

Because the efficiency of cultivating forage peas depends on their ability to cope with abiotic stresses, determining the responses of different forage pea varieties against stress factors is critical. Recognizing that need, Avcı et al. (2018) and Demirkol et al. (2019) have investigated the effects of salt stress on the germination and early seedling development of forage peas in Turkey. In other work, Okçu et al. (2005), Petrović et al. (2016), and Pereira et al. (2020) have examined the responses of different pea genotypes to salt and drought stress. In those studies, the responses of pea genotypes to salt and drought stress differed, however. In response to those findings, the study reported here involved examining how salt and drought stress affect the germination and seedling growth of six different forage pea cultivars in Turkey.

Materials and Methods

Three purple-flowered (Töre, Taşkent, and Özkaynak) and three white-flowered (Ulubatlı, Ürünlü, and Gölyazı) forage pea (*P. sativum* var. *arvense* L. Poir.) cultivars were used as seed materials.

A controlled experiment in incubators was performed in a two-factor arrangement with a completely randomized design involving four replications. The first factor was the forage pea cultivars under both stresses, while the second was salt (0, 5, 10, 15, and 20 dS/m) or drought (0, -2, and -4 bar) stress. Salinity was adjusted with a WTW 3.15i EC meter using sodium chloride (NaCl), whereas drought stress was achieved using polyethylene glycol (PEG 6000 mol.w.), as described by Michel and Kaufmann (1973).

Germination was performed in four replications of 50 seeds each on three filter papers 20 × 20 cm in size (ISTA, 2018). Once 7 mL of pure water was added to each filter paper, the papers were placed in sealed plastic bags in order to prevent evaporation. The papers were checked every 2days, and pure water was added as needed. The seeds were counted every day, and ones with a root length of 2 mm were considered to have germinated.

Germination percentage, mean germination time, seedling length, seedling fresh and dry weights, and reduction rate were evaluated to determine the tolerance of the cultivars to salinity and drought. Mean germination time was calculated with the formula of Ellis and Roberts (1980), while the reduction rate was calculated as follows: (Seedling characteristics of control plants – Seedling characteristics of stress plants) / (Seedling characteristics of control plants) ´ 100.

All data were subjected to variance analysis using MSTAT. Arcsin √x transformation was applied to percentage values (Sokal and Rohlf, 1981), and Duncan's multiple comparison test was performed to determine the differences, if any, between mean values.

Results and Discussion

In terms of salt stress, the effects of cultivars, salinity, and the interaction of cultivar and salinity on characteristics of germination and seedling growth were significant at the 1% level (Table 1). As shown in Table 2, the Töre cultivar in the control condition and in 5 dS/m of salinity, along with the Gölyazı cultivar in the control condition and in 5 and 10 dS/m of salinity, had higher germination percentages than the other cultivars. The lowest germination percentage, was recorded for the Ulubatlı cultivar, decreased rapidly once salinity reached 5 dS/m. In general, the increase in salinity began adversely affecting germination at levels of 10 dS/m and higher (Table 1). Similar to those findings, Demirkol et al. (2019) observed a significant decrease in germination rate parallel to increased salinity after 90 mM. Contrary to those findings, Okçu et al. (2005) and Avcı et al. (2018) reported that germination percentage did not vary by level of salinity.

Mean germination time was prolonged in all cultivars as salinity level increased (Table 2). Whereas Töre, Özkaynak, and Ürünlü cultivars germinated the fastest, Ulubatlı and Gölyazı cultivars were the slowest (Table 1). Such findings confirm the results of Tsegay and Andargie (2018) and Demirkol et al. (2019), who found that mean germination time increased in parallel to salt concentration. Similar findings have also been reported for lentils (Karaman and Kaya, 2017), common vetch (Ertekin et al., 2017), and Hungarian vetch (Önal Aşçı and Üney, 2016).

As salinity rose, the length of seedlings decreased, except in Özkaynak and Ulubatlı cultivars (Table 2). In those cultivars, the effect of salinity at 5 dS/m on seedling length was even positive. When the reduction rate in seedling length compared to the control at 15 dS/m was evaluated, Özkaynak, Ulubatlı, and Töre cultivars demonstrated the lowest values, in that order (Figure 1). However, a dose of 20 dS/m negatively affected nearly all varieties, for the average reduction rate of approximately 70%. Avcı et al. (2018) and Demirkol et al. (2019) also found that salinity negatively affected seedling growth, as well as that its effect on the roots of pea genotypes commenced at lower doses than on the shoots.

The highest fresh and dry weights of all seedlings were obtained in the control condition for the Gölyazı cultivar, whereas the lowest were obtained in the 20 dS/m treatment of the Taşkent cultivar (Table 2). As salinity increased, fresh and dry weights decreased in all cultivars except for Özkaynak, whose fresh and dry weights were positive affected by 5 dS/m of salinity. The lowest reduction rate in fresh weight at 15 dS/m of salinity was recorded in that cultivar as well (Figure 2). Although all reduction rates in the fresh weights of the seedlings at 20 dS/m were similar, the Taşkent cultivar was the most affected. Supporting those findings, Avcı et al. (2018), Tsegay and Andargie (2018), and Demirkol et al. (2019) have all reported that the fresh and dry weights of seedlings decreased in pea genotypes due to increased salinity.

Table 1. Analysis of variance and differences between mean values of germination and seedling growth characters of forage pea cultivars grown under different salinity stresses.

Factors	Germination (%)	Mean germination time (day)	Seedling length (cm)	Fresh weight (mg/ seedling)	Dry weight (mg/ seedling)
Cultivars					
Töre	99.60[a†]	2.85[c]	9.83[a]	138.89[bc]	13.31[cd]
Taşkent	94.80[b]	2.99[b]	9.30[a]	132.06[c]	13.03[d]
Özkaynak	95.10[b]	2.98[bc]	9.93[a]	142.37[b]	13.93[cd]
Ulubatlı	94.50[b]	3.15[a]	9.48[a]	154.14[a]	15.39[b]
Ürünlü	95.20[b]	2.96[bc]	8.45[b]	137.30[bc]	14.08[c]
Gölyazı	98.90[a]	3.18[a]	8.18[b]	161.16[a]	16.70[a]
Salinity (dS/m)					
0 (Control)	97.91[a]	2.62[d]	13.38[a]	249.21[a]	22.04[a]
5	96.50[a]	2.95[c]	12.83[a]	179.18[b]	17.27[b]
10	97.58[a]	3.06[bc]	9.52[b]	139.06[c]	13.93[c]
15	95.33[b]	3.13[b]	6.23[c]	86.77[d]	10.20[d]
20	94.41[b]	3.35[a]	4.02[d]	67.37[e]	8.58[e]
Analysis of variance					
Cultivars (A)	*	*	*	*	*
Salinity (B)	*	*	*	*	*
A x B	*	*	*	*	*

*: significant level of 1%. †: letters show different groups at 5% level.

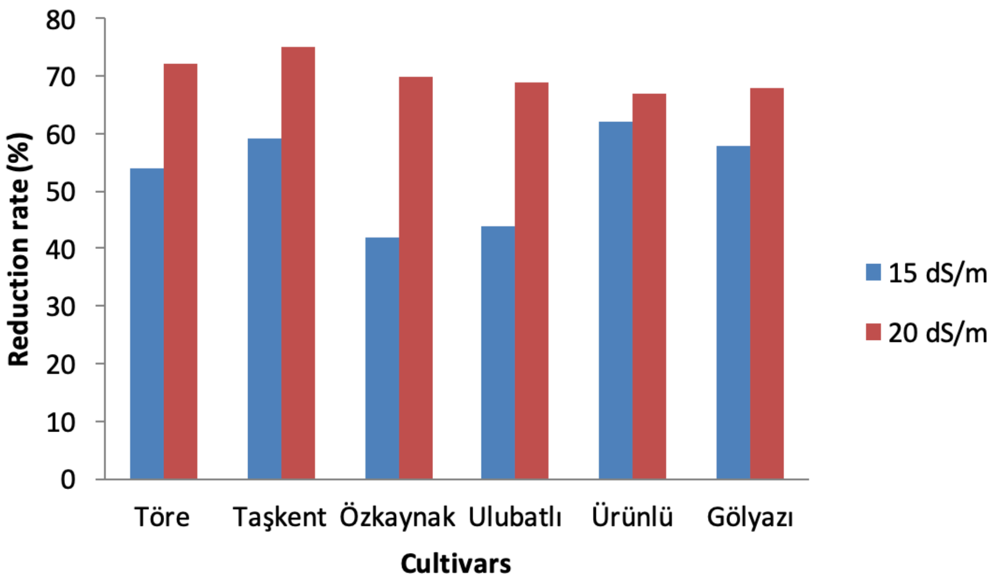

Figure 1.Reduction rates in seedling lengths of forage pea cultivars at 15 and 20 dS/m of salinity stresses

Table 2. The effect of cultivars x salinity interaction on germination and seedling growth characters of forage pea cultivars.

Cultivars	Salinity (dS/m)	Germination (%)	Mean germination time (day)	Seedling length (cm)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)
Töre	Control	100.0	2.38	14.55	229.75	20.25
	5	100.0	2.66	14.16	184.15	16.10
	10	99.0	2.93	9.66	145.02	14.22
	15	100.0	3.12	6.77	76.77	8.37
	20	99.0	3.16	4.01	58.75	7.62
Taşkent	Control	95.0	2.71	14.20	242.00	21.00
	5	90.5	2.73	14.12	171.45	15.92
	10	99.0	3.08	8.68	117.55	12.42
	15	94.5	3.00	5.89	73.80	8.80
	20	95.0	3.46	3.61	55.47	7.02
Özkaynak	Control	96.5	2.36	12.89	206.25	18.50
	5	95.0	2.85	16.04	209.28	18.92
	10	95.5	2.98	9.45	148.85	14.82
	15	95.0	3.16	7.28	89.22	10.27
	20	93.5	3.56	3.98	58.27	7.15
Ulubatlı	Control	99.5	3.04	11.81	256.75	23.50
	5	99.0	3.36	14.20	188.38	18.37
	10	95.5	3.09	10.95	152.75	14.55
	15	89.5	2.97	6.68	96.90	10.97
	20	89.0	3.32	3.76	75.90	9.55
Ürünlü	Control	96.5	2.71	13.34	263.50	22.00
	5	94.5	2.89	9.42	133.15	14.67
	10	96.5	3.00	9.92	135.75	13.32
	15	96.0	3.09	5.17	80.70	11.12
	20	92.5	3.14	4.40	73.37	9.30
Gölyazı	Control	100.0	2.52	13.46	297.00	27.00
	5	100.0	3.20	9.06	188.65	19.67
	10	100.0	3.27	8.45	134.42	14.25
	15	97.0	3.45	5.60	103.25	11.70
	20	97.5	3.48	4.35	82.50	10.87
$LSD_{\%5}$		6.39	0.46	1.63	18.07	1.76

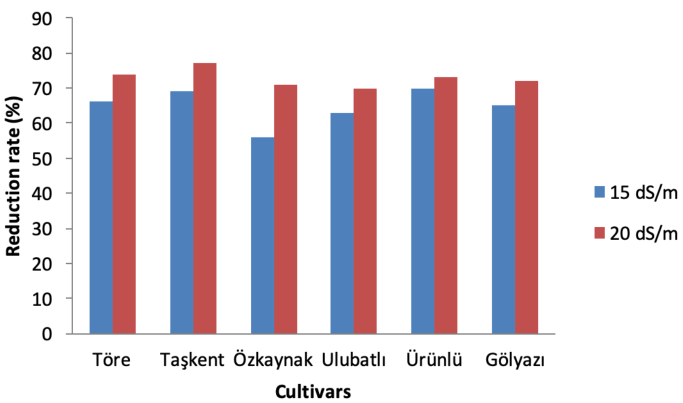

Figure 2. Reduction rates in seedling fresh weights of forage pea cultivars at 15 and 20 dS/m of salinity stresses

As in the salt stress study, the effects of cultivar, drought, and their interaction on characteristics of germination and seedling growth were significant in the drought study (Table 3). The highest and lowest germination percentages were achieved by the Töre and Gölyazı cultivars, respectively, although the germination percentages of the cultivars were not negatively affected in -2 bar of drought stress except in the Ulubatlı and Gölyazı cultivars (Table 4). Those two cultivars also showed the greatest decrease in germination percentage at -4 bar of drought stress. Drought stress caused by PEG negatively affected germination on pea genotypes in the studies of Okçu et al. (2005) and Pereira et al. (2020). Similar results have also been obtained for lentil genotypes (Muscolo et al. 2013), grass pea varieties (Aslan and Atış, 2018), and some alfalfa genotypes (Özkurt et al., 2018).

Table 3. Analysis of variance and differences between mean values of germination and seedling growth characters of forage pea cultivars grown under different drought stresses.

Factors	Germination (%)	Mean germination time (day)	Seedling length (cm)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)
Cultivars					
Töre	97.5[a†]	3.56[c]	10.74[a]	130.73[bc]	13.30[bc]
Taşkent	81.0[b]	4.32[b]	8.78[bc]	120.67[cd]	12.30[c]
Özkaynak	80.2[bc]	4.12[b]	9.33[b]	115.91[d]	12.34[c]
Ulubatlı	77.0[cd]	4.73[a]	7.91[c]	132.27[b]	13.92[b]
Ürünlü	86.5[bc]	4.15[b]	9.46[b]	140.14[ab]	13.85[b]
Gölyazı	72.8[d]	4.62[a]	8.77[bc]	145.70[a]	15.60[a]
Droughts (bar)					
0 (control)	98.75[a]	2.62[c]	13.38[a]	249.21[a]	22.04[a]
-2	95.83[b]	4.15[b]	8.89[b]	95.17[b]	11.47[b]
-4	52.91[c]	5.98[a]	5.22[c]	48.32[c]	7.15[c]
Analysis of variance					
Cultivars (A)	**	**	**	**	**
Droughts (B)	**	**	**	**	**
A x B	**	**	*	**	**

*, **: significant level of 5% and 1%, respectively. †: letters show different groups at 5% level.

The Töre cultivar achieved the fastest germination, whereas the Ulubatlı and Gölyazı cultivars germinated the slowest (Table 3). Mean germination time was prolonged in all cultivars as drought stress increased (Table 4). The fastest-germinating cultivars at -4 bar of drought stress were the Töre and Ürünlü cultivars. Such findings regarding prolonged mean germination time due to increased drought stress align with the results of Okçu et al. (2005) and Aslan and Atış (2018).

The longest seedlings, at 14.55 cm, were obtained in the control condition of the Töre cultivar, whereas the shortest ones, at 3.96 cm, emerged in the -4 bar application of the Ulubatlı cultivar (Table 4). As drought stress intensified, seedling length steadily decreased in all cultivars. The lowest reduction rates in seedling lengths at -2 bar were recorded in the Töre, Özkaynak, and Ulubatlı cultivars (Figure 3). Although the Ürünlü, Özkaynak, and Töre cultivars showed the lowest reduction rates at -4 bar, the highest reduction rates were observed in the Taşkent, Ulubatlı, and Gölyazı cultivars. Both Okçu et al. (2005) and Petrović et al. (2016) have reported that increased doses of different applications of osmatic pressure created using NaCl and PEG negatively affected root and shoot length in pea genotypes. Added to that, Pereira et al. (2020) found that a reduction in epicotyl and root length in pea genotypes occurred when osmotic potential was no more than 0.2 MPa.

The fresh and dry weights of the seedlings steadily decreased as drought stress increased (Table 4). The highest and lowest fresh and dry weights were obtained for the Gölyazı cultivar in the control condition and in the Taşkent cultivar at -4 bar of drought stress, respectively. The lowest reduction rates in fresh weight at -2 bar of drought stress were recorded for the Töre and Özkaynak cultivars, which paralleled the reduction rates in seedling length (Figure 4). Although the reduction rates of cultivars at -4 bar of drought stress were similar, as with the reduction rates of seedling length, the Töre, Özkaynak, and Ürünlü cultivars had low reduction rates, whereas the Taşkent, Ulubatlı, and Gölyazı cultivars had high ones. Okçu et al. (2005), Petrović et al. (2016), and Pereira et al. (2020) all observed a remarkable decrease in the fresh and dry weights of pea seedlings due to the decreased availability of water as doses of NaCl and PEG increased.

Table 4. The effect of cultivars x droughts interaction on germination and seedling growth characters of forage pea cultivars.

Cultivars	Droughts (bar)	Germination (%)	Mean germination time (day)	Seedling length (cm)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)
Töre	0 (Control)	100.0	2.38	14.55	229.75	20.25
	-2	100.0	3.05	11.28	111.40	12.17
	-4	92.5	5.26	6.38	51.05	7.50
Taşkent	0 (Control)	98.5	2.71	14.20	242.00	21.00
	-2	99.0	4.02	8.05	85.67	10.77
	-4	45.5	6.25	4.10	34.35	5.12
Özkaynak	0 (Control)	98.0	2.36	12.89	206.25	18.50
	-2	97.0	3.84	9.38	89.10	11.22
	-4	45.5	6.15	5.71	52.37	7.30
Ulubatlı	0 (Control)	99.5	3.04	11.81	256.75	23.50
	-2	93.0	4.65	7.95	93.62	11.47
	-4	38.5	6.52	3.96	46.42	6.80
Ürünlü	0 (Control)	96.5	2.71	13.34	263.50	22.00
	-2	96.0	4.18	8.34	97.95	10.85
	-4	67.0	5.55	6.75	58.97	8.70
Gölyazı	0 (Control)	100.0	2.52	13.46	297.00	27.00
	-2	90.0	5.18	8.37	93.32	12.32
	-4	28.5	6.17	4.48	46.77	7.50
$LSD_{\%5}$		6.39	0.46	1.63	18.07	1.76

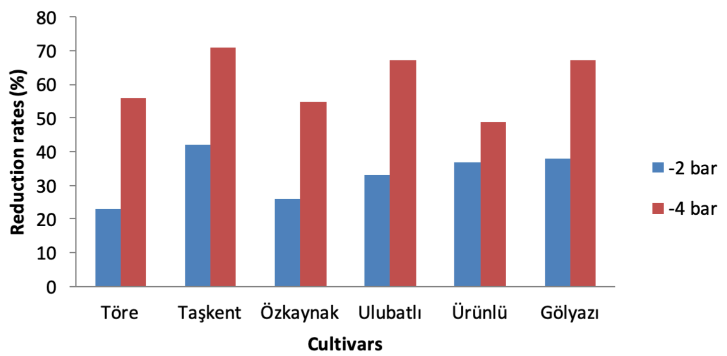

Figure 3. Reduction rates in seedling lengths of forage pea cultivars at -2 and -4 bar of drought stresses

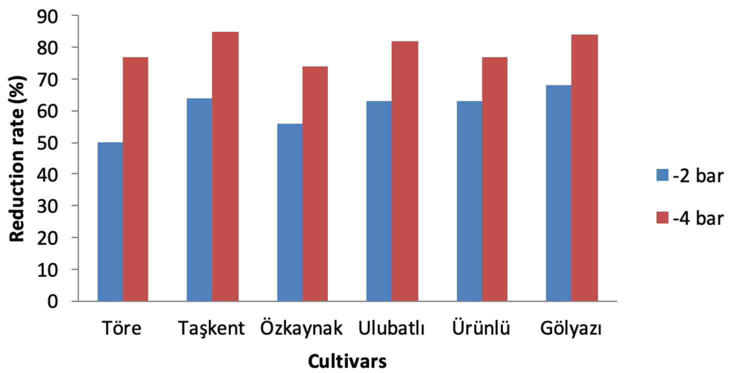

Figure 4. Reduction rates in seedling fresh weights of forage pea cultivars at -2 and -4 bar of drought stresses

Conclusion

In sum, the responses of the cultivars to salinity and drought stresses were similar. The Töre cultivar had high values in terms of germination percentage, mean germination time, and seedling length, whereas the Gölyazı cultivar had high values in the fresh and dry weights of its seedlings in both stress conditions. Depending on the intensity of the stress conditions, reduction rates in the seedling length and fresh weight of the cultivars indicated that the Töre, Özkaynak, and Ulubatlı cultivars were less affected than the Taşkent, Gölyazı, and Ürünlü ones at -2 bar of drought stress and in 15 dS/m of salinity. However, all cultivars were adversely affected in 20 dS/m of salinity and exhibited approximately similar reduction rates. Among other results, as osmotic pressure rose, the Ürünlü cultivar had the lowest reduction rate at -4 bar of drought stress, followed by the Özkaynak and Töre cultivars. The most negatively affected cultivars at that level of drought stress were the Taşkent, Ulubatlı, and Gölyazı ones. Altogether, the results revealed that the Töre cultivar can best tolerate adverse stress conditions but that the Özkaynak cultivar shows promise as well. Although parameters other than reduction rates were disappointing, it can also be focused on the Ulubatlı and Ürünlü cultivars.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

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Consent for publication

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Effects of blades types on shear force and energy requirement of paddy stem

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Abstract

This study was aimed to determine of the shearing force and shearing energy of paddy stem as a function of blade type, blade-edge angle and cutting speed. The Karacadag white paddy variety was used as plant materials. Shearing properties were measured by a universal testing machine. Depend on measured shear force and cross-section area of paddy stem, energy values were calculated by measuring the surface area under the cutting force-deformation curve for each test separately. The tests were conducted at five blade angles, five loading speed with various three different type blades. The tests results showed that the shearing force, shearing strength, shearing energy, specific shearing energy and extension at maximum load were affected significant ($p<0.01$) by blade type, cutting angle and cutting speed. While the lowest values were determined at serrated 2 blade types, followed by the serrated 1 and flat-edge blade. The paddy stalk shearing force and energy values has increased with decrease in the blade edge angle from 90° to 50°. The highest force and energy values were measured at 90° cutting edge of blade as 25.47 N and 5.8 N cm. The effect of loading speed on the cutting forces, cutting strength, cutting energy and specific cutting energy were found significant ($p<0.01$). The shearing and energy has slightly decreased with an increase of blade cutting speed. While the highest values were found at 2 m s^{-1} loading speeds, the lowest values were found at 6 mm s^{-1} cutting speed.

Keywords: Cotton, Cutting properties, Design, Shearing force, Stalk

Introduction

Paddy (Oryza sativa L.) is one of the oldest cereals and important staple food and the main source of income for about half of the world's population and it is growing in more than half of the countries in the world and it will continue to be the mainstay of life for future generations as well (Correa at al., 2007; Badawi, 2001; Esgici et al., 2016). 90% of world paddy production takes place in Asian continent. One of the paddy producer countries in terms of climate, soil and environmental conditions is Turkey. Southeastern part of Turkey is also an important paddy producer. In this region, the paddy variety known as Karacadag white widely is grown.

Harvesting and threshing operations of paddy are known as one of crucial part and influential processes on quantity, quality, grain losses and production cost. Paddy harvesting is mostly performed by worker using sickles. So, in paddy harvest stage, use of labor is intensity and also harvest cost is very high. In the recent years, paddy producers in this region have been using conventional grain harvesters that are not suitable for paddy. As a result, combine harvesters have negative impacts on quantity and quality of paddy grains which seriously affect the profitability of the crop since majority of the losses are due to improper adjustment of the machines according to paddy varieties and crop conditions. Losses in paddy production is an

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estimated ratio ranging between 25 % to 30 % (Esgici et al., 2016) in this region. This value of losses is quite high and we have to reduce a reasonable level. Because, the objective of good harvesting is to maximize grain yield while minimizing grain losses and quality deterioration. Therefore, it is very important to choose proper shearing parameters of paddy stem and kernel during harvesting processes to minimize the level of grain damage and to increase grain quality (Correa et al., 2007; Yore et al., 2002). Paddy harvesting machines and combine-harvesters are commonly equipped with reciprocating cutting mechanism. In this case, a cutting bar has some blades and counter edges which move against each other. If plants stem stands between them, it is cut due to reciprocating movement of blade or both blade and counter edge. The performance of cutting elements on harvesting mechanisms can be judged by cutting energy requirement, shearing force, shearing strength and applied force. Various parameters such as physical and mechanical properties of the stem, paddy cultivar, blade velocity and blade components are effective on the cutting force energy and the specific cutting energy (Allameh and Alizadeh, 2016). Cutting energy of a plant stem can be estimated from the relationships between the force of cutting the stem and the displacement of the blade (Chen et al., 2003).

Information on plant properties and the power or energy requirement of equipment has been very valuable for selecting design and operational parameters of the equipment (Persson, 1987; Chen et al., 2003; Jicheng et al., 2017). Such information is needed for the design of paddy harvesters and combine harvesters, assuring appropriate machine functions and an efficient use of energy. Although there is considerable interest in mechanical processing of paddy, there is little information on shearing properties depend on blade type, shearing angle and loading speed of paddy stems. This information is important to know behavior of cutting of paddy stem. We have to be made paddy harvest at high moisture contents by Combine harvesters. Because during the harvesting season, the moisture content of paddy stalk is higher than the other cereals and the strength of paddy is more. Also, the behavior is different from paddy variety to variety during cutting stage. So, both suitable blade type and edge angle are important parameters for reducing shearing force, cutting energy and increase effective cutting performance of cutter bar of machine (Koloor and Borgheie, 2006).

The main aim of this study was to determine the shearing force, shearing strength, shearing energy, specific shearing energy and extension at maximum load speed depend on blade type, blade cutting- angle and blade-loading speed in a laboratory condition with paddy stems.

Materials and Methods

The Karacadag white paddy variety (Figure 1) was used for tests. Paddy stems were collected at harvesting season from a commercial farm in Diyarbakır province in 2018, Turkey. The whole paddy plants average 0.82 m in height and were cut manually prior to the cutting testes at height of 25 cm above the soil level. After the paddy stem was harvested, it was covered and transported to University of Dicle, Department of Agricultural Machinery and Technologies Engineering Research Laboratory for tests. The tests samples stored at a temperature of 4 ºC for one month until start to shearing tests. Selected some properties of paddy are seen in Table 1.

Figure 1. Paddy stalk with panicles

Table 1. Some physical properties of paddy.

Physical properties	
Plant height, m	0.82
Panicle length, cm	18
Panicle weight, g	2.08
Moisture content of stalk, %	68.00
Moisture content of kernel, %	29.00
The weight of 1000 kernel, g	33.90
Stem diameter, mm	4.40

Moisture content of paddy stems samples were measured according to ASABE standards (ASABE, 2008). Before tests, four samples of 25 g paddy stalks were weighed and dried in an oven of 103ºC for 24 h which were then reweighed in order to determine the average moisture content of paddy stem. The average moisture content of paddy stems (stalk) was obtained as 68.00 % w.b during tests. The diameter varied greatly within the field, ranging from 4 to 4.8 mm and averaged 4.4 mm. Diameters of stalks were measured with a digital caliper. So, stem diameter was considered as 4.40 mm for the cutting experiments. The stalks diameter (mm) was converted to cross-section area in mm^2.

An Universal Materials Testing Machine, Lloyd LRX Plus, were used to measure of shearing properties of paddy stalks (Figure 2). The shear force was recorded as a function of displacement depend on selected parameters. The tests were carried out with three different types of blades (Figure 2), two of them are serrated type (serrated 1 (blade-edge thick), serrated 2 (blade-edge thin) and flat (blade-edge flat). Five blade angles (50º, 60º, 70º, 80º and 90º) and five cutting speed (2, 3, 4, 5, and 6 mm s^{-1}) were selected independent parameters. The shearing energy of paddy stalk was calculated by measuring the surface area under the force-deformation curve by an instron universal material testing machine (Chattopadhyay and Pandey, 1999; Yore et al., 2002; Chen et al., 2003; Kocabiyik and Kayisoglu, 2004; Ekinci et al., 2010; Zareiforoush et al., 2010; Ghahraei et al., 2011; Voicu et al., 2011; Ozdemir et al., 2015; Nowakowski, 2016; Pekitkan et al., 2018). A computer data acquisition system recorded all force-displacement curves during the cutting process by using a NEXYGEN computer program for each parameter.

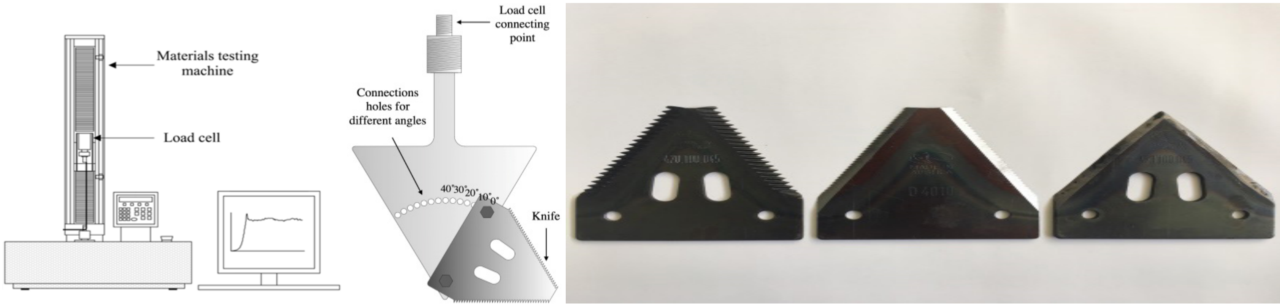

Figure 2. View of testing machine and blades

A force-deformation curve is given in Figure 3. As you seen in Figure 3, the first peak value was considered as the yield point (lower yield) at which stalk damage was initiated. The second peak value (upper yield) corresponds to maximum force (Figure 3).

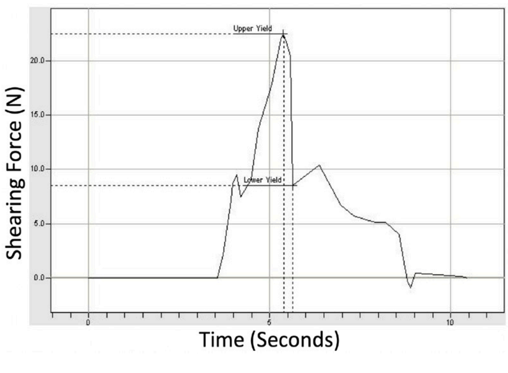

Figure 3. The time –shearing curve for cutting of paddy stalks.

The maximum shearing strength, obtained from the force values, was determined by the following equation (Mohsenin, 1986; O'Dogherty et al., 1995; Zareiforoush et al., 2010; Ekinci et al., 2010; Chandio et al., 2013; Sessiz et al., 2013; Sessiz et al., 2018):

$$\sigma_s = \frac{F_{max}}{A}$$

Where: σ_s is the maximum shearing strength in (MPa), F_{max} is the maximum shearing force in (N) and A is the cross-sectional area in (mm^2).

Specific shearing energy, Esc was calculated by:

$$E_{sc} = \frac{E_s}{A}$$

Where: E_{sc} is the specific cutting energy (J mm^{-2}) and E_s is the shearing energy (J).

Test results were subjected to variance analysis. Mean comparisons were made according to Duncan's multiple range tests .

Results and Discussion

The effect of Blade type

The shearing properties depend on blades types are given in Table 2. The analysis of variance and Duncan's test results showed that the effect of blade type was found significant ($p<0.01$) on shearing force, shearing strength, shearing energy, specific shearing energy and extension at maximum load. There were found significant differences among the three blade types ($p<0.01$) according to measured properties. The lowest values were determined at serrated 2 blade types, followed by the serrated 1 and flat type blade (Table 2). The highest shearing force, shearing strength, shearing energy, specific shearing energy and extension at maximum load values were obtained at flat blade type as 22.66 N, 1.490 N mm^{-2}, 0.045 Nm, 0.004 J mm^{-2} and 8.35 mm, respectively. A serrated blade edge gives a lower cutting force and requires lower cutting energy than a flat- edge type. So, we can argue that serrated blade types are better than the flat type blades for paddy harvesting. According to results, flat-edge blade type is not suitable for shearing paddy stem. It is concluded that the edge of serrated blades has a good grasp of the paddy stems than flat-edge type blade. This means that the energy requirement of flat edge blade for cutting of paddy stem is more than serrated blades. In contrast, Koloor and Borgheie (2006) argued that blade bevel angle and blade type had no significant effect on the shearing strength of paddy stem.

Table 2. Analysis of variance of the cutting force and cutting energy with respect to blade type.

Blade type	Shearing force (N)	Energy strength (N mm^{-2})	Energy (Nm)	SCE (J mm^{-2})	Extension at maximum load (mm)
Serrated type 1 (blade-edge thick)	18.54b*	1.220 b	0.047 a	0.003 a	18.74 a
Serrated type 2 (blade-edge thin)	13.84c	0.910 c	0.030 b	0.002 b	11.11 b
Flat type (blade-edge flat)	22.66a	1.490 a	0.045 a	0.004 a	8.35 c
Mean	18.35	1.214	0.041	0.003	12.73

*means followed by the same letter in each column are not significantly different by Duncan's multiple range test at the 1 % level.

The Effect of Blade Edge Angle

Table 3 shows the mean values of the shearing force, strength, energy, specific shearing energy and extension under maximum load at different blade- edge angle. The effect of blade cutting angle on shearing properties were found significant as statistically ($p<0.01$). The study showed that the mean shearing values gradually decreased with decrease in the blade-edge angle from 90° to 50°. The highest shearing force, shearing strength, shearing energy, specific shearing energy and extension at maximum load values were obtained at 90° angle as 25.476 N, 1.677 N mm^{-2}, 0.058 Nm, 0.004 J mm^{-2} and 11.31 mm, respectively, followed by 80°, 70°, 60° and 50° blade-edge angles. The lowest results were determined at serrated 2 blade types as 11.86 N, 0.780 N mm^{-2}, 0.0026 Nm, 0.003 J mm^{-2} and 12.05 mm, respectively (Table 3). By decreasing vertical shearing angle, the paddy stem will be bent alongside the applied force. Therefore, more energy is taken in order to complete shear operation. The similar results were observed by Kronbergs et al. (2011). They argued that shearing force and energy depends on some parameters such as the material deformation process, blades edge- angle and friction forces. These parameters cause significant increase of cutting energy. According to the results, the suitable blades edge angle is change between 25º and 45º. The decrease of shearing force and energy depend on blade- edge angle allows proper design of the cutting unit and cutting machine for paddy stem and predicting the power requirements. Hoseinzadeh et al. (2009) investigated the effect of moisture content, bevel angle and cutting speed on shearing energy for three wheat varieties. According to theirs results, the blade edge angle has significant effect on the cutting force and energy. Dowgiallo (2005) also reported that besides the cutting edge, blade edge sharpness and blade speed are effect parameters on cutting properties. The cutting force and energy required for the pigeon pea crops were investigated by Dange et al. (2011). The study investigated that the cutting energy and cutting force were directly proportional to cross-sectional area and moisture content at the time of harvesting of pigeon pea crop. 30º and 45º bevel angle were selected for the cutting experiment. According their result, the blade 45º bevel angle required 23.74 % more cutting energy than the blade with 30º bevel angle for 30 mm diameter stem. Whereas the blade with 45º bevel angle required 16.05 % more cutting force than the blade with 30 º bevel angle. Optimum blade-edge angle, shear angle, oblique angle, and rake angle were measured 25º, 40º, 40º, and 40º, respectively, for Kenaf stems (Ghahraei et al., 2011). Koloor and Borgheie (2006) studied that the effect of blade parameters on the cutting energy of paddy stem by an impact type shears tests apparatus. According their results, specific cutting energy decreased with increasing in oblique angle and it is a minimum at 30º. Yore et al. (2002) investigated the cutting properties of paddy straw to aid development of novel header system for combines. Tavakoli et al. (2010) compared mechanical properties of two Iranian varieties of paddy straw, namely Hashemi and Alikazemi. The results showed that the energy requirement for cutting of Hashemi straw is more than Alikazemi variety. Mathanker et al. (2015) investigated the effect of cutting speed and blade oblique angle on cutting energy. The results showed that the specific cutting energy increases with cutting speed. The specific cutting energy showed a close correlation with stem diameter and stem cross-sectional area. Our results indicated that shear force and energy were significantly larger for the 90º angle than the other four cutting edge angles. The mean of this situation that using 50º blade edge angle and serrated 2 blade types can minimize the shearing force and shearing energy requirements.

Table 3. Analysis of variance of the cutting force and cutting energy with respect to blade edge angle

Blade cutting angle (°)	Cutting force (N)	Energy strength (N mm^{-2})	Energy (Nm)	SEC (J mm^{-2})	Extension at Max. load (mm)
90	25.48 a*	1.677 a	0.058 a	0.004 a	11.31 d
80	23.13 b	1.521 b	0.050 a	0.003 a	13.28 b
70	17.63 c	1.159 c	0.040 ab	0.003 a	14.19 a
60	14.09 d	0.931 d	0.028 b	0.002 b	12.86 b
50	11.86 e	0.780 e	0.026 b	0.003 a	12.05 c
Mean	18.35	1.214	0.041	0.003	12.73

*means followed by the same letter in each column are not significantly different by Duncan's multiple range tests at the 1 % level.

The effect of loading speed on shearing properties of paddy stem is summarized in Table 4. The Table 4 shows that the effect of blade loading speed has been significant ($p<0.01$) on the shearing forces, shearing strength, shearing energy, specific shearing energy and extension at maximum load. There has been an inverse relationship occurred between stem loading speed and independent shearing properties such as shearing force, strength and energy. The cutting force and energy values decreased with increase loading speed from 2 mm s^{-1} to 6 mm s^{-1}. The highest shearing force, shearing strength, shearing energy, specific shearing energy and extension at maximum load were obtained at 2 mm s^{-1} as 19.48 N, 1.281 N mm^{-2}, 0.043 Nm, 0.003 J mm^{-2} and 12.49 mm, respectively. The minimum values were occurred at 4, 5 and 6 mm s^{-1}. While the significant differences were found between 2 mm s^{-1} and the other loading speed values, there were not found significant differences among 4, 5 and 6 mm s^{-1} cutting speed. The resistance of plant to lodging is closely related to the physical and mechanical properties of their stems (Alizadeh et al., 2011). According to O'Dogherty et al. (1995) the physical properties of plant materials depend on rate of loading. Similar results were reported by Yore et al. (2002) for paddy straw, by Alizadeh et al. (2011) for paddy stem. These results are in agreement with Chandio et al. (2013) who concluded that the average shear force and strength was obtained from 13 N to 15 N at 5, 10 and 15 mm min^{-1} for paddy stalk. Similar results were found by Yiljep and Mohammed (2005). They investigated that the effect of blade velocity on shearing energy and efficiency during impact of sorghum stalk. According to their results, the cutting energy requirement decreased with increase in blade velocity. The cutting energy required to cut sorghum stems showed a minimum at 2.9 m s^{-1} cutting speed and it increased as the cutting speed increased above 2.9 m s^{-1}. However, contrary to these results, our results did not in agreement with Yiljep and Mohammed (2005) results. The cutting force, strength and cutting energy increased with an increase in loading speed from 5 to 15 mm min^{-1}. Allameh and Alizadeh (2016) conducted a study on cutting properties of paddy. The results revealed that paddy cultivar and blade velocity had significant effects ($P<0.01$) on the specific cutting energy. There were significant differences among cultivars in the view of specific cutting energy so that the highest and lowest values belonged to Hashemi variety (29.29 kJ m^{-2}) and Khazar variety (16.81 kJ m^{-2}), respectively. When blade velocity increased from 1.5 m s^{-1} to 2.5 m s^{-1}, specific cutting energy raised about 77 %.

Also, to calculate and estimate the cutting force and energy values as the theoretical, a regression equation was derived from the average values for five blade-edge angle levels and five loading speeds. The linear regression equations and coefficients values are shown in Table 5 and Table 6. This derived linear relationship can be used to estimate the shearing properties to cut paddy stem.

Table 4. The relationship between cutting properties and cross-sectional area of paddy stem

Cutting speed (mm s^{-1})	Cutting force (N)	Energy strength (N mm^{-2})	Energy (Nm)	SEC (J mm^{-2})	Extension at Max. load (mm)
2	19.48 a*	1.281 a	0.043 a	0.003 a	12.49 b
3	18.94 ab	1.245 ab	0.042 a	0.004 a	13.43 a
4	18.01 b	1.185 b	0.041 a	0.003 a	12.34 b
5	17.64 b	1.160 b	0.037 b	0.003 a	12.83 b
6	17.58 b	1.157 b	0.039 b	0.002 b	12.59 b
Mean	18.35	1.214	0.041	0.003	12.73

*means followed by the same letter in each column are not significantly different by Duncan's multiple range test at the 1 % level.

Table 5. The regression equations depend on blade edge-angle.

Parameter	Regression equation	R^2
Shearing force (N)	$Y=29.322 – (3.628) X_1$	0.979
Shearing strength (N mm^{-2})	$Y=1.9284 – (0.238) X_1$	0.979
Shearing energy (Nm)	$Y=0.00662 – (0.0086) X_1$	0.969
Specific shearing energy (J mm^{-2})	$Y=0.0042 – (0.004) X_1$	0.80

X_1 is the angle of blade edge.

Table 6. The regression equations depend on blade loading speed.

Parameter	Regression equation	R^2
Shearing Force (N)	$Y=19.847 – (0.5026) X_1$	0.911
Shearing strength (N mm^{-2})	$Y=1.3055 – (0.0333) X_1$	0.910
Shearing energy (Nm)	$Y=0.0449– (0.0015) X_1$	0.969
Specific shearing energy (J mm^{-2})	$Y=0.0042 – (0.004) X_1$	0.80

X_1 is the blade loading speed.

Conclusion

The results of the analysis of variance and Duncan's test also showed that the main effect of blade type, blade angle and cutting speed and their interactions were found significant on shearing force, shearing strength, shearing energy, specific shearing energy and extension at maximum load ($p<0.01$). The lowest and best results were determined at serrated 2 blade types; 90° shearing angle and 6 mm s^{-1} loading speed, followed by the serrated 1 and flat-edge type blade. The highest values were observed at flat blade type. The cutting force and cutting energy increased with an increase in the blade edge angle from 50° to 90° edge angle. When we evaluated all measurement of interactions of Duncan test results, the peak values of shearing force, shearing strength, shearing energy, specific shearing energy and extension at maximum load were observed at flat-edge blade, 90° shearing angle and 2 mm s^{-1} cutting speed as 43.47 N, 2.86 nmm^{-2} and 0.106 Nm, 0.007 Jmm^{-2} and 24.5 mm.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Technical and Economic Features of Tractors in the Second Hand Market in Sanliurfa Province

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Abstract

In this study, it was aimed to determine various parameters of tractor use in agricultural production in Şanlıurfa province and the tendency of the farmers in the region towards tractor use by analyzing the collected data and obtaining generalizable results. In this context, an information gathering form was prepared to determine the technical and economic characteristics of tractors in the second-hand tractor market. The 450 information gathering forms were filled in face to face with the tractor dealers in the province, the dealer (broker) trading tractors and the vendors in the tractor market. The collected data were analyzed with the help of different statistical analysis programs. According to the results, the average rated engine power for tractors in the second hand market was determined as 57 kW. In addition, the average annual usage time of tractors in the second-hand market has been determined as 432.8 h, and other operating parameters related to the use of tractors include age, rated engine power, engine, gearbox, hydraulic system, cabin/hood and tires, and average annual usage. duration, estimated selling price, depreciation characteristics, etc. parameters were determined for tractors and the obtained data were evaluated by multiple regression analysis and the results were examined.

Keywords: Tractor, Operating parameters, Agricultural mechanization level, Sanliurfa

Introduction

The use of tractors in agricultural enterprises is an issue that needs to be examined in technical and economical terms. Scientific approach in making decisions about the tractor is a requirement of a rational management. Because, tractor investment has an important place in the fixed investment expenditures made in the enterprise. On the other hand, the part stemming from the tractor constitutes the leading part of the operating expenses. For these reasons, it is necessary to determine the operating parameters related to the use of tractors in order to make correct investment and management decisions.

Operating parameters of tractors related to use include age, rated engine power, engine power per unit area, loading rate, fuel and oil consumption, maintenance and repair costs, condition of engine-gearbox-hydraulic system-cabin/bodywork and tires, average annual service life, market value, depreciation characteristics, scrap value etc. parameters can be counted (Bowers 1975, Witney 1988b, Hunt 2001, Basol 2006). The values of these parameters may vary depending on the business and the user. The process, which initially started with the decision to choose suitable tractors for the business, ends in many different ways depending on the usage characteristics. From this point of view, determining the usage characteristics of tractors is important in terms of the appropriateness of the selection and the operational performance.

The aim of this study is to examine the various parameters of tractor use in agricultural production in Sanlıurfa-Harran

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region (age, rated engine power, engine, gearbox, hydraulic system, cabin/hood and tire condition, average annual usage time, estimated sales price, depreciation characteristics, etc.) obtains generalizable results by determining and analyzing the data collected for tractors. In this way, it is aimed to determine the tendency of the farmers in the region towards tractor use.

Material and Method

Material

The research material consists of the tractors found in the tractor dealers in Sanlıurfa Province and its districts and in galleries (brokers) that trade and exchange tractors. In order to reach the determined sample size, data of 450 tractors in total, which are offered for sale in 31 separate galleries, have been compiled. The general agricultural production characteristics of Sanlıurfa and its districts and the general soil structure of the districts, climate characteristics, the use of agricultural land, agricultural products production, business size and land distribution determine the types of tractors preferred in the plain.

Method

Method used to determine the sample size

An information gathering form was prepared to determine the technical and economic characteristics of tractors in the used tractor market. The 450 information gathering practices consisting of 20 questions were carried out by meeting face to face with the tractor dealers in the province, the dealer (broker) trading tractors and the vendors in the tractor market. An information gathering form was filled in for each tractor subject to sale in the market.

Conducting interviews and information gathering method

The questions in the information collection form used in the second hand tractor market research were prepared for the purpose of determining the current situation in the region in accordance with the purpose of the research. Some of the questions in the information collection form reflect the views of the authorized person interviewed on the issue. These questions were answered in line with the personal opinion of the authorized person and the general structure of the used tractor market. Before preparing the information gathering form, information about the general, technical and economic characteristics of tractors was reviewed. Particular attention was paid to selecting the most prominent and relevant questions on the subject. Some of the questions in the form include the date of the interview, the place where the interview was held, the address information of the interviewee and the company. Most of the questions in the form are related to the technical and economic characteristics of the tractor. These are engine, cabin-hood, gearbox, hydraulic system and tire condition.

Methods used in data analysis

The questions in the information collection forms showing the technical and economic characteristics of tractors were filled out separately for each tractor. The information collection forms obtained as a result of the research were grouped and numbered primarily on the basis of districts. The answers to the questions in the information collection forms were processed in a workbook created in the Microsoft Excel program. The data obtained were analyzed with the help of various statistical analysis programs (SPSS, MINITAB).

Regression relations between variables were determined by creating means, proportional values and tables with the data obtained by evaluating the answers to the questions in the information collection forms. The data classified on the basis of two or more characteristics of the tractors within the scope of the study were evaluated. Accordingly, the degree of affinity between two or more tractors of tractors was determined. By applying statistical analysis to these variables, the model giving the highest degree relation was determined.

Later, in determining the relationship between the estimated sales price of tractors in the second-hand market and other variables, the LIMDEP package program was used for model approaches based on data transformations, tabulation, linear and nonlinear regression analysis (Gül et al. 2001). In the multiple regression analysis model used within the scope of the program, the estimated sales price (ESP) parameters for the tractors in the second hand market were used as the dependent variable.

Tractor brands (New Holland, Uzel (MF), Türk Traktör (FIAT) and Ford), tractor age, number of cylinders, rated engine power, total service life, engine condition, engine revision, cabin-bodywork condition, gearbox-differential, the parameters related to the hydraulic system and tire conditions were included as independent variables in the models. By correcting the variation of error terms in the model, each observation has the feature of having equal variation.

The following formula was used for calculating the elasticity coefficients of the variables in the developed linear models (Cinemre and Ceyhan 1998).

$$e = \frac{\partial Y}{\partial X} * \frac{\overline{X}}{\overline{Y}} \quad \text{................(1)}$$

where,

e: Elasticity coefficient is used to calculate what percentage change in the independent variable creates a percentage change on the dependent variables of supply or demand (Anonymous, 2002),

$\frac{\partial Y}{\partial X}$: First order derivative of the dependent variable with respect to X (coefficient of X),

$\overline{X}$: Average value of the explanatory variable,

$\overline{Y}$: Shows the average value of the dependent variable.

In order to achieve meaningful results in this model, the elasticity coefficients were determined and evaluated in order to reveal the percentage change on the tractor purchase price (TPP), which is the dependent variable of the other independent variables (AGE, TUT and REP), which are not only dummy variables (expressed as unit 1 or zero).

Results and Discussion

Among the technical features of the tractors subject to research, the characteristics of the traction condition come first. The 87% of the second hand tractors offered for sale in the market consist of standard structured tractors called single wheel drive. Double wheel drive tractors have a proportional share of 13%. This situation encountered can be said that the enterprises in the plain have turned to the use of double-wheel drive tractors with the polyculture product pattern implemented with irrigation.

The 91% of the tractors in the second hand market have 4 cylinders. Here, it is observed that the number of cylinders of medium power tractors (50-65 kW), which is the dominant group, stands out.

One of the most important operating parameters that come to the fore in researches on tractors is the duration of use. Because the operating time of the tractor directly affects the operating costs. In Figure 1, the total service life (TSL) values of the tractors in the second hand market with reliable data are given depending on the age. The lineer equation obtained as a result of the regression analysis was placed on the scatter plot of the data.

A probability level of p <0.0001 was determined with a coefficient of indication of R2 = 0.477 in the linear model between the age and TSL parameters for tractors in the second hand market. The equation for the linear model is y = 432.8 * x. From the first derivative of the regression equation obtained, the annual average usage time can be calculated as 432.8 h (Figure 1).

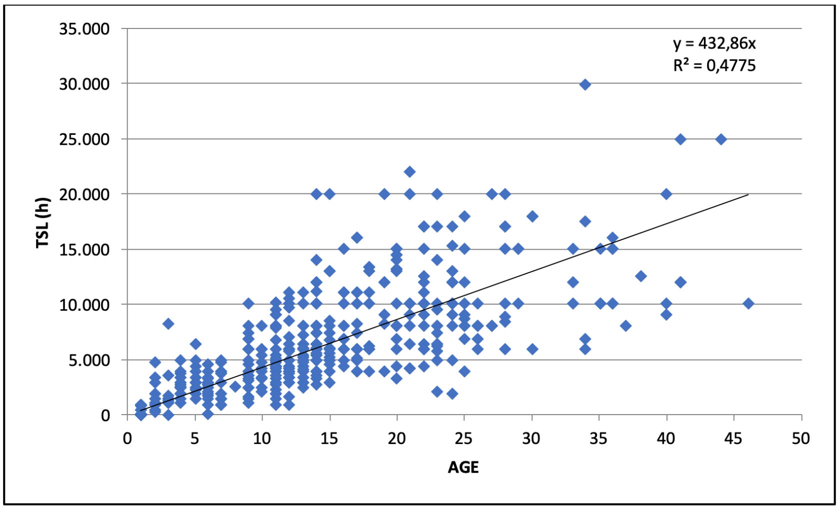

Figure 1. The change between tractor age and total service life (TSL) in the used tractor market

The graph shown in Figure 2 is obtained by taking the average of the total usage periods of tractors of the same age from the data in Figure 1. It has been determined that the observation values in the graph are suitable for a model (cubic model) that can be expressed with a third order equation with the coefficient of determination of R^2 = 0.7876 at the probability level of p <0.0001. The equation of the determined model is; y = 0.0537´3-8.0937x2 + 596.45´-65.509.

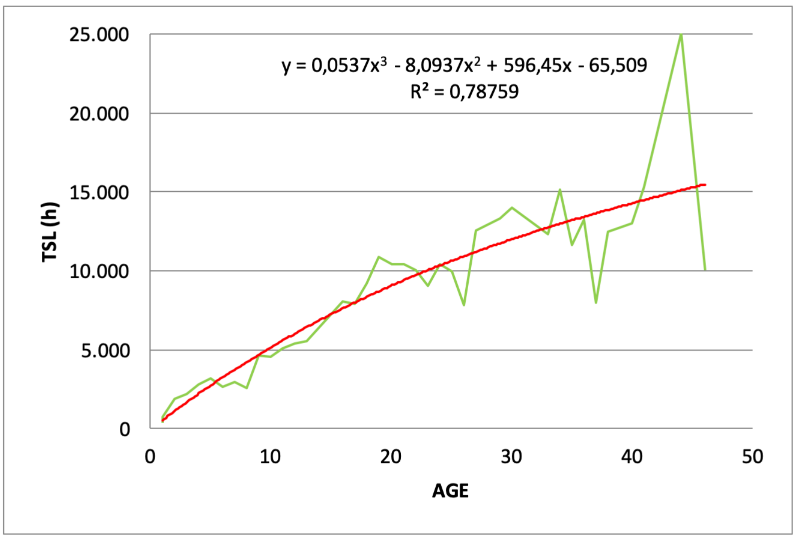

Figure 2. Change of age and total usage time values obtained according to average values

The average annual usage time of tractors in the second hand market is 432.8 hours. These values are based on the average annual usage time of tractors (365 h/year) determined for Harran District in the study conducted by Isik (1998) in Sanlıurfa-Harran Plain, and the average annual usage time determined by Sumer et al. (2008) for used tractors in Canakkale (377 hours/year) and Akinci and Canakci (2000) for the enterprises with the highest number of enterprises in the Antalya region with a land size of 5.1-10 ha values found for tractors and some in Business Administration (550.6 h/year) again, given robust and Akdemir in the literature (2002) by the average annual usage period set for Turkey's north west (479.32 h/year) high and tractors in the second hand market. The values found for (432.8 h/year) are lower than the values in the same literature.

Considering the literature information such that the hourly total costs of tractors decrease with the increase of annual usage time, hourly tractor costs are lower for enterprises with large production areas (Henderson and Fanash 1984) and the tractor usage time should be over 800 hours economically (Saral 1982), at least 650 h/year should be used and that the usage between 850-1000 h/year is the range where the tractor is used effectively (Demirci 1986), it is seen that the tractor usage time is quite low under the conditions of the Harran Plain.

The most important technical parameter of agricultural tractors is the rated engine power. This value significantly affects the machine size and operating costs that the tractor can operate. The rated engine power of tractors encountered in businesses varied between 40 kW and 86 kW. The rated engine power of tractors encountered in the second hand market varied between 35 kW and 89 kW. Examining the tractors in the used tractor market, it is understood that the power distribution is in the 50-65 kW range, which can be described as the medium power group.

It can be said that the proportional increase in the amount of tractors for sale with a nominal engine power of 50-55 kW in the second-hand market depends on the lack of power. With the widespread use of irrigated agriculture in the Harran Plain, it is observed that the tractors and the equipment operated with them have diversified and their capacities have increased in parallel with the applied product pattern and the tractors with a rated engine power below 45 kW are decreasing in the region. Accordingly, it is observed that there is an increase in the use of high powered tractors. In the example taken from the second hand tractor market, the average rated engine power value of all tractors was calculated as 57 kW.

However, the average tractor power for Turkey was 44 kW according to Agricultural Machinery and Equipment Manufacturers Association (TARMAKBIR) report for 2007 (Anonymous 2007). Sabanci et al. (2003a) Evcim et al. (2010) reported it as 43,6 kW and 43 kW, respectively. According to these values, the average power determined to track tractors scope of work in the second hand market (57 kW) seems higher than the average in Turkey. However, it is lower than the average tractor engine power (74 kW) determined for EU countries (Anonymous, 2007).

In order to determine the average annual service life of tractors in the second hand market, the total usage time (TUT) value was proportioned to the tractor age (AGE). The data obtained are given in 4.11 depending on the rated engine power. The results of both the shape examination and the statistical analysis revealed that the average annual usage time is independent of the rated engine power for tractors in the second hand market.

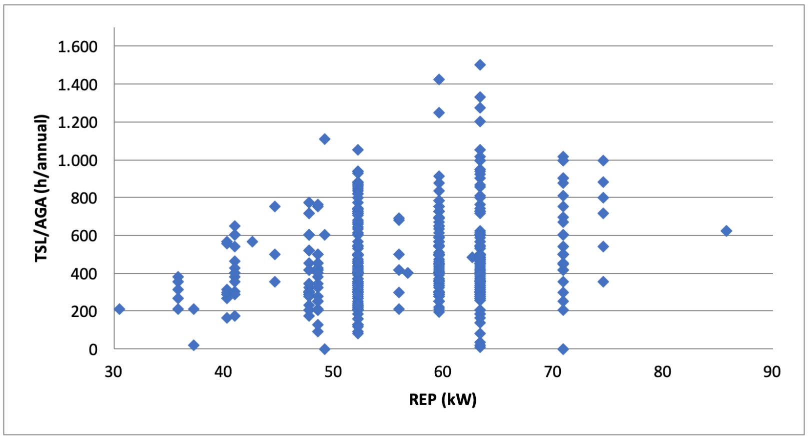

Figure 3.Variation of the average annual service life of tractors in the second hand market depending on the rated engine power (REP)

The 30% of the total tractors evaluated within the scope of the study are tractors that have not undergone engine revision before (0 revision number). Considering that the average age of the tractors in the example is approximately 15, it is seen that the remaining tractors have undergone at least one engine overhaul. On the other hand, by examining the number of revisions depending on age, it is seen that there is no tractor that underwent revision before the age of 8. In the 8-10 age range, it is observed that the first or the second engine revision took place. Tractors between the ages of 10-18 have at least 1 engine revision, and this number can go up to 5. If the age of 18 and over is, the average number of engine revisions is 2.6 per tractor.

It is noteworthy that the engine, gearbox and hydraulic system repair requirements of tractors in the used market show age-related repetitive changes. This situation, which shows periodic changes, is thought to stem from the use and the importance of these three organs for the tractor. In other words, it is absolutely necessary to eliminate the malfunctions of these organs in terms of the tractor's function.

However, the change obtained in terms of the cabin/body repair requirement is different from these. This need for repair arises both at a later age and is considered to be a delayed repair requirement from the farmer's perspective. We have the impression that the reason for the proportional highness of 28-year-old tractors in the second-hand market in terms of the need for cabin/hood repair is due to the fact that for the first time such a requirement arises at the mentioned age is a result of the said delay. While there are periodic renewals that can be encountered at an early age due to use in the tire renewal requirement of tractors in the used tractor market, a proportional increase at the age of 14 and 22 is noteworthy. The reason for this is thought to be due to the general aging of the tractor.

Since the tractors encountered in the second hand market have not been sold yet, the monetary values demanded by the sellers have been named as the estimated sales price and used in order to determine the value of the tractor. In determining the estimated sales price, the opinions of the experts of the organizations operating in the market were also used. Figure 4a and 4b shows the distribution of estimated sales price (ESP) values depending on the model year of the tractors in the example. Figure 5 shows the distribution of estimated sales price values depending on the age of the tractor.

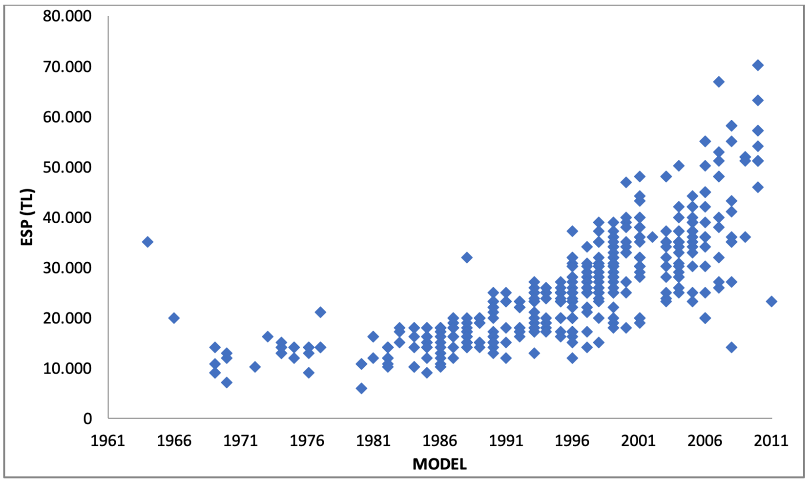

Figure 4a. The change of the estimated sales price (ESP) of tractors in the second-hand market depending on the model year.

As a result of the analyzes made by taking the average for the purpose of modeling the distribution, it was determined that the age-related estimated sales price values correspond to a third order (cubic) function at a high degree ($R^2 = 0.961$) and at the probability level of $p < 0.01$. The equation of the cubic model obtained is y = -0.495x3 + 60.06x2-2562x + 49932. Figure 5 shows the average values and the graph of the cubic model obtained depending on them.

The decrease in the estimated sales price (ESP) of tractors with the increase in age in tractors in the second hand market is similar to the results given in the literature (Isik et al. 1995, Guher 2008, Basol 2006).

As a result of the statistical analysis made with the average estimated sales price values of tractors with the same usage hours, the distribution of the estimated sales value of tractors in the second-hand market based on the total operating time (TOT) is second-order ($R^2 = 0.4858$, $p < 0.01$).) a relationship has been identified. The equation of the model obtained is y = 0.000005x2-2.4593x + 39792 (Figure 6).

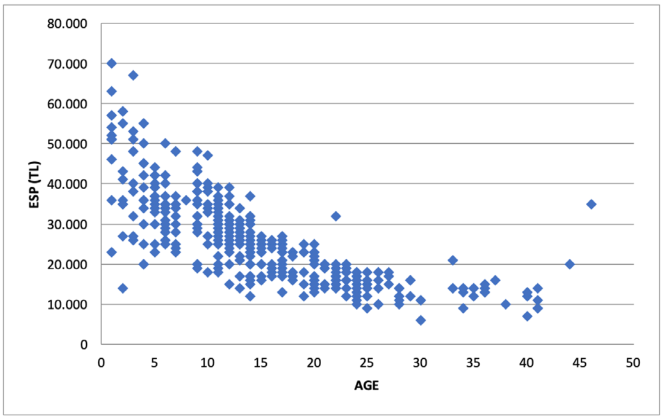

Figure 4b. Age-related change in the estimated sales price (TSB) of tractors in the second hand market

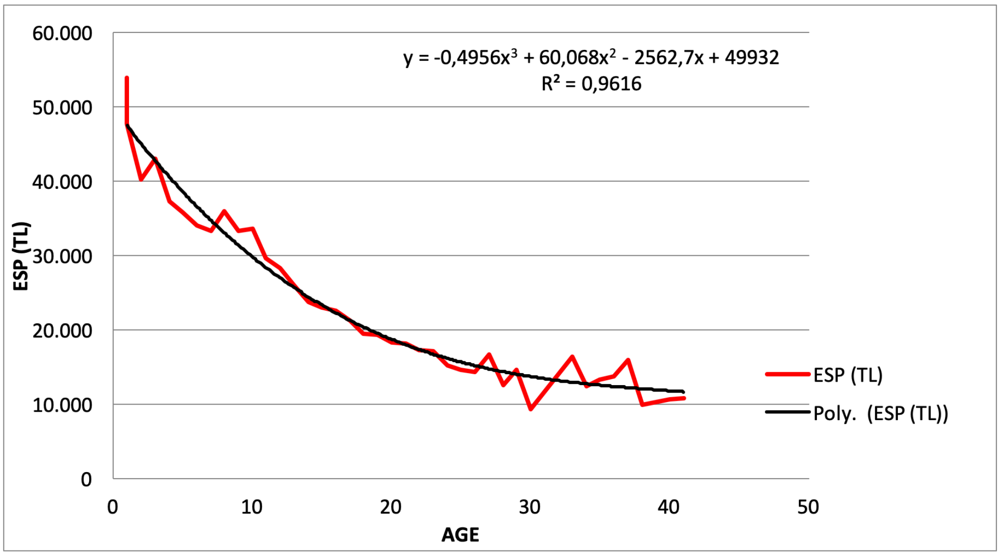

Figure 5. Changes in the age-estimated sales price (ESP) values of tractors in the second-hand market according to average values.

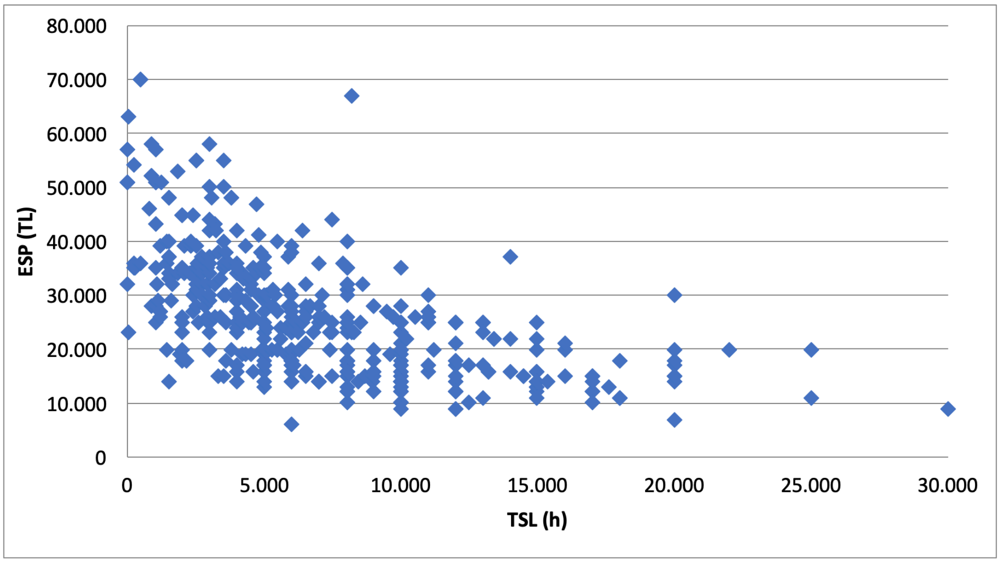

Figure 6.Variation of the estimated sales price (ESP) of tractors in the second hand market depending on the total service life (TSL)

In the study, the average annual life of the tractor was obtained by proportioning the total usage time (TUT) read from the tractor meter to the age. Figure 7 shows the distribution of estimated sales price (ESP) values depending on the TUT/AGE parameter.

Statistical analysis revealed that there was no relationship between the TUT/AGE parameter and TSB that could be subject to the model. The estimated sales price (ESP) of the tractor in the second-hand market is determined by the effects of various factors. The first factor that comes to mind among these is the tractor's rated engine power (REP).

As a result of the analyzes made for the purpose of modeling the relationship between the estimated sales prices of tractors in the second-hand market and the nominal engine power, the estimated sales price values depending on the nominal engine power can be converted to a third-order (cubic) function (R^2 = 0.821, p <0, 01) level.

The equation of the cubic model obtained is y = 0.163x3-13.94x2-532.5x + 11242. The result obtained here shows that the rated motor power factor has a high effect, approximately 82%, on the estimated sales price (Figure 8).

On the other hand, it was previously determined that the age-related estimated sales price values fit a third-order (cubic) function with a high degree (R^2 = 0.961), at the level of p <0.01. Accordingly, the age parameter has a higher effect on the estimated sales price than the nominal motor power parameter. These values are similar to the values given in the literature (Basol, 2006). As a result of the analyzes aimed at determining the effects of both parameters on the market value together, the following equation has been obtained:

TSP (TL) = 20109,187 - 835,793 ´ *AGE* + 325,623 ´ *REP* (kW)

In this equation, R^2 = 0.637 and p <0.01, while the standard error of the estimation made using the equation is ± 6719.26 TL. The data obtained within the scope of the study are in great agreement with the results obtained in the study of Isık et al. (1995) in the literature and previously reported.

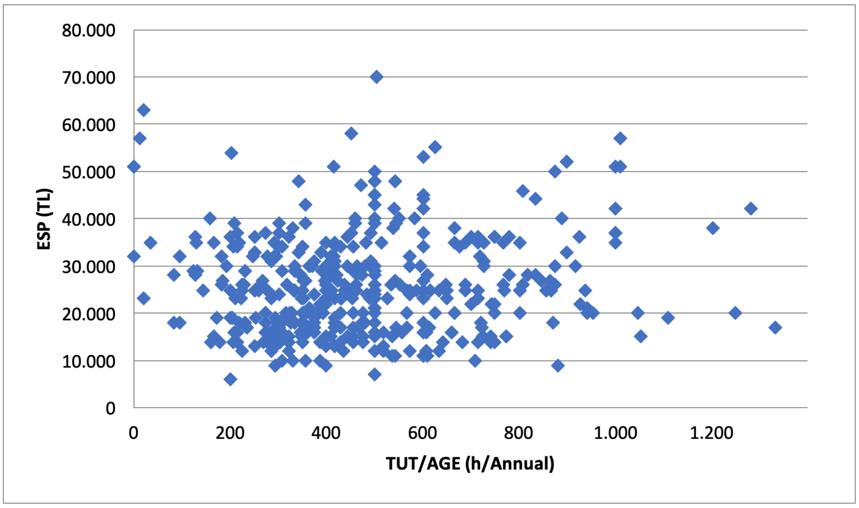

Figure 7. Variation in the estimated sales price (ESP) of tractors in the second hand market depending on the average annual usage period (TUT/AGE)

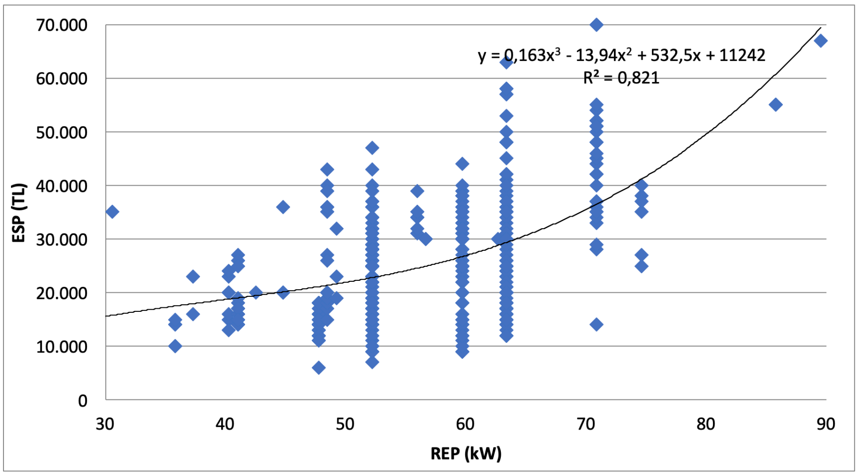

Figure 8. Variation of the estimated sales price of tractors in the second hand market depending on the average rated engine power.

Likewise, when the estimated sales price of the unit rated engine power of tractors is shown with the ratio of TSP/REP, the change of these values depending on the age is seen in Figure 9. In this graph, the average of the TSP/REP values of different tractors of the same age was obtained, and the observation values in Figure 10 were obtained. It was determined that the observation values in this way fit a third-order model with an indication coefficient of R^2 = 0.563 and a probability level of p <0.01.

The equation of the cubic model obtained is y = 0.020x3-1.364x2 + 16.07x + 470.9. In other words, it is observed that due to the increase in tractor age, the estimated sales price (ESP) for unit power decreases within the framework of the determined model.

In order to have information about the depreciation characteristics of tractors in the second-hand market, used tractors, of which new ones are still produced and sold, were also analyzed. For this purpose, the ratio of estimated sales price (ESP) values to the sales price (SP) of the new one of the same tractor was used as the analysis parameter. The change of the values obtained is given in Figure 11.

Analyzes for tractors in the second-hand market highlighted two different model types. The first of these is the linear model and it has a coefficient of indication of R^2 = 0.483 and a probability level of p <0.01. The equation for the linear model is y = -1,352X + 64.11. The second model is the cubic model with a third order equation. In this model, R^2 = 0.856 and p <0.01. The equation for the cubic model is y = -0.017X3 + 0.879X2-13.34X + 89.66. Analyzes made by Hunt (2001) for US conditions based on the age-dependent value (the value of the tractor at the beginning of the year - the depreciation amount for that year) also showed a very strong (R^2 = 0.924, p <0.001) cubic model between these two parameters. revealed that it was found. A similar result can be obtained with the values given by Witney (1988b).

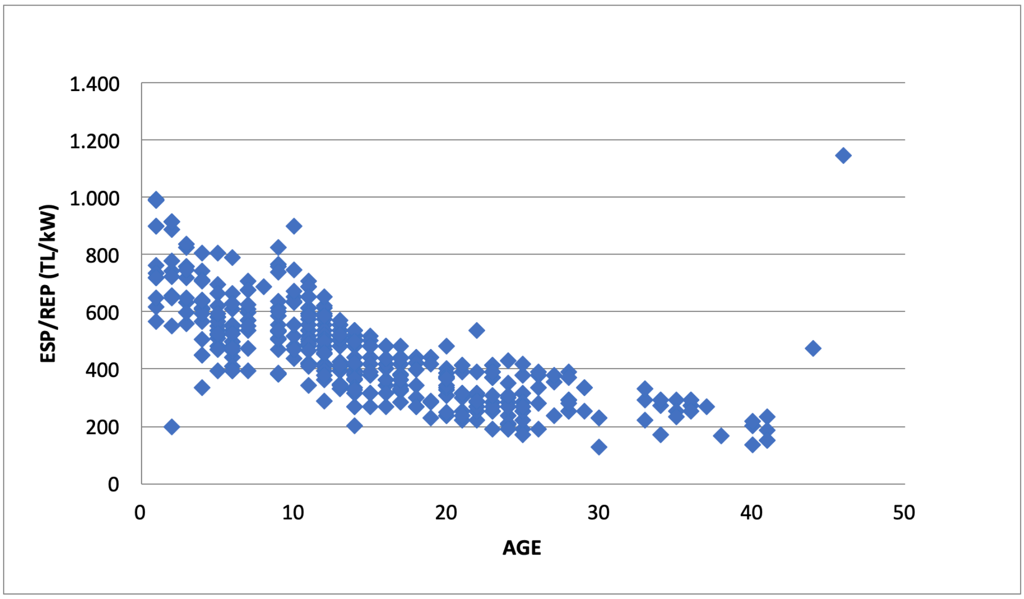

Figure 9. Age-dependent variation of the estimated purchase price (TSP/REP) of the unit rated engine power of tractors in the second hand market

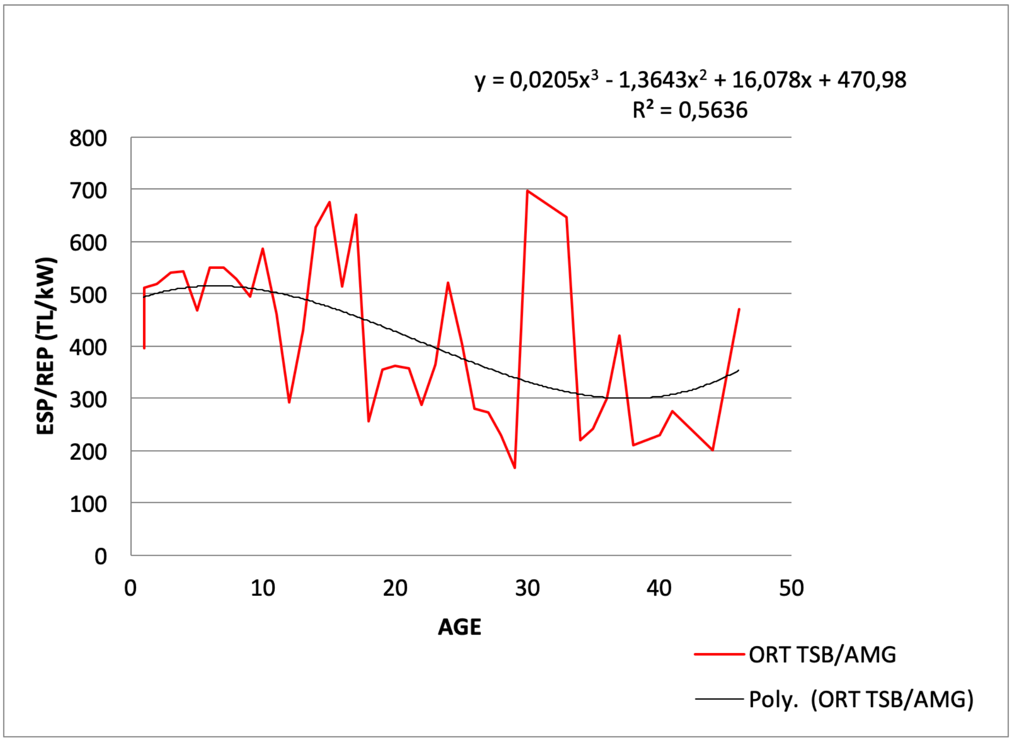

Figure 10. The change of age-related TSP/REP values obtained according to the mean values and the graph of the cubic model obtained accordingly

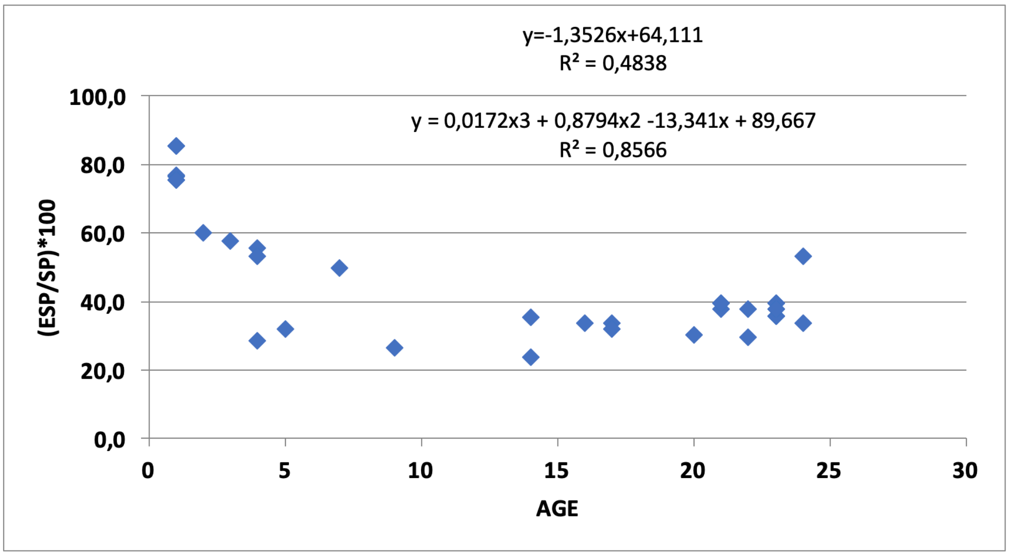

Figure 11. Age-related TSP /SP values of tractors in the second hand market

Results and Conclusions

With this study, the properties of used tractors for sale in Sanlıurfa Harran Plain (Central, Harran and Akcakale Districts) were tried to be determined. These features and some other factors that are thought to be effective were analyzed and their degree of influence was investigated. Studies conducted have revealed the results summarized below. According to this:

1) The average rated engine power of tractors in the second hand market in the research area is 57 kW, while the rated engine power of the tractors varies between 35-89 kW, while the power distribution is in the range of 50-65 kW, which is also described as the medium power group.

2) The average age of tractors in the second-hand tractor market is approximately 15, and tractors in the 0-15 age group constitute approximately 60% of the total tractors and 30% in the 16-30 age group. It is seen that 43% of the tractors in the second hand market are 15 years old and older tractors. Considering the 15-year economic life given in the literature for tractors (Sabancı et al. 2003a, Sabancı et al. 2003b), this situation reveals that the use of tractors that have completed their economic life is quite significant. However, when we look at the sales reasons for the tractors in the second hand market, 48% of the tractors are due to model aging, 2% to wear and again 2% to lack of capacity. This shows that more than half (52%) of second-hand tractors in the market have been put up for sale for upgrades; this reveals the renewal potential of the tractor park in Şanlıurfa Harran Plain.

3) The average annual usage time of tractors in the second hand market is 432.8 hours. When the product projections proposed for the region in both the GAP Master Plan (Anonymous 1989) and the GAP Regional Development Plan (Anonymous 2001b) are implemented, these values for the average tractor usage periods are calculated as the tractor used economically (650 h/year) or effectively (850-1000 h / year). years). In order to increase this period, it is necessary to increase the use of agricultural machinery foreseen by irrigated agriculture depending on the variety of product patterns of the enterprises.

4) As a result of the analysis of the regression analysis between the total usage time (TUT) of the tractors in the second hand market and the age parameters, it was observed that the total usage time of the tractor increased depending on the age of the tractor.

5) It has been observed that there is a strong negative relationship between the age-related market value of tractors in the second-hand market and the age-related estimated sales price. The price determination for used tractors is based on market conditions. Average prices are determined by model (age) and rated engine power and brand. Price values, which are highly affected by the general economic situation, are adopted by almost all market players. Other features of the tractor may have little effect on the actual price.

6) For tractors in the second hand market, it has been determined that the average annual service life is independent of the rated engine power.

7) Approximately 49% of the variation in the estimated sales price (ESP) of tractors in the second hand market can be explained by the variation in the total usage period.

8) It has been revealed that there is no relationship between the average annual service life (ASL/AGE) parameter of tractors in the second hand markets and the estimated sales price (ESP) that can be subject to the model. It is thought that the low annual average usage time of tractors is effective on this result.

9) The 82% of the variation in the estimated sales price of tractors in the second-hand market can be explained by the change in the rated engine power (REP) of the tractors. In this context, it has been determined that the rated engine power (REP) is very effective in price formation, especially for tractors in the second hand market.

10) The 56% of the change in unit power price (TSP/REP) in tractors in the second hand market is due to the age factor.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Data availability

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Consent for publication

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