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## RESEARCH ARTICLE

### The Silicon Effects on Antioxidant System of Wheat Cultivars under Pb Stress

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Payame Noor University, Faculty of Basic Sciences, Department of Biology, Tehran/Iran

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#### ABSTRACT

Pb poses a major threat to plant growth and silicon can reduce its toxicity. This work was conducted hydroponically as a completely randomized factorial design to study the effect of Si (70 and 140 ppm) on *Triticum aestivum* cultivars Chamran and Shiroudi under Pb stress (150 ppm). Pb caused significant increases in the H<sub>2</sub>O<sub>2</sub>, free amino acids and proline contents of wheat cultivars and MDA content of cv. Chamran. Furthermore, Pb stimulated the activities of SOD and APX in cv. Chamran and POD in cv. Shiroudi. Si application significantly increased the free amino acid content of cultivars and proline content of cv. Chamran in absence of Pb. The protein content of wheat cultivars significantly increased at 70 ppm of Si in absence of Pb and at both levels in presence of Pb. In cv. Chamran, Si application significantly decreased the H<sub>2</sub>O<sub>2</sub> content and the activities of SOD, POD and APX at both levels, free amino acids and proline contents at 70 ppm and MDA content at 140 ppm in presence of Pb. In cv. Shiroudi, Si application significantly decreased the proline content at both levels, H<sub>2</sub>O<sub>2</sub> and free amino acids contents at 70 ppm and MDA content at 140 ppm.

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#### Introduction

Silicon is an abundant element in soils and consists about 28% of the earth's crust (Emamverdian and Ding, 2017). Plants normally absorb silicon in the form of monosilicic and polysilicic acids. Even though, this element is not traditionally considered as an essential element for plants, but it has positive effects on plant's growth and could alleviate the environmental stresses by changing the extracellular matrix (apoplast), improving the water transport and water status of plant, affecting the ion transport, increasing the plant's antioxidant activities and reducing the lipid peroxidation (Liang et al., 2007; Emamverdian and Ding, 2017; Kim et al., 2017).

Lead (Pb) is an abundant and ubiquitous toxic element that is present in soils, seawaters, lakes and rivers. Due to low

solubility and strong binding capacity with soil colloids, Pb has long residence time in soil, causing a large number of direct and indirect effects on plants growth and metabolism (Britto et al., 2011).

Wheat contributes more calories and proteins to the world diet than any other cereal crops and is considered as a good source of protein, minerals, B-group vitamins and dietary fiber (Kumar et al., 2011). The aim of this work was to study the antioxidant system responses in two wheat cultivars (Chamran and Shiroudi) to Si application under lead stress.

#### Materials and Methods

The seeds of wheat (*Triticum aestivum* L.) cultivars (cvs.) Shiroudi and Chamran were obtained from the Agricultural Research Center of Tabriz, Iran.

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### **Plant Growth Condition**

The experiment was conducted in a growth chamber with a temperature regime of 28/20°C, photoperiod of 16 h and relative humidity of 70%. After disinfection, seeds were germinated in petri-dishes and uniform seedlings were transferred to pots containing one liter of Hoagland solution. One week after transferring, Pb (150 ppm, as lead acetate) and Si (70 and 140 ppm, as potassium silicate) were applied through root medium. Plant's shoots were harvested two weeks after treatments and frozen in liquid nitrogen until assays.

### **Antioxidant Enzymes Assays**

To obtain the crude extract, 0.5 g of leaves were homogenized in 5 mL of 50 mM potassium phosphate buffer (pH 7), containing 0.2% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12000 g at 4°C for 20 min. The resulting supernatant was used to measure the activities of antioxidant enzymes. Each enzyme assay was tested for linearity between the volume of crude extract and the measured activity. Changes in the absorbance of substrates or products were measured using spectrophotometer.

The activity of superoxide dismutase (SOD) was measured according to its capacity to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture contained 2.65 mL of 67 mM potassium phosphate buffer (pH 7.8), 0.2 mL of 0.1 mM EDTA solution containing 0.3 mM sodium cyanide, 0.1 mL of 1.5 mM NBT, 50 mL of 0.12 mM riboflavin and 0.5 µL of enzyme extract. One unit of SOD was defined as the amount of enzyme that caused 50% inhibition of NBT reduction (Winterbourn et al., 1976).

Guaiacol peroxidase (POD) assayed following the method of Chance and Maehly (1955). The reaction mixture contained 1.5 mL of 100 mM citrate-phosphate-borate buffer solution (pH 7.5), 50 µL of 15 mM guaiacol, 25 µL enzyme extract and 50 µL of 3.3 mM H<sub>2</sub>O<sub>2</sub>. The polymerization of guaiacol was initiated by adding H<sub>2</sub>O<sub>2</sub> and an increase in absorbance at 470 nm was recorded for 3 min. POD activity was calculated using the extinction coefficient, 26.6 mM<sup>-1</sup>.cm<sup>-1</sup>, for guaiacol. The generation of 1 µM of tetraguaiacol per min was catalyzed by the amount of enzyme that was introduced as one unit of POD.

The activity of ascorbate peroxidase (APX) was measured according to Nakano and Asada (1987). The reaction mixture contained 25 µL of enzyme extract with 2.5 mL of phosphate buffer (pH 7) containing EDTA 0.1 mM, H<sub>2</sub>O<sub>2</sub> 1 mM and ascorbic acid 0.25 mM. The decrease in absorbance at 290 nm for 1 min was recorded and the amount of ascorbate oxidized was calculated using extinction coefficient of 2.8 mM<sup>-1</sup>.cm<sup>-1</sup>. One unit of APX was defined as the amount of enzyme that oxidized 1µM of substrate.min<sup>-1</sup>.

The catalase (CAT) activity was determined by monitoring the absorbance decrease due to H<sub>2</sub>O<sub>2</sub> dismutation at 240 nm for 3 min. The reaction mixture contained 1.5 mL of 100 mM citrate-phosphate-borate buffer solution (pH 7.5), 50 µL enzyme extract and 13 µL of 10 mM H<sub>2</sub>O<sub>2</sub>. The amount of enzyme for dismutation of 1 µM H<sub>2</sub>O<sub>2</sub> per min was expressed as

one unit. Extinction coefficient for H<sub>2</sub>O<sub>2</sub> was considered 39.4 mM<sup>-1</sup> cm<sup>-1</sup> (Obinger et al., 1997).

### **Proline Assay**

The proline was extracted with 10 mL of 3% sulfosalicylic acid solution. 2 mL of liquid was reacted with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid for 1 h in 100°C and reaction was terminated at ice bath. The reaction mixture was extracted by 4 mL toluene. The absorbance of chromophore containing toluene was read at 520 nm (Bates et al., 1973). Proline concentration of samples was determined from a standard curve.

### **Free Amino Acid Assay**

Free amino acids were extracted by 80% ethanol and centrifuged at 5000 g for 10 min. Supernatants were taken in to test tubes and 1 mL of ninhydrin reagent and 0.2 mL of citrate buffer added to them. The mixtures were incubated at 100°C in a water bath for 10 min. The absorbance of samples was measured at 570 nm and free amino acid contents were calculated using a standard curve of glycine (Yemm and Cocking, 1955).

### **H<sub>2</sub>O<sub>2</sub> and MDA assays**

To obtain the crude extract, 0.1 g of leaves were homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12000 g at 4°C for 15 min.

The H<sub>2</sub>O<sub>2</sub> content was assayed according to the Harinasut et al. (2003). To 0.5 mL of the supernatant, 0.5 mL of 10 mM phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide was added. The mixture was incubated at 25°C for 15 min. The absorbance was measured at 390 nm. The H<sub>2</sub>O<sub>2</sub> content was calculated using a standard curve of H<sub>2</sub>O<sub>2</sub>.

For malondialdehyde (MDA) assay to 1 mL of the supernatant, 4 mL of 20% TCA containing 0.5% thiobarbituric acid was added. The mixture was incubated at 95 °C in a water bath for 30 min, and then quickly cooled on ice. The mixture was centrifuged at 10000 g for 15 min and the absorbance was measured at 532 nm. MDA levels were calculated from 1,1',3,3'-tetraethoxypropane standard curve (Heath and packer 1968).

### **Total Protein Assay**

Total protein content was measured by the method of Bradford (1976) using bovine serum albumin as a standard.

### **Statistical Analysis**

All assays were carried out in triplicate and the results were presented as mean values ± SD. Statistical analyses were performed using a one-way analysis of variance test and the significance of the differences between means was determined by Student's multiple range test. The InStat (3.0) software was used to perform statistical analysis.

**Results and Discussion**

**Antioxidant Enzymes**

In cv. Chamran, Pb induced the significant ( $p < 0.05$ ) increases in the activities of SOD (10.87%) and APX (46.19%). Si application had not significant effect on the activities of antioxidant enzymes in absence of Pb in this cultivar, but significantly decreased the SOD (43.42% and 32.44%), POD

(49.11% and 35.76%) and APX (28.47% and 30.9%) activities at 70 and 140 ppm in presence of Pb (Table1).

In cv. Shiroudi, Pb significantly increased the activity of POD (24.06%). Si application could not change the activities of antioxidant enzymes in absence of Pb, while its application at 140 ppm significantly decreased the activities of SOD (27.49%), POD (54.23%) and APX (28.84%) in presence of Pb (Table 1).

**Table 1.** Effect of Si on the activities of antioxidant enzymes of wheat cultivars under Pb stress.

Cultivar	Treatments	CAT (U g <sup>-1</sup> FW)	SOD (U g <sup>-1</sup> FW)	POD (U g <sup>-1</sup> FW)	APX (U g <sup>-1</sup> FW)
Chamran	Control	0.14±0.02 <sup>bc</sup>	287.09±4.37 <sup>b</sup>	3.37±0.47 <sup>a</sup>	1.97±0.03 <sup>b</sup>
	Pb 150	0.15±0.02 <sup>b</sup>	318.32±2.21 <sup>a</sup>	3.97±0.12 <sup>a</sup>	2.88±0.13 <sup>a</sup>
	Si 70	0.13±0.03 <sup>bc</sup>	276.7±6.21 <sup>b</sup>	3.64±0.21 <sup>a</sup>	2.03±0.11 <sup>b</sup>
	Si 140	0.16±0.03 <sup>b</sup>	299.4±9.11 <sup>ab</sup>	2.94±0.71 <sup>ab</sup>	2.11±0.04 <sup>b</sup>
	Pb150, Si 70	0.13±0.01 <sup>bc</sup>	180.08±7.73 <sup>c</sup>	2.02±0.11 <sup>b</sup>	2.06±0.02 <sup>b</sup>
	Pb 150, Si 140	0.16±0.05 <sup>b</sup>	215.05±7.29 <sup>bc</sup>	2.55±0.15 <sup>b</sup>	1.99±0.14 <sup>b</sup>
Shiroudi	Control	0.16±0.01 <sup>A</sup>	238.16±7.37 <sup>AB</sup>	4.28±1.00 <sup>B</sup>	2.22±0.29 <sup>A</sup>
	Pb 150	0.14±0.02 <sup>AB</sup>	281.72±9.70 <sup>A</sup>	5.31±0.29 <sup>A</sup>	2.60±0.47 <sup>A</sup>
	Si 70	0.17±0.03 <sup>A</sup>	232.11±6.14 <sup>AB</sup>	4.22±1.1 <sup>B</sup>	2.32±0.33 <sup>A</sup>
	Si 140	0.15±0.02 <sup>A</sup>	229.17±8.36 <sup>AB</sup>	3.98±0.96 <sup>B</sup>	2.29±0.49 <sup>A</sup>
	Pb 150, Si 70	0.12±0.01 <sup>B</sup>	221.28±9.45 <sup>AB</sup>	3.64±1.16 <sup>AB</sup>	2.48±0.12 <sup>A</sup>
	Pb 150, Si 140	0.17±0.02 <sup>A</sup>	204.26±16.4 <sup>B</sup>	2.43±0.87 <sup>B</sup>	1.85±0.08 <sup>A</sup>

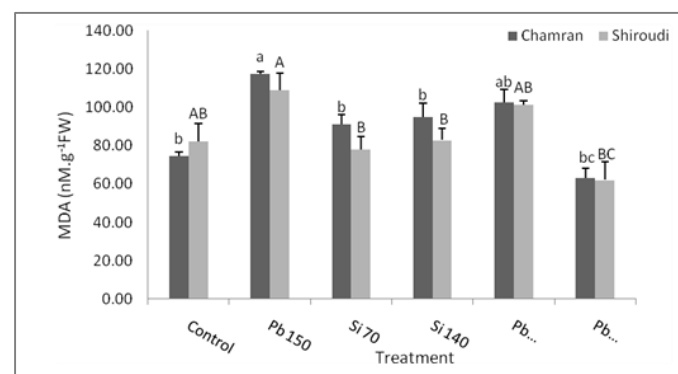
Values are means ± SD of three replicates; different letters in each column indicate significant differences at  $p < 0.05$ .

Exposure to heavy metals enhances the ROS production and promotes oxidative stress in plants. The induction of antioxidant enzymes activities is necessary for protection the plants tissues and organelles against the produced ROS species under stressful conditions. The responses of antioxidant enzymes to heavy metals is varying among plant species, cultivars and tissues (Shu et al., 2012). SOD is considered as the first defense against oxidative stress and catalysis the transformation of free superoxide radicals to H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> (Pourrut et al., 2011). The product of SOD, H<sub>2</sub>O<sub>2</sub>, is the substrate for peroxidases and catalase (Fendereski et al., 2015). In this study, Pb induced the notable increases in the activities of SOD and APX in cv. Chamran and POD in cv. Shiroudi. Catalase is an antioxidant enzyme that isn't active in low concentrations of H<sub>2</sub>O<sub>2</sub> (Shu et al., 2012). Therefore, the concentration of produced H<sub>2</sub>O<sub>2</sub> in wheat cultivars is not adequate to up-regulation the catalase activity under Pb stress. It seems that the alleviated activity of APX in cv. Chamran and POD in cv. Shiroudi are sufficient to remove the produced H<sub>2</sub>O<sub>2</sub>.

Silicon application under Pb stress could moderate the activities of up-regulated antioxidant enzymes in wheat cultivars. The stimulatory and also inhibitory effects of Si on antioxidant enzymes activities in different plant species have been reported by authors under heavy metals stresses (Shi et al., 2010; Tripathi et al., 2015). For example, similar to results of this study, Pontigo et al. (2017) reported the significant reductions in the activities of antioxidant enzymes by Si application in Al- treated plants of ryegrass. It seems that there are obvious differences between plants species and varieties from the viewpoint of antioxidant enzymes activities in response to Si application under various environmental stresses.

**MDA and H<sub>2</sub>O<sub>2</sub>**

The MDA content of cv. Chamran increased significantly ( $p < 0.05$ ) in response to Pb, but did not affect significantly in cv. Shiroudi. Si application had not notable effect on MDA content of cultivars in absence of Pb, but at 140 ppm significantly decreased this metabolite content in presence of Pb (Figure 1).

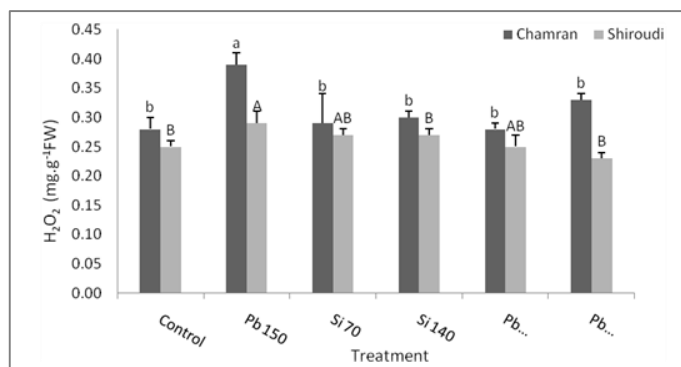


**Figure 1.** Effect of Si on MDA content of wheat cultivars under Pb stress

The H<sub>2</sub>O<sub>2</sub> content of wheat cultivars significantly increased in response to Pb stress. Application of Si in absence of Pb, hadn't significant effect on H<sub>2</sub>O<sub>2</sub> content of cultivars, but its application at both levels in cv. Chamran and at 140 ppm in cv. Shiroudi considerably reduced this metabolite content in presence of Pb (Figure 2).

Increasing in the MDA and H<sub>2</sub>O<sub>2</sub> contents of plants under Pb stress is common among plant species (Britto et al., 2011; Hasanuzzaman et al., 2017). Heavy metals are able to induce the overproduction of ROS, which can react with macromolecules and cause lipid peroxidation and oxidative

stress (Abu-Muriefah, 2015). In studied wheat cultivars, Si could alleviate the Pb-induced accumulation of MDA and H<sub>2</sub>O<sub>2</sub>. This could be partially related to the Si ability in improving the plant's defense capacity against oxidative damages. It has been demonstrated that Si could protect the plant from oxidation damages by regulating the general mechanisms of cellular redox cycle and decreasing the permeability of membranes (Abu-Muriefah, 2015; Hasanuzzaman et al., 2017). Results obtained from this study showed that, in both cultivars, the MDA contents of Pb-treated plants had the lowest values at 140 ppm of Si. Vice versa, the application of 70 ppm of Si was more effective in controlling the H<sub>2</sub>O<sub>2</sub> contents in Pb-treated plants.

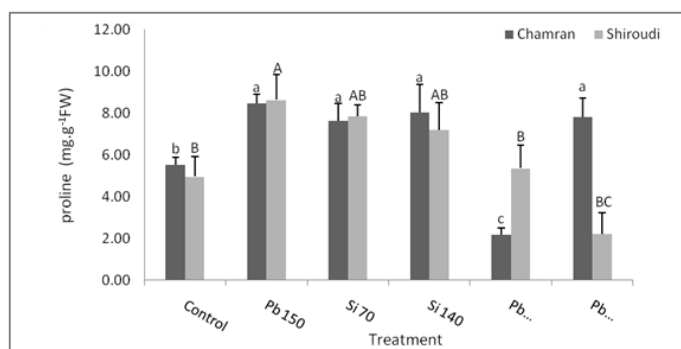


**Figure 2.** Effect of Si on H<sub>2</sub>O<sub>2</sub> content of wheat cultivars under Pb stress

### Proline

Pb caused a significant ( $p < 0.05$ ) increase in the proline content of wheat cultivars. In cv. Chamran, Si application at both levels significantly increased the proline content of plants in absence of Pb, but significantly decreased this metabolite in presence of Pb at 70 ppm.

In cv. Shiroudi, Si application had not significant effect on the proline content of plants in absence of Pb, but significantly decreased this metabolite at both levels in presence of Pb (Figure 3).



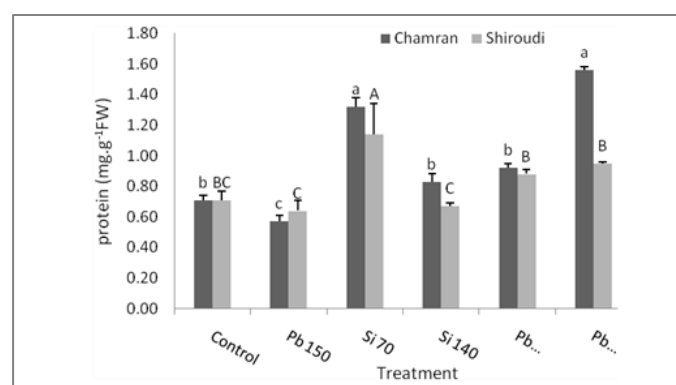
**Figure 3.** Effect of Si on proline content of wheat cultivars under Pb stress

Proline is a compatible osmolyte and is known to accumulate in response to various abiotic stresses (Abu-Muriefah, 2015). Accumulation of proline in plant tissues has been suggested to result from: (a) a decrease in proline degradation, (b) an increase in proline biosynthesis, (c) a decrease in protein synthesis or proline utilization, and (d)

hydrolysis of proteins (Britto et al., 2011). Proline had a protective role in lipid peroxidation and possesses the ability to mediate osmotic adjustment, stabilize subcellular structures and scavenge free radicals. Silicon application reduced the proline content in wheat cultivars. This may be due to increased activities of proline degradation enzymes or incorporation of proline in the protein structure in plants received Si. Application of 70 ppm of Si in cv. Chamran and 140 ppm of Si in cv. Shiroudi were more efficient in moderating the proline level.

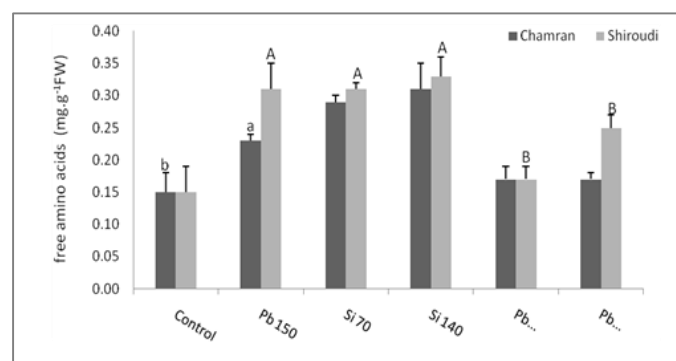
### Total Protein and Free Amino Acids

Lead stress reduced the soluble protein content of plants significantly in cv. Chamran and non-significantly in cv. Shiroudi. Si application at 70 ppm in absence of Pb and at both levels in presence of Pb significantly increased the soluble protein content of wheat cultivars (Figure 4).



**Figure 4.** Effect of Si on total protein content of wheat cultivars under Pb stress

Free amino acid content of wheat cultivars significantly increased in response to Pb and Si application in absence of Pb. While Si application significantly decreased the free amino acid content of plants in presence of Pb (Figure 5).



**Figure 5.** Effect of Si on free amino acid content of wheat cultivars under Pb stress

There is a consensus that proteins are key targets of heavy metals and heavy metals can bind to native proteins and inhibit their biological activity (Tamas et al., 2014). The quantitative decreases in the total protein content could be attribute to several Pb effects such as oxidative stress, modification in gene expression, increased ribonuclease activity, protein utilization by plants for the purposes of its detoxification and increased hydrolysis of protein (Alia et al., 2015). In this study,

silicon application improved the protein contents of cultivars, especially in cv. Chamran, that was accompanied with obvious decreases in the free amino acids and proline contents. It has been proposed that Si has a positive effect on protein synthesis from free amino acids precursors and inhibits the protein hydrolysis under stress conditions (Soundararajan et al., 2017).

### Conclusion

From this study, it could be concluded that the antioxidant response of wheat plants to lead stress was relatively different between studied cultivars. Silicon application in plants that were not under Pb stress could not affect the antioxidant enzymes activities, but caused to increase in the protein and free amino acid contents of plants. Silicon application in plants that were under Pb stress could control the free radical formation and moderate the up-regulated activities of antioxidant enzymes more effectively in cv. Chamran.

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## RESEARCH ARTICLE

### Experiences on Establishment of Scots Pine (*Pinus sylvestris* L.) Plantation in Ash Dump Sites of Reftinskaya Power Plant, Russia

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#### ABSTRACT

Since the middle of the last century in the Urals of Russia there has been a problem of environmental pollution by man-made emissions. The purpose of this investigation has been to summarize the recultivation experience of Reftinskaya power plant ash dump. The station was put into operation in 1970 and it is the largest one in Russia. Specific feature of the used coal is high content of ash (47%). Daily consumption of coal in winter period constitutes 48 thousand tons. Yearly emissions of the station constitute 400 thousand tons. The main components of the emissions are sulphureous anhydride (up to 40%) solid stuff (up to 50%) and nitric oxides. The Scots pine (*Pinus sylvestris* L.) plantations on ash dumps have shown good adaptation and growth. The plantations have formed 143 m<sup>3</sup>/ha total volume at the age of 20 in 1<sup>st</sup> site index of the recultivation site of the ash dump with ash layer up to 7 m. Weakly alkaline reaction of the ash spread by wind promoted soil dioxidation that results in soil fertility increasing significantly on territory of adjacent stands. Recultivation process includes two main stages which are ash dump surface covering with 25-40 cm soil layer, planting with 2-year old Scots pine (*Pinus sylvestris* L.) seedling.

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#### Introduction

Extraction and processing of mineral resources is inseparably connected with soil withdrawal for sand pits overburden rock dump wastes of ores processing and enriching as well as routes communication (Stanturf, 2015; Martinyuk, 2016). In particular, solid fuel ashes, deposited near thermal power stations, are harmful to people living in nearby areas and the environment.

Natural regeneration of disturbed lands the most often lasts for many dozens and even hundred years that calls forth

necessity of artificial recultivation. However, the latter requires significant labor and financial expenditures.

There are about 4.5 million km<sup>2</sup> of man-made landscapes in the world that require radical reclamation (Belyakova et al., 2003). Many studies have been carried out on technogenic pollutions, especially in Russia and the Urals (Zavyalov et al., 2018; Mohnachev et al., 2018; Makhniova et al., 2019; Menshikov et al., 2019; Potapenko et al., 2019). Regretfully, in spite of works significant number on artificial recultivation many problems of their carrying out are still remains to be debated. Among the destroyed lands there are ash dumps -

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places where ash is stored after burning coal. They are man-made terrains (hills) that rise above the surrounding surface.

Due to the high concentration of industrial production and insufficient waste treatment since the middle of the last century in the Urals and in some regions of Russia there has been a problem of environmental pollution by man-made emissions. Scientific and practical interest in the subject of remediation is determined by the extent of disturbed land, resulting in more than 300-year history of the development of metallurgy and the whole industry in Russia. Research on biological recultivation of territories in the Urals has a long history. Since the late 50-ies of the 20<sup>th</sup> century began to actively develop methods of biological reclamation of disturbed lands, which were more effective than technical reclamation. In the following years, institutes of technical and biological profiles have been developed and successfully implemented research and practical projects on land reclamation in Russia (Makhnev, 2002; Barannik, 2005; Martinyuk, 2006; Lukina, 2008; Androkhonov and Kurachev, 2010; Kapelkina, 2013; Zhukov, 2016).

In this study it was focused on effects of Reftinskaya power plant, thermal power plant working on coal, Sverdlovsk region in Ural Region. The investigation area is immediately to the disturbed lands of ash dumps. The purpose of the investigation was to summarize the recultivation experience of Reftinskaya power plant ash dump and present certain recommendations for improving rehabilitation works.

### Materials and Methods

The investigation area of this research is the site of Reftinskaya power plant ash dump № 1 (Figure 1). The station was put into operation in 1970 and it is the largest one in Sverdlovsk region of the Russian Federation. Nowadays the ash dump № 1 covering the territory of 440 ha is fully filled up. The second ash dump on territory of 860 ha is being filled up, and the process of ash storing by dry method has been doing.

The fixed electric capacity of the station constitutes 3800 thousand kwt, thermal - 350 Hcal/hour. Republic of Kazakhstan Ekibastuzsky deposit coal serves as raw material

for the station. Specific feature of this coal is high content of ash; it can constitute 47%. Daily consumption of coal in winter period constitutes 48 thousand tons; besides that, 150 tons of fuel-oil are also used by the station. Yearly emissions of the station constitute 400 thousand tons. The main components of the emissions are sulphureous anhydride (up to 40%) solid stuff (up to 50%) and nitric oxides.

The investigations carried out have shown that ash in the ash dump № 1 is characterized by heightened as compared with soil, content of microelements as well as available nutrition elements of plants ( $P_2O_5$  and  $K_2O$ ). Weak alkaline reaction of ash spread by wind promoted soil dioxidation that results in soil fertility increasing significantly on territory of adjacent stands. The forest management plans data of Sukholorhsky forest district testify to the fact that growth site index capacity in the forest district where Reftinskaya power plant is located has been changed from 1970 to 2000. If in 1970 the Scots pine (*Pinus sylvestris* L.) stand share of I<sup>a</sup> and I classes of growth site index capacity constituted 17.9%, in 2000 the Scots pine plantations share in these growth site classes shows portion of 43.6% (Table 1).



Figure 1. Map of the study area

Table 1. Scots pine plantations areas and percentages according to site indexes (bonitet index) in Sukholorhsky forest district

Year	Unit	Area of stands according to bonitet index (ha/%)								Total
		I <sup>a</sup>	I	II	III	IV	V	V <sup>a</sup>	V <sup>6</sup>	
1970	ha	12	7906	23605	10210	819	1090	590	-	44232
	%	0.03	17.87	53.37	23.08	1.85	2.47	1.33	-	100
1990	ha	-	16527	17285	3626	605	956	540	79	39618
	%	-	41.72	43.63	9.15	1.53	2.41	1.36	0.20	100
2000	ha	402	17805	15749	5432	954	954	378	43	41717
	%	0.96	42.68	37.75	13.02	2.29	2.29	0.91	0.10	100

The Institute of Vegetation and Animal Ecology together with the Ural Forest Experimental Station expressed the typology of forest stands as shown in table 2 (Anonymous, 1984).



Table 2. Characteristic of forest type groups

№ of forest type groups	Forest type groups	Characteristics of the dominant living ground cover of forest types <i>Pinus sylvestris</i>
1	Pineta cladinosa	<i>Cladonia</i> spp Mountainous.
2	Pineta fruticulosa	<i>Vaccinio vitis-idaeae</i>
3	Pineta fruticuloso-hylocomiosa	<i>Vaccinium myrtillus</i> , <i>Vaccinium myrtillus</i> + <i>Rubus saxatilis</i> , <i>Vaccinium myrtillus</i> + <i>Vaccinium vitis idaea</i>
4	Pineta composita (nemoro-boroherbosa)	<i>Tilia cordata</i> + <i>Oxalis acetosella</i> + <i>Carex rhyzina</i>
5	Pineta mesoxerophiloherbosa (multiherbal)	<i>Calamagrostis</i> spp. <i>Rubus saxatilis</i> + <i>Calamagrostis arundinacea</i>
6	Pineta parviherboso-hylocomiosa	<i>Oxalis acetosella</i> + <i>Rubus saxatilis</i> , <i>Pleurozium schreberi</i>
7	Pineta magnoherbosa	<i>Filipendula ulmaria</i>
8	Pineta eutropho- uliginosoherbosa	<i>Equisetum sylvaticum</i> + <i>Sphagnum</i> spp.
9	Pineta sphagnosa	<i>Eriophorum vaginatum</i> + <i>Sphagnum</i> spp.; <i>Carex lasiocarpa</i> + <i>Sphagnum</i> spp.; <i>Sphagnum girgenzohnii</i> + <i>Sphagnum</i> spp.

From 1993 beginning the works on the ash dump № 1 on biologic recultivation are being carried out, the artificial forest stands (plantations, pine cultures) are supposed to be established on its surface. The research carried out showed that light recultivation effect is achieved only when a definite technic stage is achieved, the one that implicates covering ash by soil thick layer of 24-40 cm. Then the biologic stage of recultivation is carried out. In fact, 2-year old seedlings of Scots pine are being planted with the forest planting machine LMD-81.

Scots pine forest cultures planting has been carried out by blocks of 500 × 50 m. The blocks were arranged in chess board order and altered with blocks of analogous size sowed with herbaceous mixtures. The soil layer thickness for sowing constituted 10-15 cm, and for forest cultures 30-40 cm.

In the process of investigations, we have succeeded in analyzing the history of artificial stands establishment of the ash dump № 1. The method of permanent quadrates (pq) has been laid into the base of the investigations. All the permanent quadrates have been laid and processed according to approved methods being used nowadays (Dancheva and Zalesov, 2015). In addition, the woody species composition and terrestrial biomass of live ground vegetation have been studied.

The process of the establishment of plantation on the ash disposal area № 1 at Reftinskaya power plant is pointed-out in brief, as follows (Menshikov et al., 2019):

- In 1992, 1.3 ha - planting of Scots pine in trenches filled with a mixture of peat and soil (1:1), depth of trenches 0.25; 0.40 and 0.65 m.

- In 1993, 3.7 ha - planting of Scots pine, Siberian larch spruce (*Larix sibirica* Ledeb.), birch (*Betula pendula* Roth and *Betula pubescens* Ehrh.), as well as balsam poplar (*Populus balsamifera* L.) 2-year-old seedlings produced in wide bulk bands, the thickness of which was 0.25; 0.40 and 0.65 m. In addition, these bands were planted with unrooted cuttings of balsam poplar and native species of willows (*Salix viminalis* L. and *Salix dasyclados* Wimm. Syn. *Salix gmelinii* Pall.), and a collection of ornamental woody and fruit species.

- In 1994, 1.1 ha of plantations of Scots pine, spruce, larch and white birch (*Betula pubescens* Ehrh.) trees on an artificial strip with a width of 3-4 m and the wide bulk of the strip, spread a drainage layer.

## Results and Discussion

Forest management works carried out in Sukholozhsky forest district as concerns the above mentioned typology have shown that 30 years after the station was put into operation unprecedented changing of forest growing conditions has taken place. The latter became apparent in berry live ground cover decreasing and multi herbal layer of forest types increasing (Table 3). In other words, in field-layer (herb and small shrub layer) cover (forest type group 3) - the share of berry undershrub has decreased and the share of forest small reeds and some other kinds of cereals has increased (forest type group 5).

**Table 3.** Scots pine stands distribution according to forest type groups in Sukholozhsky

Year	Unit	Pine stands area according to forest type groups, ha/%							Total
		2	3	4	5	6	7	8	
1970	ha	806.0	24489	66	16366	129	-	2376	44232
	%	1.82	55.37	0.15	37.00	0.29	-	5.37	100
1990	ha	636.3	5360.9	18.8	31130.1	134.1	144.1	2193.6	39617.9
	%	1.61	13.53	0.05	78.58	0.34	0.36	5.53	100
2000	ha	1269	15297	63	22895	45	10	2138	41717
	%	3.04	36.67	0.15	54.88	0.11	0.02	5.13	100

Stands' share of multi-herb group of forest type increasing has complicated reforestation process and imposed wildfire risk in autumn and spring seasons. Due to these reasons, on the account of podzol soils deoxidation their fertility has increased and furthermore, volume increment for every square unit has also increased. Zavyalov et al. (2019) emphasized that the annual growth rings of Scots pine on fertile soils were significantly higher than those of on poor soils. Also, soil fertility allows better adaptation of Scots pine to low temperatures, and more actively grow on sites with increasing precipitation in technogenic areas. However, Makhniova et al. (2019) stated that the level of soil technogenic pollution predominantly contributes to the formation and growth of sprouts and seedlings i.e. their morphometric characteristics. Most likely, under natural conditions, the level of soil technogenic pollution is a particularly strong factor in restricting plant seed reproduction.

Recultivation experiments on the first ash dump were started in 1992, due to the process of air dusting. Ash from the ash dump was spread by wind to adjacent territories.

In the process of the first experiments carrying out on the ash dump the tranches of 0.7 m width and 0.25 depth; 0.45 and 0.65 cm have been laid, followed by filling with sandy soils and turf mixture (1:1), and then with the process of Scots pine and Sukhacheva larch (*Larix sukaczewii* Dylis Syn. *Larix sibirica* Ledep.) planting. The space between the tranches to prevent

dusting has been covered with the soil layer of 0.1 m thickness. Unfortunately, the results of recultivation were unsatisfactory and most of the plant layer has been lost.

In 1993 recultivation experiments were kept on under direction of Makhnev. Namely, 4 ha square has been covered with soil layer of 25, 40, and 60 cm (Makhnev, 2002). The process was followed by 3-year old seedlings of Scots pine, Siberian spruce (*Picea obovate* Ledeb.), Sukhacheva larch, silver birch (*Betula pendula* Roth.) and white birch, balsam poplar, as well as Russian broom (*Salix rossina* Nas.), willow branched (*Salix viminalis* L.), willow woolly sprouted (*Salix dasyclados* Wimm.) planting. This carried out research made it possible to start reclamations in industrial scale.

During 1996 - 2001, an area of 172 ha of Scots pine cultures have been established. Research carried out in 2001 showed that the forest cultures planted on ash dumps are characterized by good adaptation and mean increment in height as well as by high preservation (Table 4). Zavyalov et al. (2018) and Mohnachev et al. (2018) stated that despite the pollution in the technogenic soils, the success of natural regeneration is effected by the stand density and the occurrence of undergrowth of Scots pine. Soil remediation can positively affect the natural regeneration process. Moreover, seed supplementation can play a major role in successful natural regeneration.

**Table 4.** Scots pine growth on the ash dump after planting

The year of planting	Area (ha)	Number of trees (psc)	Height (m)	Diameter at root collar (cm)	Current increment (cm)	Capacity for survival (%)
1996	0.8	100	1.69±0.03	4.03±0.09	48±1.0	86.2
1996	3.2	100	1.76±0.05	4.10±0.13	44±1.0	79.4
1996	0.1	100	1.18±0.06	2.74±0.13	33±3.0	73.0
1997	19.9	200	1.32±0.05	3.19±0.12	41±1.0	89.7
1998	30.7	150	0.63±0.03	1.72±0.07	26±6.0	64.3
1999	4.2	100	0.76±0.03	2.11±0.09	26±7.0	72.5
1999	23.1	300	0.75±0.03	2.16±0.11	24.3±1.3	89.3
2000	20.0	150	0.47±0.02	1.38±0.07	6.0±0.1	73.0
2000	28.0	200	0.81±0.03	2.11±0.07	22.0±0.1	96.2
2001	10.0	100	0.47±0.02	1.30±0.05	8.5±0.1	91.7
2001	10.0	100	0.48±0.02	1.42±0.06	11.0±0.1	91.7
2001	22.0	100	0.45±0.01	1.19±0.09	7.0±0.0	84.0

High preservation and growth in height indices, as well as possibility of mechanized planting, favors Scots pine as main species for establishment of forest stands on the ash dump.

In 2011, Reftinskaya power plant ash dump № 1 recultivation was entirely completed. On its territory, a total of 360.2 ha of pine stands have been established (Figure 2, 3).



**Figure 2.** Establishment of Scots pine plantation on the ash dump of Reftinskaya power plant (Photo by: S. Zalesov)

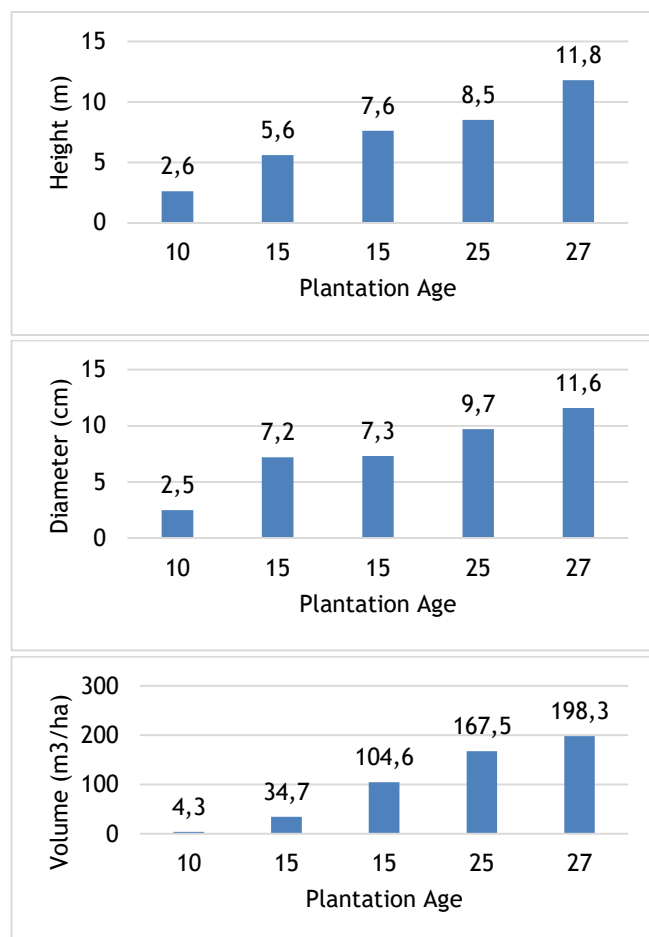
We have made inventory of artificial stands planted on the ash dump and have established permanent quadrates to determine the main inventory indices and subsequent monitoring according to their growth and development. The main taxation indices of the Scots pine plantations established on ash dump are shown in table 5.

Our results testify to the fact that if at the age of up to 8 years' artificial stands are characterized by II class of forest capacity site then at the age of more than 10 years by the first (1) class. The latter, to our opinion, is explained by competition from field-layer during the first years after forest cultures are being planted.

At the age of 20 artificial stands are characterized by 143 m<sup>3</sup>/ha stem volume that corresponds to yearly increment of 7.15 m<sup>3</sup>/ha. In other words, artificial stands established on the ash dump exceed the stands of analogous age established on felled sites of the most productive forest types (Table 6). In addition, it can be seen that the stand volume, diameter and height change according to plantation age (Figure 4).



**Figure 3.** 20-years old Scots pine plantations on the ash dump of Reftinskaya power plant (Photo by: A. Opletaev)



**Figure 4.** The stand volume, diameter and height change according to plantation age

**Table 5.** The main fixation indices of artificial pine stands established on Reftinskaya power plant ash dump

№ of order	Composition (%)	Thickness, p/ha	Biologic age, years	Average		Basal area (m <sup>2</sup> /ha)	Volume (m <sup>3</sup> /ha)	Site Index
				Height (m)	d <sub>1,30</sub> (cm)			
7	100 C	3016	7	2.4	2.4	1.423	3.36	II
	99.8 C	3675	8	2.5	2.5	1.884	4.58	
6	0.2Oc	13	6	2	2	0.004	0.01	
	Total	3688				1.888	4.59	
5	99.8 C	2142	10	5.4	5.4	4.85	18.67	I <sup>a</sup>
	0.2Oc	53	8	1.6	2	0.016	0.03	
	Total	2195				4.866	18.7	
4	100 C	4377	13	6.4	6.4	14.069	61.51	I <sup>a</sup>
	-Oc	23	11	2	2	0.007	0.01	
	Total	4400				14.076	61.52	
3	99.9C	3632	15	7.8	7.9	17.821	88.15	I <sup>a</sup>
	0.1Oc	72	13	2	2.3	0.03	0.05	
	Total	3704				17.851	88.2	
2	99.2 C	2149	16	8.8	9	13.739	75.16	I <sup>a</sup>
	0.8 Oc	104	14	4.5	4.5	0.171	0.61	
	Total	2253				13.91	75.77	
1	98.5C	3390	20	11.5	9.1	22.113	140.74	I <sup>a</sup>
	1.3 Б	133		9	5.6	0.337	1.79	
	0.2Лц	29		8.5	4.9	0.053	0.29	
	- Oc	19		4	2	0.006	0.02	
	Total	3571					22.509	

C: Scots pine (*Pinus sylvestris* L.), Oc: Aspen (*Populus tremula* L.), Б: Silver birch (*Betula pendula* Roth.), Лц: Siberian larch (*Larix sibirica* Ledeb.)

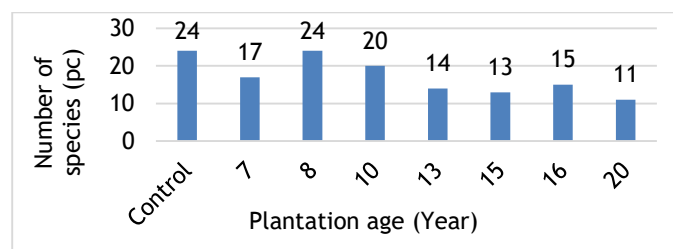
**Table 6.** The main taxation indices of Scots pine plantations created on the felled sites

№ of order	Composition (%)	Age, years	Average		Thickness (p/ha)	Basal area (m <sup>2</sup> /ha)	Volume (m <sup>3</sup> /ha)	Site Index
			Height (m)	d <sub>1,30</sub> (cm)				
1/12	100C	10	2,6	2,5	3420	1,67	4,3	II
2/12	100C	15	5,6	7,2	2604	10,56	34,7	I
3/12	100C	15	7,6	7,3	5989	25,91	104,6	I
4/12	100C	25	8,5	9,7	3405	26,66	167,5	II
5/12	100C	27	11,8	11,6	2650	30,48	198,3	I

C: Scots pine (*Pinus sylvestris* L.)

In addition, beside the woody vegetation planted on the recultivated ash dump thrives herbaceous (grassy) vegetation. In the process of investigations, it has been established that 43 species of field-layer grow on the territory. With artificial stands age increasing species of field-layer are decreasing (Figure 5).

If in the first years after planting forest crops, the above-ground phytomass of living soil cover amounted to 415.9 kg/ha. After 20 years it did not exceed 12.8 kg/ha in absolutely dry condition

**Figure 5.** Change of herbaceous vegetation species composition on the recultivated ash dump according to plantation age

Protective measures against forest fire on the recultivated ash dump are very important. To stop forest cover fires during the first years after establishing plantations, it is recommended to make flat field-layer in spaces between rows in rainy weather or immediately after rainfall. The established plantation strips should be planned in a manner that delays fire progress and facilitates firefighting. To decrease fire risk, some counter fire measures should be taken. Particularly, to prevent forest cover fires developing into crown fires, it is recommended low pruning of tree branches up to 2,5 m height to be carried out (Figure 6).

The state of the path network, the removal of living ground cover and trimming of the lower branches excludes the possibility of the transfer of grassroots ground fire to the tree crowns.



**Figure 6.** 20-years old Scots pine plantation with trimmed branches (Photo by: S. Zalesov)

## Conclusion

The station emissions during the period of its exploitation brought to unprecedented change on forest growing conditions that results in forest type changing on more than 40 thousand ha of adjacent territories. Scots pine plantations has formed 143 m<sup>3</sup>/ha volume at the age of 20 in 1<sup>st</sup> site index of the recultivation area of the ash dump with ash layer up to 7 m.

Experiences with the Scots pine plantations established at the ash dump site are as follows: 1) On the ash dumps obtained from burning coal, the most promising species is the Scots pine plantations, 2) Recultivation process includes 2 main stages: The 1<sup>st</sup> stage consists of ash dump surface covering with soil layer of 25-40 cm thickness; The 2<sup>nd</sup> is establishment of plantation with 2-year old Scots pine seedling, 3) The establishing of artificial pine stands eliminates the spread of ash dust, which improves the environmental state; Also, the pine plantations on ash dumps are superior in productivity i.e. Scots pine stands growing in natural conditions, 4) The weed growth is short-term and, 20 years after planting Scots pine plantations, the aboveground phytomass of the weed does not

exceed 13 kg/ha; In addition, depending on the age of the plantation and the crown closure (canopy), the number of weed species gradually decreases, 5) Taking into account potentially high fire risk in the establishment of the plantations on the ash dump, effective counter fire measures are necessary on recultivation areas, 6) On the territory of the ash dump, in addition to planting, it is of high importance to conserve the self-regenerating process of the vegetation in successional dynamics, which can be effectively used to level-up into uneven-aged plantations that are much more stable concerning wildfires and forest protection.

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## RESEARCH ARTICLE

### Olive Marketing Analysis in Northern Iran: Marketing Margins and Indices

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#### ABSTRACT

This study investigated olive marketing channels, margins and indices at Iran's olive trade center namely Guilan province, northern Iran. For this purpose, marketing margin, share of marketing agents, marketing cost coefficient and different types of efficiency in olive market were calculated. The required data set were collected through survey using a questionnaire and simple random sampling on 2017. Results revealed that there were eight different marketing channels in the olive market of Guilan province. Average wholesale and retail margins were 11500 and 31870 Rials, respectively. The average wholesalers' share from the retail price was 9.59%. Also, the average retailers' share from the final product price (retail price) was 26.57%. The average marketing cost coefficient was 20.29%. The highest and lowest overall efficiency in olive marketing channels were 49.78% and 27.56%. Policy solutions should lead to increased marketing services and significant impact of these costs on the marketing margin in the olive market of Guilan province.

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#### Introduction

The olive tree (*Olea europaea*) is widely cultivated for the production of both oil and table olives. Olive and its products have significant economic value (IOC, 2019). Olive and olive oil, a traditional food product with thousands of years of history, are the essential components of the Mediterranean diet and are largely consumed in the world (Uyulaşer & Yildiz, 2014). Olive farming is a multifunctional activity. In particular, it has much positive social effects in rural areas depending on plantation characteristics and farming practices (Marangon & et al., 2008). Iran is one of the 24 countries with significant olive production (Mohammadi & et al., 2019). The most important olive producing provinces in Iran are Fars, Zanjan, Guilan, Qazvin and Kermanshah provinces. Olive is one of the strategic products of Guilan province and especially Rudbar

County. Olive orchards are located in the central district of Rudbar county. The region's economy is tied to olive and there are about 22,000 olive producers in this county (Statistics and ITC office of Iran's Jihad-Agriculture Ministry, 2017). South Rostam Abad, Rahmat Abad, Manjil, Ali Abad and Lushan areas with subtropical Mediterranean climate are among the most important olive hubs in Rudbar region.

Marketing is the last link in the production chain which any inefficiency can destroy the ability of production. With the expansion of the urbanization, the importance of marketing services has increased, and today marketing is seen as an essential activity. Improving the marketing system by introducing new methods of warehousing, grading, packing, shipping and standardizing reduce waste as well as enhances market transparency (Eslami, 2015). One of the factors

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contributing to the desirable degree of development is the reduction of transaction and marketing costs in goods and services markets. Marketing involves the different stages of processing, packaging, transportation and warehousing. The value of the product increases with each of these steps, so the marketing process is a flow of value added. Going through the above steps is costly and consequently causes a discrepancy between the price at the consumer level and the price at the producer level. This price gap is called marketing margin (Park, 2009).

Marketing margin or price spread is a well-known performance measure in marketing systems (Abbott & Makeham, 1991). The difference between the prices that are taken by producers and prices that are paid by consumers called marketing margin (Askan, 2019). Marketing margins are the result of the demand and supply factors, marketing costs, marketing agencies, technological changes in marketing process and the degree of the marketing channel competition (Marsh & Gary, 2004; Rahim and et al., 2018). Marketing margin is the sum of costs or benefits created from harvesting the product until it reaches the consumer (Wohlgenant & Mullen, 1987). Marketing margin has remained an important tool in analyzing the performance of marketing systems. The study of marketing margin could help policy-makers and managers to improve marketing efficiency (Dinesh and Sharma, 2019). Marketing costs and profit margins which make up marketing margins can be both indicators of marketing systems efficiency. The benefits that accrue to the individual participants may be incentives or disincentives to continue in the business. Proper computation, understanding and interpretation of marketing margin value in relation to prevailing circumstances can reveal a lot about performance in the marketing channels (Achike & Anzaku, 2010). Marketing margins are the result of the demand and supply factors, marketing costs, and the degree of the marketing channel competition (Marsh & Gary, 2004). Thus, margins reflect the aggregate processing and retailing firm behavior which influence the level and variability of farm prices and may influence the farmer's share of the consumer food dollar (Gardner, 1975; Wohlgenant & Haidaicher, 1989; Tomek & Robinson, 1990). According to Cramer & Jensen (1982) marketing margin is the percentage of the final weighted averages selling price taken by each stage of the marketing chain. The total marketing margin is the difference between what the consumer pays and what the producer/farmer receives for his product. In other words, it is the difference between retail price and farm price (Mendoza & Rosegant, 1995). Since marketing costs affect retail prices, reducing them increases the welfare of the community. In order to achieve this goal, studying the market margins is essential. Few areas of agricultural economics have received as much public scrutiny as marketing margins. Until now, there is little consensus on the sources of changes in margins and whether such changes over time have led to a deterioration or improvement in the welfare of farmers and consumers (Wohlgenant, 2001).

In agricultural markets, the shorter marketing channels and fewer marketing agents involved between production and

consumption is more efficient. On the other words, shorter marketing channel may lead to less waste costs as well as the other costs of marketing. In Iran, agricultural marketing system is traditional and inefficient (Koopahi, 2013). Price can be an effective means of providing the necessary incentives for farmers to increase production if an efficient marketing system that is compatible with the characteristics of agricultural commodities supply has been developed. This system can support farmers, increase the income and employment levels in agriculture sector. Considering the needs and preferences of consumers, improving the olive marketing system and reducing the marketing margins of this product is necessary. Therefore, investigating olive marketing issues, problems and indicators in Rudbar County as well as evaluating marketing channels and market agents is essential.

There have been many studies on the marketing of agricultural products. For instance, Achike & Anzaku (2010) studied the performance of the marketing system of benniseed in Nasarawa State by using marketing margin models. The results showed that the mean marketing margin was 18.2%, marketing costs 12.8%, net profit 8.3% and farmer's share 78.9% of the retail price. Kızılaslan & Elmalı (2012) analyze marketing margins of grape in Tokat Province, Turkey. Results showed that the margin of mediator was 77.05%.

Kohansal & Dogani (2013) studied the economic marketing of olive at Fars province of Iran and presented inherent techniques for steam lined market of this product. Their results revealed that 20 and 25 percent of canned and oily olive price were related to marketing costs. Adegbola & et al. (2016) analyzed the functioning of the marketing systems of Jew's mallow (*Corchorus olitorius*) produced in Agbédranfo (Dogbo), Southwest Benin. The net margin for jew's mallow produced in Agbédranfo was 3.24 for producers, 9.67 for retailers and 8.37 for wholesalers. Tesfaw (2017) investigated market structure and chain analysis of haricot bean in Ethiopia. Following the marketing chains, 7 marketing channels were identified. Gross marketing margin was maximum for city wholesalers (38.60%) and minimum for farmer traders (13.22%) of the consumers' price. Net marketing margin was maximum (11.52%) for processors and minimum (7.36) for rural assemblers. Jassam & et al. (2018) studied the efficiency and marketing margins of the main vegetable crops in Baghdad province, Iraq. Results revealed that marketing efficiency of marketers was 63.22%, 65.58%, and 60.31% for tomato, eggplant, and cucumber crops, respectively. Also, the total marketing margins were 212 IQD/Kg, 235 IQD/Kg, and 125 IQD/Kg for tomato, eggplant, and cucumber, respectively.

One of the aims of this study is to examine the different marketing channels of olive in the existing market structure and analyze the economic criteria of marketing in each path. To achieve this, it is important to identify marketing agents. The product eventually reaches the consumer, but the path that product reaches the customer is sometime long and in other cases short, which are illustrated by charting the marketing channels. Investigating marketing channels and margins, identifying factors affecting marketing margins, determining the share of different market agents, and



analyzing the marketing efficiency in the olive market are the most objectives of this study.

### Materials and Methods

To investigate the marketing margin thoroughly and exactly, it was divided into two smaller portions, the retailer margin and wholesale margin. The wholesale margin is the difference of the price at which wholesalers sell their product and the price which they pay to the farmers as they buy the product from them, and the retailer margin refers to the difference of the price at which the retailers sell the acquired products to the consumer and the price they pay to the wholesalers (Toure & Wang, 2013). The criteria used to determine marketing margins are the relationships of retail, wholesale and total margins (Digby, 1989; Mendoza & Rosegant, 1995):

$$MM_W = P_W - P_F \quad (1)$$

$$MM_R = P_R - P_W \quad (2)$$

$$MM_M = MM_W + MM_R \quad (3)$$

Where  $MM_W$ ,  $MM_R$  and  $MM_M$  are the wholesale, retail and total olive marketing margins, respectively. Also,  $P_R$ ,  $P_W$  and  $P_F$  are the weighted average of retail, wholesale and farm prices, respectively.

The Shefferd & Futrell (1959) method was used to determine the shares of olive market agents (producer, wholesaler and retailer) from final consumer price at Guilan province:

$$SH_F = (P_F / P_R) \times 100 \quad (4)$$

$$SH_W = (P_W - P_F) / P_R \times 100 \quad (5)$$

$$SH_R = (P_R - P_W) / P_R \times 100 \quad (6)$$

Where  $SH_F$  is the olive producer share,  $SH_W$  is the wholesaler share and  $SH_R$  is the retailer share.

The marketing cost coefficient ( $r$ ) reflects the share of marketing costs from the retail price (Eslami, 2015):

$$r = (MC / P_R) \times 100 \quad (7)$$

Where, the  $MC$  is olive marketing costs. The  $MC$  is the sum of all marketing services costs in the olive market like transportation, labour, energy, tax, tariff, and the opportunity cost of capital.

Efficiency is the most important issue in marketing analysis (Thakur, 1992). Prices in an efficient market must always fully reflect available information (Fama, 1970). Profit in marketing is directly related to its efficiency. Inefficient and backward marketing system leads to higher costs, widespread losses, high waste of products, and unreasonable prices. In order to determine the marketing efficiency of olives in Guilan province, the proposed relationship by Shefferd & Futrell (1959) was used. A marketing system operates efficiently when it generates 1\$ for 1\$ marketing service costs.

$$M_E = MV / MC \quad (8)$$

Where,  $M_E$  is the efficiency of olive marketing channel and  $MV$  is olive marketing Value-added in Rudbar County. The  $MV$  is the difference between retail and wholesale price of olive.

In this study tree types of marketing inefficiencies, including technical inefficiency ( $I_T$ ), price inefficiency ( $I_P$ ), and total inefficiency ( $I_o$ ) introduced by Shrivatava & Randhir (1995) were used:

$$I_T = C_w / MM_M \quad (9)$$

$$I_P = MC / MM_M \quad (10)$$

$$I_o = (MC + C_w) / MM_M \quad (11)$$

Where,  $C_w$  is the cost of wastes which is calculated based on marketing agents reports. In the above equations, if the marketing and waste costs equal zero, the total inefficiency equals zero and the efficiency equals one (100%), which indicates the overall efficiency of the marketing system. If these costs are equal to the marketing margin, the marketing system is completely ineffective.

From social welfare point of view, it is desirable for the marketing system to generate 1\$ value added per 1\$ marketing services costs. The following equation was used to calculate social welfare efficiency index ( $M_R$ ):

$$M_R = (MC + C_w) / (MM_M - MC - C_w) \quad (12)$$

Improving the market environments should be a priority for improving the supply and satisfying the market demand of olive. In order to improve the marketing system linked with the markets at the studied area, the role of market-actors, market channels and the existing constraints and opportunities along the olive chain need to be identified. Thus, this study was initiated to investigate the different marketing channels and analyze the marketing indicators. In this study, a questionnaire with 10 components was designed to investigate olive marketing problems. The design questions were about the amount of olive waste, the price of the olive product at three levels of production, wholesaler, retailer and marketing service costs. The interviewed population were 30 stakeholders (producers, wholesalers and retailers) from the different stages of the olive value chain.

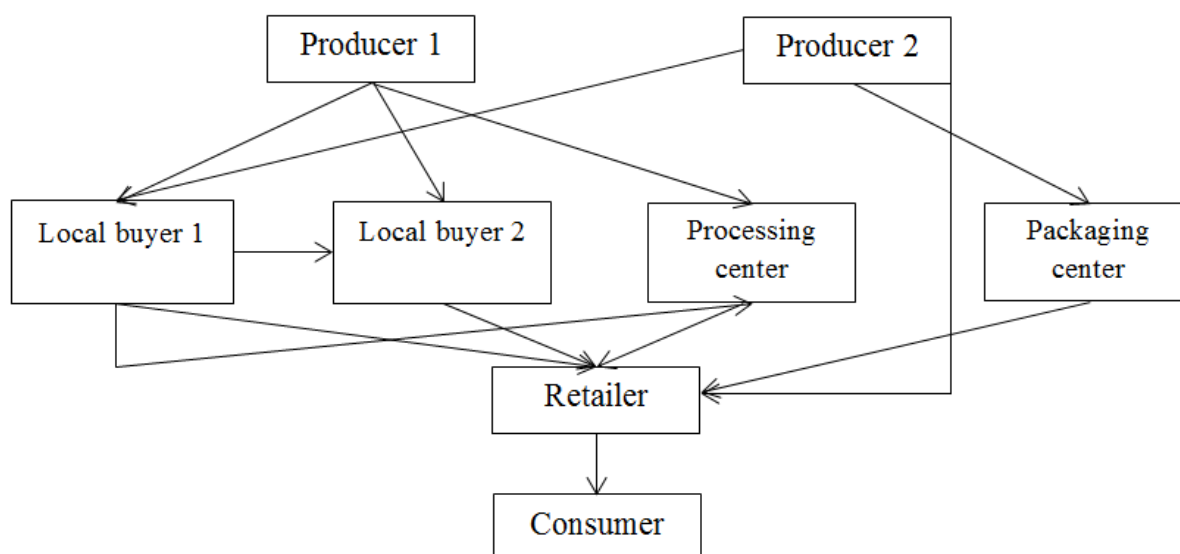
### Results and Discussion

Our survey revealed that olive production in Rudbar County is mainly carried out by traditional gardeners, but in recent years, private production companies have started producing olives in this region. After harvest, the olives are sold in two ways. In the first case, the producer sells the product immediately after harvesting. In the latter case, the producer breaks down the harvested olives and then sells them. Hence, two types of local buyers are defined. The first type of local buyer buys and sells broken (in order to make the olive, bitter and sweet, after being washed, it is beaten to bring more salt and brine into the fruit) or unbroken olives. The second type of local buyer buys unbroken olives, then breaks it down and sells it to other marketing agents. Field survey of the olive marketing channels in Rudbar County showed that the marketing agents at different levels of the market can be defined as follows:

**Table 1.** Marketing agents at different level of olive market in Rudbar County

Producers	Wholesalers	Retailers
<p><b>Producer 1:</b> Local gardeners or agribusinesses that sell olives immediately after harvest.</p> <p><b>Producer 2:</b> Local gardeners or agribusinesses who break olives and then sell them.</p>	<p><b>Local buyer 1:</b> Individuals who buy olives from the producers (broken or unbroken) and sell them to other agents.</p> <p><b>Local buyer 2:</b> Individuals who buy unbroken olive from the producers then breaks it and sell the broken olives to other agents.</p> <p><b>Processing center:</b> A center that buys olives (broken or unbroken) and produce canned olives, processed olives, etc.</p> <p><b>Packaging center:</b> A center that buys broken or unbroken olives and packs them up.</p>	<p><b>Retailer:</b> Includes all shopkeepers, stores and supermarkets that supply olive and its products to end-consumers.</p>

Field survey showed that the following marketing channels exist in the olive market of Rudbar County:



**Figure 1.** Marketing channels of olive in Rudbar County

Channel #1: Producer 1 → Local buyer 1 → Local buyer 2 → Retailer → Consumer

Channel #2: Producer 1 → Local buyer 2 → Retailer → Consumer

Channel #3: Producer 1 → Local buyer 1 → Processing center → Retailer → Consumer

Channel #4: Producer 1 → Local buyer 1 → Packaging center → Retailer → Consumer

Channel #5: Producer 1 → Processing center → Retailer → Consumer

Channel #6: Producer 2 → Retailer → Consumer

Channel #7: Producer 2 → Packaging center → Retailer → Consumer

Channel #8: Producer 2 → Local buyer 1 → Retailer → Consumer

Field survey showed that channels 1, 3 and 4 were the longest channels with 5 agents. The cost of waste and transportation in these paths is higher than the rest. The shortest marketing channel is channel 6 with 2 agents. Except for breaking the olives, all marketing costs is borne by the retailer. Also, channel 6 has the lowest waste and transportation costs.

Marketing margins were calculated based on the average sales prices of olives at three levels of garden, wholesaler and retailer.

**Table 2.** Marketing agents at different level of olive market in Rudbar County

Marketing Agents	Farm level (Producer)		Wholesale level				Retail level
	Producer 1	Producer 2	Local buyer 1	Local buyer 2	Processing center	Packaging center	Retailer
Price (Rials)	70000	80000	75000	80000	90000	100000	120000

**Table 3.** Marketing margin of different olive marketing agents in 2017 (Rials)

Channel	Wholesale margin				Retail margin	Marketing (total) margin
	Local buyer 1	Local buyer 2	Processing center	Packaging center		
#1	5000	5000	0	0	40000	50000
#2	0	10000	0	0	40000	50000
#3	5000	0	15000	0	30000	50000
#4	5000	0	0	25000	20000	50000
#5	0	0	20000	0	30000	50000
#6	0	0	0	0	40000	40000
#7	0	0	0	20000	20000	40000
#8	5000	0	0	0	35000	40000

Channels 1 to 5 have the highest marketing (total) margin of 50000 Rials and retailers in these channels (except channel #4) had the highest margin and share of the final product price. The maximum retail margin was 40000 Rials which belongs to channels # 1, 2 and 6. On channel #4, the packaging center with 25000 Rials had the most margins among the marketing agents. The lowest margin was for local buyer 1 (on channel #1) and local buyer 2 (on channels # 1, 3, 4 and 8). On channels #6 and 8, retail had the largest marketing margin, but on

channels #7, the retail margin was equal to the wholesale margin (packaging center). On channels # 1 and 2 the retail margin was 300% higher than the wholesale margin. On channel # 3 and 5 this was 50% more than the wholesale margin but on channel #4 the wholesale margin was 50% more than the retail margin. On channels #8 the retail margin was 600% more than the wholesale margin. The average wholesale and retail margin were 11500 and 31870 Rials, respectively.

**Table 4.** Share of marketing agents from retail price (%)

Channel	Producer		Wholesaler				Retailer	Total share of Wholesaler and Retailer
	Producer 1	Producer 2	Local buyer 1	Local buyer 2	Processing center	Packaging center		
#1	58.33	0	4.16	4.16	0	0	33.35	41.67
#2	58.33	0	0	8.33	0	0	33.34	41.67
#3	58.33	0	4.16	0	12.6	0	25	41.67
#4	58.33	0	4.16	0	0	20.85	16.66	41.67
#5	58.33	0	0	0	16.67	0	25	41.67
#6	0	66.66	0	0	0	0	33.34	33.34
#7	0	66.66	0	0	0	16.67	16.67	33.34
#8	0	66.66	4.16	0	0	0	29.18	33.34

The maximum share of the wholesalers from the retail price (20.85%) belonged to packaging center on channel #4. The minimum share of the wholesalers from the retail price (4.16%) belonged to local buyer 1 (channels # 1, 3, 4 and 8) and local buyer 2 (channels #1). The average wholesalers' share from the retail price was 9.59%. Also, the average retailers' share from the final product price was 26.57%. In channels # 1 and 2, the retailers' share from the final product price is 300% higher than the wholesalers share. On channel #3, the retailers share was 49.16% higher than the wholesalers. On channel #4

the wholesalers' share of the retail price was 12.12% more than the retailers share. At channel #5, the retailers' share was 49.97% higher than that of the wholesalers. On channel #7 the wholesalers and retailers share of the final product price were equal (16.67%). On channel #8, the retailers' share was 601.44% higher than the wholesalers' share. In channel # 1 to 5, Producer 1's share of the retail price was 39.98% higher than the total retailers and wholesalers shares. On channel # 6 to 8, producer 2's share of retail price was 99.94% higher than total retailers and wholesalers shares.

**Table 5.** Olive marketing indicators on 2017 by marketing channels

Channel	MC (Rials)	C <sub>w</sub> (Rials)	TC (Rials)	r (%)	M <sub>R</sub> (%)	M <sub>E</sub> (%)	I <sub>P</sub> (%)	I <sub>T</sub> (%)	I <sub>O</sub> (%)
#1	26600	6000	32600	22.16	262.90	38.03	59.11	13.33	72.44
#2	23600	4000	27600	19.66	212.50	63.04	52.44	8.88	61.33
#3	26600	6000	32600	22.16	262.90	38.03	59.11	13.33	72.44
#4	26600	6000	32600	22.16	262.90	38.03	59.11	13.33	72.44
#5	23600	4000	27600	19.66	212.50	63.04	52.44	8.88	61.33
#6	20600	2000	22600	17.16	120.58	99.11	45.77	4.44	50.22
#7	23600	4000	27600	19.66	212.50	63.04	52.44	8.88	61.33
#8	23600	4000	27600	19.66	212.50	63.04	52.44	8.88	61.33

In Table 5, total marketing services cost (TC) of each channel was calculated by summing marketing costs (MC) and cost of waste ( $C_w$ ). The marketing cost coefficient (r) for channels # 1, 3 and 4 was equal (22.16%). This means that 22.26% of the olive's retail price in these three channels was related to marketing costs. The maximum value of r is also related to these three channels. The marketing cost coefficient on channels # 2, 5, 7 and 8 is 19.66%, indicating that 19.66% of the olive retail price was spent on marketing services costs (TC). The minimum marketing cost coefficient belonged to channels #6 (17.16%). Also, the average marketing cost coefficient of all understudy channels was 20.29% which mean on average 20.29% of the olive retail price was spent on marketing services costs in these channels.

According to the different calculated inefficiency types, the highest overall efficiency ( $I_o$ ) with 49.78% was for channel #6. This is due to the low number of marketing agents along this channel. The least  $I_o$  was for channels # 1, 3 and 4 (27.56%). Price inefficiencies ( $I_p$ ) on channels were higher than technical inefficiencies ( $I_T$ ) because the waste cost was much lower than the marketing services cost.

The results of marketing channel efficiency ( $M_E$ ) showed that channel #6 had the highest efficiency and a unit cost on marketing in this channel created more value-added (0.99 unit) in compare with other channels. The channels with high efficiency indices had lower social welfare efficiency index ( $M_R$ ) which means the necessity of promoting and supporting these channels for improving consumers' welfare.

## Conclusion

The price shares of the producers, wholesalers and retailers indicate that gardeners have a higher share in the final price of olives. Low processing in order to achieve greater value-added was the main reason for these results. The calculations showed that, on average, the margins of retailers were greater than those of wholesalers. This can be attributed to retailers' higher share of marketing costs, poor marketing services, and more retailers bargaining power. Chegini & et al. (2015) showed that the development of olive processing activities and the production of byproducts can bring significant value-added to the region's economy as well as creating employment in the field of marketing services. Also, Linking the olive value chain to rural and agro-tourism activities, could diversify and increase farmers income.

Market price efficiency of Guilan province olive is lower than technical efficiency. A better monitoring of prices will give more precise information about the performance of the marketing system and will improve its effectiveness. One of the reasons for the low price efficiency in the province is the heterogeneous and inadequate demand structure for fresh olives, which tends to increase marketing costs. It seems that the development of the olive-related food industry and the completion of its supply chain rings could help to alleviate these problems. Inadequate storage practices and non-implementation of olive manufacturing practices were serious challenges in the region.

In recent years, there has been an emphasis on using the value-chain framework in agricultural organization to increase efficiency and expand the sector. The value chain framework is characterized as "a range of activities that are required to bring a product from its conception, through its designing, sourcing of raw materials and intermediate inputs, marketing and distribution, to the final consumer." As such, the value chain creates linkages between the different phases in agriculture, enabling relevant stakeholders understand how best to deliver products efficiently and innovatively, how to reduce costs of production and increase financial gains, and how to ensure successful marketing, food safety, and widespread distribution. It seems that olive value-chain in Guilan province is a necessity. Olive producers and marketing agents could benefit from its advantages. Also, regional economy bloom would happen with this strategy.

Olive market development in Guilan province needs production increase but one of the main challenges was high cost of production. High cost of production and low productivity lead to minimal farmers' profit margin that finally limits farmers' incentives for production increase and investing in orchards development. A major production cost is harvesting cost. The use of mechanical harvester has helped farmers reduce cost, significantly. Also, significant cost of production is link to the small scale of production. Cooperative structures can play a key role to reduce cost by creating common procurement schemes as well as, when feasible, common application of capital inputs.

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## RESEARCH ARTICLE

### Evaluation of the Erythrocyte Fragility, Haematological Parameters and Antioxidant Properties of *Platanus orientalis* Leaf Infusion Against Ethanol Toxicity in Rats

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#### ABSTRACT

The aim of this study was to evaluate the protective effects of the leaf infusion of chinar (*Platanus orientalis* L.) on erythrocyte fragility, haematological parameters and antioxidant status against ethanol-induced oxidative stress in rats. Thirty male rats were divided into five groups: Control, Ethanol, Ethanol+Silymarin (10 mg kg<sup>-1</sup>), Ethanol+PO-20 mg mL<sup>-1</sup> infusion, and Ethanol+PO-60 mg mL<sup>-1</sup> infusion. According to the results, in the Ethanol group, erythrocyte counts, red cells distribution, plateletcrit, platelet and lymphocyte levels significantly decreased compared to the Control group, while PO-60 dose-fed group showed a significant increase in haematocrit and haemoglobin values compared to the Ethanol group. There were significant changes in erythrocyte fragility of Ethanol and Ethanol-treatment groups at different NaCl concentrations of 0.3 and 0.6 according to Control group. It was observed that PO Leaf infusion reduced the hemolysis caused by ethanol at a concentration of 0.3% NaCl, thus reducing the values to the control values. In addition, PO leaf infusion caused a significant increase in total antioxidant status against ethanol toxicity and a significant decrease in total oxidative status and oxidative stress index. It was concluded that PO leaf infusion may have antihematotoxic effect, reducing erythrocyte fragility and increase antioxidant capacity against ethanol toxicity.

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#### Introduction

Reactive oxygen species (ROS) or free radicals are substances generated by oxygen (O<sub>2</sub>) metabolism which is balanced by the proportion of oxidant formation and the proportion of oxidant elimination (Sinha and Dabla, 2015). Oxidative stress (OS) results in the formation of free radicals or ROS due to insufficient antioxidant defence systems. There are many endogenous and exogenous factors causing OS. Ethanol (EtOH) is one of the exogenous factors causing OS. The majority of EtOH is metabolised in the liver by alcohol

dehydrogenase, aldehyde dehydrogenase and catalase (Zakhari, 2006; Pari and Suresh, 2008). The activation of O<sub>2</sub> by cytochromes P450 generates superoxide (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which contributes to oxidative tissue damages (Lu and Cederbaum, 2008). Free radicals are among the by-products of EtOH metabolism and are known to cause cellular and tissue damage, unless the body can use antioxidants to clean them up (Varga et al., 2017). Ethanol-induced oxidative stress increases the production of ROS which cause damage to the RBC membrane via lipid peroxidation (Wrońska-Noferd et al., 1991). Additionally, it causes a deteriorating effect on the

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membrane integrity by increasing fluidity as well as the deactivation of membrane-bound receptors and enzymes (Arihan et al., 2016).

To the best of our knowledge, the effects of PO leaf infusion on hematological parameters have not been investigated so far. It was one of our main goals to uncover the possible effects of PO leaf infusion consumption in eliminating ethanol-induced hematological problems. Since, haematological parameters, erythrocytes, leukocytes and platelets, are commonly used to diagnose various diseases such as anemia, inflammatory, autoimmune, allergic, infectious, cancer, neutrophil and lymphocyte-related parameters as well as some further common disorders (Gao et al., 2019). The osmotic fragility test is one of the additional screening tests for evaluates the red blood cells ability to associate hypotonic solutions (King et al., 2015). The excess of hemolysis means that the structure of the cell membrane is destroyed.

Antioxidant mechanisms protect cells and tissues against free radicals. The total oxidative status (TOS), total anti-oxidative status (TAS) ratio and oxidative stress index (OSI) are indicative parameters of the degree of OS (Tamura et al., 2016). TAS and TOS are well known indicator parameters for the analysis of the oxidative stress in many diseases such as cancer, diabetes, cardiovascular and organ damage.

*Platanus orientalis* (Plantanaceae), also known as Chinar or Oriental plane, is commonly used in folk medicine against tooth and knee pain, wounds, inflammation, liver, kidney and stomach diseases (Dogan and Anuk, 2019). In previous studies it has been reported that *P. orientalis* contains important compounds such as kaempferol and kaempferol derivatives, caffeic acid, platanoside, tiliroside, flavonol and proanthocyanidin glycosides, phytol derivatives, benzaldehyde, palmitic acid, 2,4-ditert-butylphenol, stearic acid, octadecanoic acid, linoleic acid, and linolenic acid (Dogan and Anuk, 2019; Khan, 2017).

The purpose of this study was to determine the possible effects of PO-leaf infusion on the erythrocyte fragility, haematological parameters and antioxidant/oxidant capacity in erythrocyte of rats with ethanol-induced oxidative stress.

## Materials and Methods

### Plant Material and Preparation of the Infusion

The *Platanus orientalis* leaves were collected by Abdulhad Dogan in Haci Hamza hamlet, district of Dargeçit, city of Mardin, in the south-eastern Anatolian region of Turkey (GPS coordinates: 37° 33' 19.7"N; 41° 47' 43.3"E) in August, 2017. The identification of the samples was confirmed by Dr. Abdullah Dalar at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Van Yuzuncu Yil University, Turkey, and a voucher specimen was deposited in the university's herbarium (Herbarium code: 340 and Collector No: A.D-761, Van Yuzuncu Yil University Faculty of Pharmacy Herbarium).

The infusion of PO-leaf was prepared according to the study by Dogan and Anuk (2019). Briefly, the fresh PO- leaf samples were washed under tap water and dried at room temperature

in the dark until dryness. The powdered samples were kept in boiling water (100 °C) for about 2 min. Then, the heating was stopped and the ground leaves were allowed to remain in the water for about 15 min. Subsequently, the liquid in the container was first filtered through a gauze cloth (rough-hew) and then through a 0.45-µm hydrophilic filter (Millipore) using a disposable injector.

### Chemicals

Ethanol, silymarin, adenosine, hydrogen peroxide solution (H<sub>2</sub>O<sub>2</sub>, %30), ammonium sulphate, phenol, o-dianisidine, ethylenediaminetetraacetic acid (EDTA), sodium hypochlorite solution, potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>), sodium chloride (NaCl), and sodium dihydrogen citrate anhydrous (C<sub>6</sub>H<sub>7</sub>NaO<sub>7</sub>) were supplied by Sigma Chemical Co. (St. Louis, MO, USA). TAS and TOS kits were supplied by Rel Assay Diagnostics Laboratories Ltd.

### Animals

The male rats (*Wistar albino*) of ~ 2 months of age and an average weight of 200 g were provided by the Experimental Animal Research Centre, Van Yuzuncu Yil University (Van, Turkey). The animals were housed at 25 ± 2 °C at a daily light/dark photoperiod of 10:14. All of the animals received water and a wheat-soybean-based diet *ad libitum* in stainless steel cages, and received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. The ethic regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. This study was approved by the Ethics Committee of Van Yuzuncu Yil University (Protocol number: 27552122-604.01.02-E.70881).

### Experimental Design

After toxicity test, the rats were randomly divided into 5 groups, with each containing 6 rats and the study was continued for 28 days.

**Control group:** The rats were allowed to receive tap water and a standard pellet diet *ad libitum*.

**Ethanol group:** The rats were allowed to receive 20% ethanol and a standard pellet diet *ad libitum*. The dose of ethanol was selected on the basis of a 20% concentration that was administered orally, which caused OS (Dogan and Anuk, 2019).

**Ethanol + Silymarin:** The rats were allowed to receive 20% ethanol and silymarin (10 mg kg<sup>-1</sup>, single dose per day) and were treated orally during the experimental period.

**Ethanol + PO-20 group:** The rats were allowed to receive 20% ethanol and *P. orientalis* (20 mg mL<sup>-1</sup>) leaf infusion during the experimental period.

**Ethanol + PO-60 group:** The rats were allowed to receive 20% ethanol and *P. orientalis* (60 mg mL<sup>-1</sup>) leaf infusion during the experimental period.

### Measurement of Erythrocyte Fragility

Blood samples were incubated for 24 hours at room temperature. 30  $\mu\text{L}$  of the samples with erythrocytes were added to solutions containing  $\text{NaH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$  buffers in order to achieve suitable pH and NaCl concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9%. After 30 minutes of incubation at room temperature, the solutions were centrifuged at 3000 rpm for 5 minutes. Absorbance of supernatant fractions was evaluated with a spectrophotometer at 546 nm (Dogan, 2018).

Calculation: Haemolysis (%) = (OD of Test Solution)/(OD of Standard Solution)  $\times$  100

### Haematological Parameters

At the end of the 28 days of experiment, blood samples were obtained the cardiac puncture by using syringe for the determination of hematological constituent. Red blood cells counts (RBC), red cells distribution width (RDW), haematocrit (HCT), haemoglobin (HGB), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean platelet volume (MPV), plateletcrit (PCT), platelet (PLT), platelet distribution width (PDW), platelet large cell ratio (PLCR), white blood cell (WBC), lymphocyte (LYM), monocytes (MON) and granulocyte (GRA) were measured by an automatic hematological assay analyzer (Coulter LH 780 Analyzer, US).

### Measurement of TOS, TAS and OSI Parameters

The levels of TOS in plasma were assessed spectrophotometrically at 530 nm using kits developed by Erel (2005). The TAS levels in plasma were measured spectrophotometrically (AE-S90-MD UV/VIS spektrofotomet) at 660 nm using kits developed by Erel (2004). The percent ratio of TOS to TAS level was considered as the oxidative stress index (OSI) and this value was calculated according to the study by Tülüce et al (2017).

### Statistical Analysis

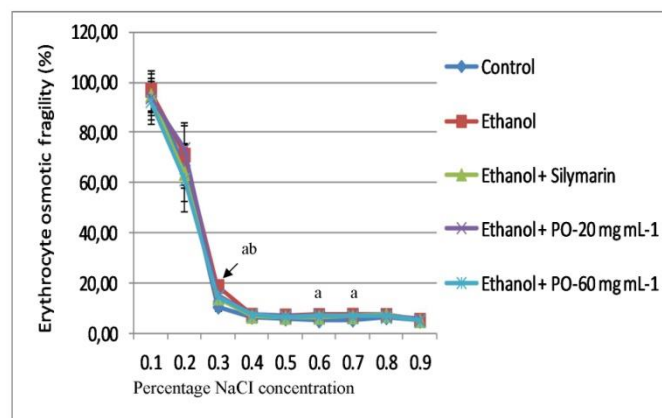
All of the obtained data were expressed as the mean  $\pm$  standard deviation (SD). The statistical analyses were made using the Minitab 14 packet program for Windows. The one-

way analysis of variance (ANOVA) was used to determine the differences between the means of the experimental groups and p value  $\leq$  0.05) was considered statistical significance.

## Results

### PO-Leaf Infusion Effects on the Erythrocyte Osmotic Fragility

As shown in Table 1 and Figure 1, there were no significant differences in the degree of erythrocyte hemolysis of groups at NaCl concentrations of 0.1, 0.2, 0.8 and 0.9 g  $\text{L}^{-1}$ . However, there were significant changes in erythrocyte fragility of Ethanol, silymarin and PO-treatment groups at NaCl concentrations of 0.3, 0.6 and 0.7 (Ethanol and Ethanol + PO-20 group) mg  $\text{mL}^{-1}$  compared to the Control group. However, at the NaCl concentration of 0.3 Ethanol-treated groups (silymarin and PO-leaf infusion doses) exhibited a significant decrease compared to the Ethanol group.



**Figure 1.** Effects of silymarin and PO-leaf infusion on the erythrocyte osmotic fragility against ethanol-induced oxidative stress in rats [Data were expressed as the mean  $\pm$  SD. One-way ANOVA followed by the Tukey test, when appropriate ( $n = 6$  animals for each of the 5 groups). <sup>a</sup> Difference between the Control group and the other groups was significant ( $p \leq 0.05$ ). <sup>b</sup> Difference between the Ethanol group and the other groups was significant ( $p \leq 0.05$ )

**Table 1.** Effects of silymarin and PO-leaf infusion on the erythrocyte osmotic fragility against ethanol-induced oxidative stress in rats

Groups	Percentage NaCl concentration									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	
Control	95.35 $\pm$ 8.08	67.06 $\pm$ 8.94	10.54 $\pm$ 1.33	6.51 $\pm$ 0.93	5.82 $\pm$ 1.00	5.01 $\pm$ 0.24	5.39 $\pm$ 0.81	6.31 $\pm$ 0.73	4.86 $\pm$ 0.28	
Ethanol	97.07 $\pm$ 7.98	71.21 $\pm$ 11.81	18.81 $\pm$ 1.65 <sup>a</sup>	7.37 $\pm$ 0.49	7.08 $\pm$ 0.93	7.23 $\pm$ 0.44 <sup>a</sup>	7.36 $\pm$ 0.48 <sup>a</sup>	7.30 $\pm$ 0.74	5.37 $\pm$ 0.71	
Ethanol + Silymarin	95.04 $\pm$ 9.99	63.90 $\pm$ 11.32	14.20 $\pm$ 2.65 <sup>ab</sup>	6.88 $\pm$ 0.71	6.49 $\pm$ 0.58	6.53 $\pm$ 0.70 <sup>a</sup>	6.67 $\pm$ 0.76	7.26 $\pm$ 0.64	5.15 $\pm$ 0.29	
Ethanol + PO-20 mg $\text{mL}^{-1}$	94.21 $\pm$ 6.23	73.63 $\pm$ 10.55	15.28 $\pm$ 0.82 <sup>ab</sup>	7.39 $\pm$ 0.51	6.78 $\pm$ 0.57	7.05 $\pm$ 0.35 <sup>a</sup>	7.00 $\pm$ 0.22 <sup>a</sup>	7.18 $\pm$ 0.24	5.43 $\pm$ 0.69	
Ethanol + PO-60 mg $\text{mL}^{-1}$	92.69 $\pm$ 9.27	61.40 $\pm$ 12.81	14.66 $\pm$ 1.33 <sup>ab</sup>	7.20 $\pm$ 0.42	6.58 $\pm$ 1.07	6.76 $\pm$ 0.66 <sup>a</sup>	6.67 $\pm$ 0.75	6.61 $\pm$ 0.31	5.00 $\pm$ 0.34	

Data were expressed as the mean  $\pm$  SD. One-way ANOVA followed by the Tukey test, when appropriate ( $n = 6$  animals for each of the 5 groups)

<sup>a</sup> Difference between the Control group and the other groups was significant ( $p \leq 0.05$ ).

<sup>b</sup> Difference between the Ethanol group and the other groups was significant ( $p \leq 0.05$ ).

<sup>c</sup> Difference between the Ethanol + Silymarin group and the other groups was significant ( $p \leq 0.05$ ).

<sup>d</sup> Difference between the Ethanol + PO-20 mg  $\text{mL}^{-1}$  group and the Ethanol + PO-60 mg  $\text{mL}^{-1}$  group was significant ( $p \leq 0.05$ ).



### PO-Leaf Infusion Effects on the Haematological Parameters

The levels of the erythrocyte and platelet parameters were given in Table 2. The levels of RBC and RDW<sub>a</sub> in the Ethanol group were significantly increased compared to the Control group; however, the HCT and HGB levels in the Ethanol + PO-leaf 60 group were significantly increased compared to the Ethanol group. The levels of PCT and PLT in the all groups were significantly reduced compared to the Control group. MPV and PDW values were significantly decreased in Ethanol + Silymarin group compared to Ethanol + PO treated groups. Additionally,

PDW and LPCR levels in the Ethanol + Silymarin group were significantly lower than Ethanol group.

The levels of WBC, LYM, MON and GRA were given in Table 3. The LYM levels in Ethanol group were significantly lower than Control group. MON (%) and GRA (%) levels in Ethanol + Silymarin group were significantly higher than Control group. Additionally, the MON ( $10^9 L^{-1}$ ) and GRA ( $10^9 L^{-1}$ ) levels in the Ethanol + Silymarin group were significantly increased compared to the almost all groups. The level of GRA (%) in the ethanol group was significantly increased compared to all other groups except for the Ethanol + PO-20 group.

**Table 2.** Effects of silymarin and PO-leaf infusion on the on erythrocytic and platelets parameters against ethanol-induced oxidative stress in rats

Parameters	GROUPS				
	Control	Ethanol	Ethanol + Silymarin	Ethanol + PO-20 mg mL <sup>-1</sup>	Ethanol + PO-60 mg mL <sup>-1</sup>
RBC ( $10^{12} L^{-1}$ )	8.69 ± 0.53	7.82 ± 0.61 <sup>a</sup>	8.36 ± 0.39	8.48 ± 0.45	8.35 ± 0.66
RDW (%)	15.10 ± 0.42	13.53 ± 0.68	14.32 ± 0.45	14.88 ± 0.89	14.68 ± 0.99
RDW <sub>a</sub> (fL)	33.76 ± 0.34	31.70 ± 1.94 <sup>a</sup>	33.00 ± 1.43	33.36 ± 1.52	31.98 ± 2.06
HCT (%)	48.22 ± 2.88	44.78 ± 2.61	47.36 ± 2.97	47.22 ± 2.29	48.75 ± 1.35 <sup>b</sup>
HGB (g dL <sup>-1</sup> )	15.72 ± 0.91	14.87 ± 0.50	15.36 ± 0.78	15.40 ± 0.76	15.80 ± 0.16 <sup>b</sup>
MCH (pg)	18.12 ± 0.43	18.50 ± 0.37	18.40 ± 0.07	18.20 ± 0.35	18.98 ± 1.27
MCHC (g dL <sup>-1</sup> )	32.66 ± 0.49	32.85 ± 1.00	32.54 ± 0.47	32.68 ± 0.36	33.02 ± 1.54
MCV (fL)	55.42 ± 0.62	56.40 ± 1.12	56.43 ± 0.70	55.76 ± 0.95	55.50 ± 2.03
MPV (fL)	6.52 ± 0.24	6.58 ± 0.13	6.36 ± 0.18	6.58 ± 0.11 <sup>c</sup>	6.64 ± 0.21 <sup>c</sup>
PCT (%)	0.49 ± 0.09	0.28 ± 0.05 <sup>a</sup>	0.32 ± 0.04 <sup>a</sup>	0.33 ± 0.03 <sup>a</sup>	0.30 ± 0.04 <sup>a</sup>
PLT ( $10^9 L^{-1}$ )	689.00 ± 54.25	445.50 ± 57.25 <sup>a</sup>	508.00 ± 40.55 <sup>a</sup>	496.60 ± 59.62 <sup>a</sup>	458.00 ± 41.04 <sup>a</sup>
PDW (fL)	8.46 ± 0.23	8.53 ± 0.13	8.26 ± 0.18 <sup>b</sup>	8.48 ± 0.11 <sup>c</sup>	8.56 ± 0.21 <sup>c</sup>
LPCR (%)	5.08 ± 1.03	5.88 ± 0.90	4.42 ± 0.61 <sup>b</sup>	5.66 ± 0.76	5.68 ± 1.16

Data were expressed as the mean ± SD. One-way ANOVA followed by the Tukey test, when appropriate (n= 6 animals for each of the 5 groups)

<sup>a</sup> Difference between the Control group and the other groups was significant (p ≤ 0.05).

<sup>b</sup> Difference between the Ethanol group and the other groups was significant (p ≤ 0.05).

<sup>c</sup> Difference between the Ethanol + Silymarin group and the other groups was significant (p ≤ 0.05).

RBC: Red Blood Cells counts; RDW: Red cells Distribution Width; HCT: Haematocrit; HGB: Haemoglobin; MCH: Mean Cell Haemoglobin; MCHC: Mean Cell Corpuscular Haemoglobin Concentration; MCV: Mean Corpuscular Volume; MPV: Mean Platelet Volume; PCT: Plateletcrit; PLT: Platelet; PDW: Platelet Distribution Width; PLCR: Platelet Large Cell Ratio.

**Table 3.** Effects of silymarin and PO-leaf infusion on the different leukocyte parameters against ethanol-induced oxidative stress in rats

Parameters	GROUPS				
	Control	Ethanol	Ethanol + Silymarin	Ethanol + PO-20 mg mL <sup>-1</sup>	Ethanol + PO-60 mg mL <sup>-1</sup>
WBC ( $10^9 L^{-1}$ )	3.73 ± 0.75	4.60 ± 1.20	5.04 ± 1.08	4.12 ± 0.72	5.20 ± 1.12
LYM ( $10^9 L^{-1}$ )	4.23 ± 1.06	3.28 ± 0.71	3.74 ± 0.90	3.12 ± 0.87	3.58 ± 0.43
LYM (%)	82.23 ± 0.94	75.93 ± 4.10 <sup>a</sup>	74.38 ± 7.98	80.54 ± 4.69	81.68 ± 3.87
MON ( $10^9 L^{-1}$ )	0.35 ± 0.06	0.33 ± 0.10	0.60 ± 0.16 <sup>ab</sup>	0.38 ± 0.08 <sup>c</sup>	0.35 ± 0.06 <sup>c</sup>
MON (%)	8.60 ± 1.63	8.93 ± 1.68	11.16 ± 1.87 <sup>a</sup>	8.12 ± 1.43 <sup>c</sup>	8.65 ± 1.98
GRA ( $10^9 L^{-1}$ )	0.33 ± 0.05	0.40 ± 0.08	0.62 ± 0.13 <sup>ab</sup>	0.46 ± 0.05 <sup>ac</sup>	0.43 ± 0.06 <sup>a</sup>
GRA (%)	9.68 ± 1.54	15.15 ± 3.23 <sup>a</sup>	14.46 ± 3.17 <sup>a</sup>	11.34 ± 2.58	9.68 ± 1.90 <sup>bc</sup>

Data were expressed as the mean ± SD. One-way ANOVA followed by the Tukey test, when appropriate (n= 6 animals for each of the 5 groups)

<sup>a</sup> Difference between the Control group and the other groups was significant (p ≤ 0.05).

<sup>b</sup> Difference between the Ethanol group and the other groups was significant (p ≤ 0.05).

<sup>c</sup> Difference between the Ethanol + Silymarin group and the other groups was significant (p ≤ 0.05).

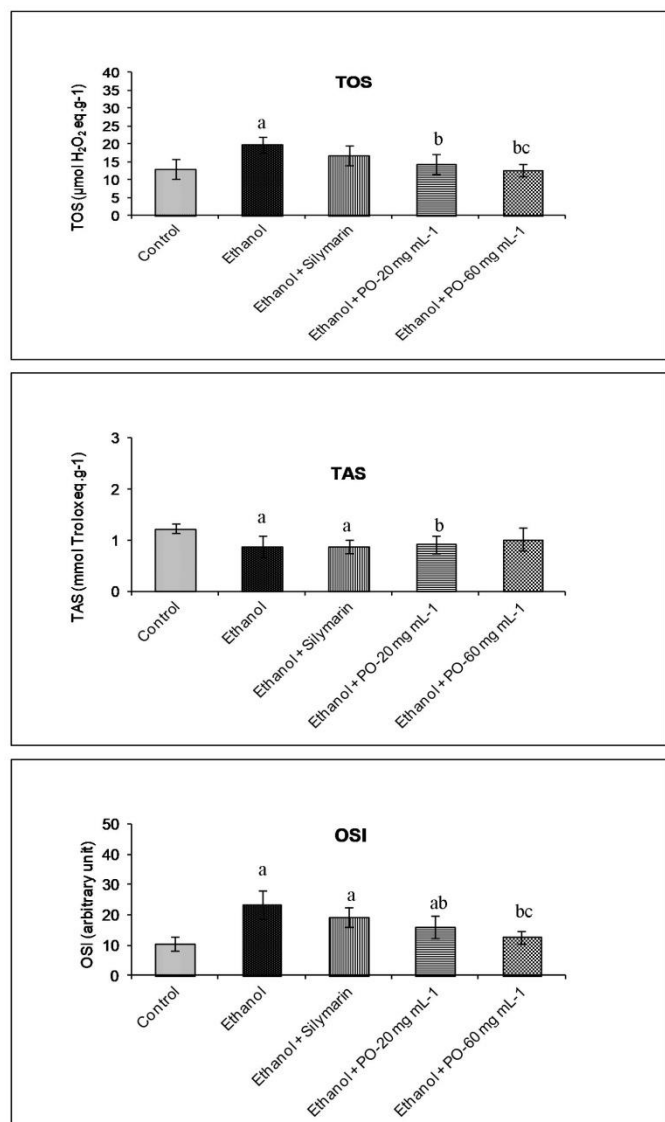
WBC: White Blood Cell; LYM: Lymphocyte; MON: Monocytes; GRA: Granulocyte.

### PO-Leaf Infusion Effects on the TOS, TAS and OSI Parameters

The effects of TOS, TAS and OSI in plasma were presented in the Figure 2. The plasma level of TOS in the Ethanol group was significantly higher than in the Control and Ethanol + PO-treated groups. Furthermore, TOS activity in plasma showed a significant decrease in the Ethanol + PO-60 group compared to

the Ethanol + PO-20 group. On the other hand, Ethanol and Ethanol + silymarin group were decreased significantly compared to the control group, similarly in TAS levels. The Ethanol group showed a significant decrease compared to the Ethanol + PO-20 group. OSI levels in Ethanol, Ethanol + Silymarin and Ethanol + PO-20 groups were significantly decreased compared to the control group while OSI levels in

Ethanol group were significantly increased compared to Ethanol + PO-treatment groups and OSI levels of Ethanol + PO-60 administration group were significantly reduced compared to the Ethanol + PO-20 group.



**Figure 2.** PO-leaf infusion effects on the TOS, TAS and OSI parameters against ethanol-induced oxidative stress in rats [Data were expressed as the mean  $\pm$  SD. One-way ANOVA followed by the Tukey test, when appropriate ( $n = 6$  animals for each of the 5 groups). <sup>a</sup> Difference between the Control group and the other groups was significant ( $p \leq 0.05$ ). <sup>b</sup> Difference between the Ethanol group and the other groups was significant ( $p \leq 0.05$ ). <sup>c</sup> Difference between the Ethanol + Silymarin group and the other groups was significant ( $p \leq 0.05$ ). <sup>d</sup> Difference between the Ethanol + PO-20 mg mL<sup>-1</sup> group and the Ethanol + PO-60 mg mL<sup>-1</sup> group was significant ( $p \leq 0.05$ )]

## Discussion

Different parts of *Platanus orietalis* have been used in various diseases for many years worldwide. For example; various researchers have reported that the use of PO-leaves in folk medicine such as in ophthalmia (Haider et al., 2012) as well as in liver, kidney damages and obesity (Dogan and Anuk,

2019). Chopra et al. (1956) have reported that the use of PO-barks boiled in vinegar for toothache, diarrhea and dysentery. Another study has reported the use of PO-buds for urinary tract antimicrobial and antiseptic agents (Mitrokotsa et al., 1993), Platanoside and Tiliroside isolated from PO-fruits have been used for anti-aging and cell toxicity (Chatzigeorgiou et al., 2017). However, the effects of PO-leaf infusion on the erythrocyte fragility, haematological and TOS/TAS index parameters have not been investigated against ethanol-induced OS in experimental rats model. In addition, silymarin was used as a positive control group; because of preventing effect of on liver damage caused by various toxic substances such as ethanol and carbon tetrachloride (Dogan and Anuk., 2019; Shaker et al., 2010).

EtOH is used to create toxicity models in rats. Chronic alcohol consumption leads to liver and kidney damage and an increase in biochemical parameters such as gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatinine, lipid peroxidation and decreases in antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, catalase and glutathione reductase (Dogan and Celik, 2012; Bati et al., 2015). EtOH can cause the suppression of the immune system and the development of various infections. The previous study showed that EtOH suppressed several leukocyte functions, phagocytic function of circulating neutrophils, as well as other neutrophil functions including adhesion, chemotaxis and oxygen metabolism (Chiu et al., 2018). Increased oxidative stress caused by EtOH leads to a decrease in reduced glutathione (GSH) levels, which has a significant protective role in erythrocytes against to protein thiol oxidation. This negative condition is responsible for decreased resistance to hemolysis (Padmini and Sundari, 2008). The EtOH, a highly cytotoxic chemical, has been reported to be responsible for the oxidation of proteins, erythrocyte abnormalities and hemolysis (Tyulina et al., 2006). It has also been reported that RBCs, membrane lipids, are very sensitive to metabolites that induce oxidation of proteins and increase fragility and this fragility is further increased in ethanol consumption (Alimi et al., 2012). In the present study, we evaluated for the first time the effect of PO-leaf infusion against ethanol-induced OS and osmotic fragility of rats erythrocytes. PO-leaf infusions against EtOH toxicity showed significant changes in hemolysis especially at 0.3 NaCl % concentration. We assume that this protective property may be a result of the effect of the plant content. In our previous study, we have reported that PO-leaf contains a variety of natural antioxidants including kaempferol and kaempferol derivatives, benzaldehyde, palmitic acid, 2,4-ditert-butylphenol, stearic acid, octadecanoic acid, linoleic acid and linolenic acid (Dogan and Anuk, 2019). The extracts containing kaempferol or kaempferol derivatives have been shown to inhibit erythrocyte hemolysis (Olchowik et al., 2012; da Cunha et al., 2016). These results were similar to the results obtained in this study.

The TOS, TAS and TOS/TAS index are important indicator parameters for the assessment of oxidative status. It has been reported that ethanol causes a decrease in TAS and an increase in TOS (Ozkol et al., 2017; Erkec et al., 2018). The cause of

this effect is uncertain, but is probably the result of various kaempferol and fatty acids contents in the plant.

## Conclusion

When all of the obtained data were evaluated, it was determined that *P. orientalis* leaves possess antioxidant properties and protective effects on erythrocyte fragility and various haematological parameters against ethanol-toxicity in rats. We are planning to evaluate the effects of PO leaf infusion immunotoxicity and neurotoxicity in further studies.

## Acknowledgements

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## RESEARCH ARTICLE

### The Influence of Turmeric Powder (*Curcuma longa*) on Fatty Acid Composition and Shelf Life of Broiler Chicken Meat

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#### ABSTRACT

The objective of this study was to determine the appropriate concentration of dietary supplementation of turmeric powder, and its effect on thiobarbituric acid reactive substance (TBARS) and fatty acid composition in thigh and breast meat of broiler chickens. Three hundred fifty (175 male and 175 female), one day old Ross-308 broiler chicks were used in this study. A corn-soybean meal based diet containing different levels of turmeric powder (0, 2, 4, 6, 8, 10 g/kg) and a single dose of chlortetracycline (10 mg/kg) was used. The result revealed that dietary supplementation of 2, 4, 6, 8 and 10 g/kg of turmeric powder decreased TBARS in thigh meat at 5<sup>th</sup> day when compared with control. The addition of 4 g/kg turmeric powder to the basal diet increased DHA, SFA and omega-3 in breast meat. DHA and SFA were increased by dietary 2 g/kg turmeric powder in thigh meats. Under the conditions of this experiment, it was concluded that turmeric powder may positive effects on tissue fatty acid compositions and shelf life of meat (TBARS). As a result, it was observed that there were positive effects on tissue fatty acid compositions and shelf life of meat (TBARS) by adding 4 g/kg turmeric powder.

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#### Introduction

*Curcuma longa*, which is a tropical plant of Zingiberaceae family, was used as a feed additive. Curcumin is the most important active ingredient of *Curcuma longa*, and has been widely used in Asian and middle-Eastern (Gowda et al. 2009; Chattopadhyay et al. 2004). *Curcuma longa* has been reported to perform a number of biological activities, like antioxidant, antimicrobial, antifungal, antimutagenic and antidiabetic (Araujo and Leon, 2001; Gowda et al. 2009). It has also been reported to increased weight gain, and improved nutrients digestibility in farm animals (Al-Sultan and Gameel, 2004; Mehala and Moorthy, 2008, Urusan and Bolukbasi, 2017). On the other hand the use of turmeric in broiler diets has been successfully demonstrated with antimicrobial activity on *E.coli*

and coliform bacteria (Samarasinghe et al. 2003; Urusan and Bolukbasi, 2017). In recent years, researchers have focused their studies on phytochemicals. It was determined with the studies that alfalfa (Ponte et al. 2004), and thyme (Bolukbasi et al. 2006) decreased the serum cholesterol and lipoproteins; mentha pulegium had positive effects on TBARS (Erhan et al. 2015); and adding turmeric decreased the SFA rates in tissues (Daneshyar et al. 2011). It is also turmeric had strong antioxidant effects and were more powerful in preventing lipid oxidation than Vitamin E (Jayaprakasha et al. 2005). Again, it was determined by many authors that curcumin has an antioxidant activity that was comparable with that of Vitamin C and E (Sharma, 1975; Shukla et al. 1997; Thiyagarajan and Sharma, 2004; Karami et al. 2011). Turmeric also prevents the

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formation of peroxide in foods and increases the preservation period.

The purpose of this study was designed to investigate the possible effect of turmeric as feed additive on the amount of oxidation in tissues and on the fatty acid composition.

## Materials and Methods

### Experimental Design and Animals

In this study, a total number of 350 (175 male-175 female) one-day-old broilers (Ross 308) were randomly allocated to 7

treatments with 5 replicates and each replicate contained 10 birds. The study was conducted with 7 groups (1 control, and 6 treatment groups) each of which included 50 chicks. Treatments were 0, 2, 4, 6, 8, 10 g/kg of turmeric and 10 mg/g antibiotic added into the feeds during experiment (42 days). Turmeric (70.79% Beta Tumerone, 9.65% Alpha-Tumerone, 2.06% Isocumene, 2.04% BetaSesquiphellandrene, and 2% Zingiberene) was purchased from a commercial company (Erzurum, Turkey). Composition of the experimental diets is presented in Table 1. Feed and water were given ad libitum.

**Table 1.** The composition of the experimental diet (g/kg)

Item	Starter diet (1-21 d)	Finisher diet (22-42 d)
Corn	562.00	556.00
Soybean Meal	189.00	120.00
Full-Fat Soybean	160.00	229.35
Poultry Meal	35.00	35.00
Meat and Bone Meal	34.00	34.00
Vegetable Oil	4.00	12.00
Salt	1.80	1.85
Lysine	3.00	2.10
Methionine	2.00	1.30
Limestone	2.00	2.20
Vitamin Mixture	2.00	2.00
Mineral Mixture	1.50	1.50
Soda	1.50	2.20
DCP	2.20	0.50
TOTAL	1000	1000
<b>Calculated composition (%)</b>		
ME (kcal/kg)	3040	3240
Ca	1.05	0.99
P	0.56	0.53
Methionine	1.22	0.94
Lysine	1.50	1.25
<b>Analysed composition (%)</b>		
Crude Protein	22.70	20.99
Crude Fat	8.13	10.15
Crude Fiber	3.96	4.21
Crude Ash	5.24	5.29
Dry Matter	88.94	90.08

1: In each 2 kg mixture; 12 000 000 IU Vitamin A., 3 500 000 IU Vitamin D3, 100 g Vitamin E., 3 g Vitamin K3. 2.5 g. Vitamin B1, 6 g Vitamin B2, 25 g Niacin. 12 g Ca-D-Pantothenate, 4 g Vitamin B6., 15 mg Vitamin B12., 1.5 g Folic Acid, 150mg D-Biotin., 100 g Vitamin C., 450 g Colin chloride. 2: each 1.5 kg 100 mg Mangan., 25 g Iron., 65 g Zink., 15 g Copper., 0.25 g Cobalt., 1 g Iodine., 0.2 g Selenium.

### TBARS (Thiobarbituric Acid Reactive Substance) Analysis

At the end of the study, ten birds (5 females and 5 males) selected randomly from each treatment were slaughtered and the thigh and breast meats of the animals were separated. Four samples were taken from each sub-group and stored at ± 4°C; and the TBARS (Thiobarbituric Acid Reactive Substance) values were examined on 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> days (Lemon, 1975). In addition, the fatty acid composition was examined in the samples that were taken from thigh and breast (Anonymous, 2000).

### Fatty Acid Analysis

Fatty acid analyses were performed at the Biotechnology Application and Research Centre. After extracting (Folch et al. 1957) meat samples were methylated for gas chromatographic analysis (GC- Agilent 6980 Mass, a fused silica capillary column, and film thickness of 0.25 µm). Oven temperature was from 165 °C to 200 °C at 5 °C/min. detector temperature was 200 °C; head pressure was 5 psi.

### Statistical Analysis

Differences between groups were analysed with one-way analysis of variance (ANOVA) by using the statistical package SPSS for Windows (1999), version 10.0. Mean values that

significantly differ were separated by Duncan's multiple comparison test at  $\alpha = 0.01$  and  $0.05$  levels, respectively.

### Results and Discussion

It was reported that TBARS analysis in food is an important quality criterion showing the oxidation of fat. TBARS value should also be less than 3 mg per kilogram of food quality (Cadun et al. 2005). When the TBARS values of the thigh were examined on the first day, it was observed that the highest TBARS value was obtained in the group which had 10 mg/kg antibiotic added to the ration, and the lowest TBARS values were observed in the group which had control, 2, 4, 6, 8 and 10 g/kg turmeric powder added to the ration, and this difference was found to be significant ( $P < 0.001$ ) statistically (Table 2). It was also observed that the highest value was obtained in the antibiotic group on the 3<sup>rd</sup> day of the storage,

and the lowest value was obtained in the groups whose ration had 6 and 8 g/kg turmeric powder added in the ration, and the difference between groups was significant ( $P < 0.001$ ). On the 5<sup>th</sup> day of the storage, which was the last day, when we examined the TBARS values of the thigh, we determined that the highest value was in the control group, and the lowest level was in the groups whose rations were added 2, 4, 6, 8 and 10 g/kg turmeric powder, and this difference was very significant ( $P < 0.001$ ). Many studies have been conducted in recent years to reveal the antioxidant characteristics of the turmeric (Pulla Reddy and Lokesh, 1994; Sreejayan et al. 1997; Asai et al., 1999; Suvanated et al., 2003; Basavaraj et al., 2011; Hosseini-Vashan et al. 2012). Attia et al. (2017) reported that turmeric is a good unsaturated fatty acid source that is 60 % of the antioxidant activity.

**Table 2.** The influence of dietary turmeric supplementation on TBARS values (mg MDA/kg tissue) in thigh and breast of the broilers

Groups	TBARS					
	Thigh			Breast		
	1 <sup>st</sup> Day	3 <sup>rd</sup> Day	5 <sup>th</sup> Day	1 <sup>st</sup> Day	3 <sup>rd</sup> Day	5 <sup>th</sup> Day
Control	0.91 <sup>bc</sup>	2.01 <sup>bc</sup>	4.71 <sup>a</sup>	0.71	2.27	3.12
Turmeric 2 g/kg	1.19 <sup>b</sup>	2.61 <sup>ab</sup>	3.12 <sup>bc</sup>	0.82	1.65	2.84
Turmeric 4 g/kg	0.85 <sup>bc</sup>	1.42 <sup>cd</sup>	2.95 <sup>c</sup>	0.91	1.67	3.09
Turmeric 6 g/kg	0.77 <sup>c</sup>	1.25 <sup>d</sup>	2.38 <sup>c</sup>	0.91	1.76	2.92
Turmeric 8 g/kg	0.65 <sup>c</sup>	1.05 <sup>d</sup>	2.18 <sup>c</sup>	0.85	1.67	2.72
Turmeric 10 g/kg	0.71 <sup>c</sup>	1.53 <sup>cd</sup>	3.03 <sup>bc</sup>	0.34	1.50	3.09
Antibiotics 10 mg/kg	1.81 <sup>a</sup>	3.12 <sup>a</sup>	4.17 <sup>ab</sup>	0.68	1.81	2.92
SE	0.082	0.154	0.206	0.055	0.113	0.068
P value	0.000 <sup>**</sup>	0.000 <sup>**</sup>	0.001 <sup>**</sup>	ns	ns	ns
Day		0.000 <sup>**</sup>			0.000 <sup>**</sup>	
Group x Day		0.003 <sup>**</sup>			ns	

a, b, c, d: The differences between the group average values shown with different letters on the same column  
SE: The standard error of the difference between the averages <sup>\*\*</sup>: $P < 0.01$ , ns: not significant.

In this study, it was determined that adding turmeric powder, which had antioxidant effects, to the ration decreased the amount of the oxidation in thigh at a significant level. It is considered that this decrease in the lipid oxidation rate is caused by curcumin, which is the main component of turmeric, and which was reported to be a phenolic antioxidant (Sreejayan et al. 1997).

It was reported by Fellenberg and Speisky (2006) that the shelf life of meat products extended due to the continuing effects of some antioxidant at postmortem period. We indicated in the current study that meat shelf life increased and lipid oxidation rates reduced as a result of the postmortem effects of turmeric dietary supplementation. This effect may be due to the accumulation of the main components of turmeric in tissues. Several researchers reported decreased TBARS value when turmeric was added to the diet (Botsoglou et al. 2002; Daneshyar, 2012; Hosseini-Vashan et al. 2012). Hosseini-Vashan et al. (2012) claimed that adding 0.4 and 0.8% turmeric to broiler feed decreased the TBARS values in the serum at a significant level.

The effect of the applications on the TBARS values of the breast on the 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> days was found to be insignificant ( $P > 0.05$ ). This situation may stem from the total fat amount in

breast being lower (Table 3). The TBARS values of the breast and thigh tissue showed an increase in all the study groups with the increase in storage period and was found to be very significant statistically ( $P < 0.01$ ).

### Fatty Acid Composition

When Table 3 is examined, it is observed that the addition of turmeric powder to broiler rations at different levels did not affect the lipid contents of the breast meat; however, changed the fatty acid composition at a significant level. Compared with the control group, almost all the SFAs in breast were significantly altered in the groups which were given dietary turmeric, except for the arachidic acid (20:0). Broilers fed with 4 g/kg turmeric had significantly higher palmitic acid (16:0), heptadecanoic acid (17:0) and total SFA in breast compared with the other groups. The stearic acid (18:0) rate in the breast meat was increased to a certain dosage by the addition of turmeric powder to the rations, and the highest value was observed in the group which received 6 g/kg turmeric powder in the ration; and as the dosage increased the stearic acid rate decreased (Table 3).

Adding 6 g/kg Turmeric to the ration decreased the total MUFA rate at a significant level ( $P < 0.05$ ). The highest

myristoleic acid (14:1), heptadecenoic acid (17:1) and eicosenoic acid (20:1n-9) rate, which are among the mono-unsaturated fatty acids, were observed in the control, 4g/kg and 8g/kg turmeric-consuming group, respectively. No influence of adding turmeric on the palmitoleic acid (16:1n-7) and linoleic acid (18:2n-6) rate was observed (Table 3).

It was determined that adding turmeric did not affect the total PUFA rate at a significant manner; however, the lowest numerical values were determined in the 4 g/kg turmeric group. The highest linoleic acid (18:2n-6) rate was observed in the group whose basal feed contained 10 g/kg turmeric powder, and the lowest linoleic acid rate was observed in the group which received 4 g/kg *Curcuma longa* to the basal feed. In terms of linoleic acid (18:3n-3), the highest rate was determined in 10 g/kg turmeric group, again; and the lowest rate was determined in the group which received 4 and 6 g/kg turmeric. It is consistent with some studies that reported there was increase in breast meat linoleic acid level (Coetzee et al.

2002). Unlike linoleic acid, eicosadienoic acid (20:2n-9) and arachidonic acid (20:4n-6) rate was determined as being the highest in the group which received turmeric powder to basal feed at a rate of 4 and 6 g/kg, and as being the lowest in the group which received 10 g/kg turmeric powder.

Supplementation of 10 g/kg turmeric powder in the diet of broilers increased the ratios of linoleic and decreased the ratio of arachidonic acid in breast tissue. This mechanism may be explained as  $\Delta$ -6 desaturase, as a rate-limiting enzyme in the conversion of linoleic acid to arachidonic acid, is inhibited by the main components of turmeric.

It was determined that the eicostrienoic acid (20:3n-6) rate was the highest in the group which received 6 g/kg turmeric powder to the basal feed. It was observed that adding 4 g/kg turmeric powder to the basal feed increased the DHA (22:6n-3), total omega 3 ( $\Sigma \omega$ -3) and omega-3/omega-6 rate at a significant level ( $P < 0.05$ ) (Table 3).

**Table 3.** Effect of dietary supplementation of turmeric powder (*Curcuma longa*) on fatty acid composition in breast meats of broiler

Fatty acids	Control	Turmeric 2 g/kg	Turmeric 4 g/kg	Turmeric 6 g/kg	Turmeric 8 g/kg	Turmeric 10 g/kg	Antibiotics 10 mg/kg	SE	P
Lipid contents	1.70	1.73	1.68	1.79	1.64	1.60	1.80	0.02	ns
Myristic acid (14:0)	2.00 <sup>a</sup>	0.38 <sup>c</sup>	0.80 <sup>b</sup>	0.31 <sup>c</sup>	0.42 <sup>c</sup>	0.74 <sup>b</sup>	0.41 <sup>c</sup>	0.154	0.000**
Myristoleic acid (14:1n-5)	1.50 <sup>a</sup>	0.39 <sup>b</sup>	0.29 <sup>b</sup>	0.05 <sup>b</sup>	0.11 <sup>b</sup>	0.17 <sup>b</sup>	0.29 <sup>b</sup>	0.132	0.000**
Palmitic acid (16:0)	18.18 <sup>bc</sup>	18.24 <sup>bc</sup>	20.85 <sup>a</sup>	19.95 <sup>ab</sup>	15.94 <sup>cd</sup>	15.14 <sup>d</sup>	16.55 <sup>cd</sup>	0.574	0.006**
Palmitoleic acid (16:1n-7)	2.87	2.39	1.72	1.91	3.19	2.88	4.02	0.238	ns
Heptadecenoic acid (17:0)	0.21 <sup>b</sup>	0.45 <sup>b</sup>	1.08 <sup>a</sup>	0.34 <sup>b</sup>	0.18 <sup>b</sup>	0.28 <sup>b</sup>	0.21 <sup>b</sup>	0.086	0.003**
Heptadecenoic acid (17:1n-7)	0.25 <sup>b</sup>	0.56 <sup>b</sup>	2.11 <sup>a</sup>	0.45 <sup>b</sup>	0.28 <sup>b</sup>	0.32 <sup>b</sup>	0.31 <sup>b</sup>	0.187	0.012*
Stearic acid (18:0)	6.21 <sup>bc</sup>	6.25 <sup>bc</sup>	6.81 <sup>ab</sup>	8.10 <sup>a</sup>	5.44 <sup>bc</sup>	4.50 <sup>c</sup>	4.78 <sup>c</sup>	0.351	0.020*
Oleic acid (18:1n-9)	27.38	24.55	23.81	21.98	24.69	28.89	29.23	0.819	ns
Linoleic acid (18:2n-6)	30.44 <sup>ab</sup>	31.37 <sup>ab</sup>	21.08 <sup>c</sup>	27.62 <sup>bc</sup>	26.53 <sup>bc</sup>	36.27 <sup>a</sup>	32.89 <sup>ab</sup>	1.402	0.027*
Linolenic acid (18:3n-3)	2.76 <sup>ab</sup>	2.17 <sup>bc</sup>	1.51 <sup>c</sup>	1.43 <sup>c</sup>	1.83 <sup>bc</sup>	3.48 <sup>a</sup>	2.73 <sup>ab</sup>	0.213	0.022*
Arachidic acid (20:0)	0.23	0.18	0.16	0.16	0.19	0.11	0.16	0.016	ns
Eicosenoic acid (20:1n-9)	0.31 <sup>c</sup>	0.41 <sup>bc</sup>	0.58 <sup>ab</sup>	0.44 <sup>bc</sup>	0.78 <sup>a</sup>	0.22 <sup>c</sup>	0.43 <sup>bc</sup>	0.051	0.015*
Eicosadienoic acid (20:2n-9)	0.80 <sup>bc</sup>	0.70 <sup>bc</sup>	1.73 <sup>a</sup>	1.47 <sup>a</sup>	0.86 <sup>b</sup>	0.28 <sup>c</sup>	0.64 <sup>bc</sup>	0.136	0.003**
Eicosatrienoic acid (20:3n-6)	0.43 <sup>c</sup>	0.78 <sup>b</sup>	0.35 <sup>c</sup>	1.15 <sup>a</sup>	0.79 <sup>b</sup>	0.46 <sup>bc</sup>	0.54 <sup>bc</sup>	0.094	0.001**
Arachidonic acid (20:4n-6)	3.65 <sup>bc</sup>	5.24 <sup>ab</sup>	6.37 <sup>a</sup>	6.26 <sup>a</sup>	5.60 <sup>ab</sup>	2.58 <sup>c</sup>	3.76 <sup>bc</sup>	0.416	0.027*
EPA (20:5n-3)	0.56	0.87	0.49	0.48	0.62	0.32	0.29	0.060	ns
DHA (22:6n-3)	0.78 <sup>b</sup>	1.12 <sup>b</sup>	2.87 <sup>a</sup>	1.49 <sup>b</sup>	0.91 <sup>b</sup>	0.59 <sup>b</sup>	0.68 <sup>b</sup>	0.216	0.004**
SFA	26.84 <sup>b</sup>	25.49 <sup>b</sup>	29.70 <sup>a</sup>	28.87 <sup>b</sup>	22.17 <sup>c</sup>	20.76 <sup>c</sup>	22.12 <sup>c</sup>	1.439	0.000**
MUFA	32.31 <sup>a</sup>	28.30 <sup>ab</sup>	28.51 <sup>ab</sup>	24.81 <sup>b</sup>	29.06 <sup>ab</sup>	32.47 <sup>a</sup>	34.28 <sup>a</sup>	0.762	0.038*
PUFA	40.13	42.25	35.41	39.89	37.13	43.99	41.54	0.961	ns
n-3 (omega3)	4.10 <sup>abc</sup>	4.15 <sup>abc</sup>	4.88 <sup>a</sup>	3.40 <sup>c</sup>	3.35 <sup>c</sup>	4.39 <sup>ab</sup>	3.70 <sup>bc</sup>	0.161	0.044*
n-6 (omega6)	36.20	38.10	30.53	36.50	33.78	39.59	37.83	0.953	ns
n-3/n-6	0.11 <sup>b</sup>	0.11 <sup>b</sup>	0.16 <sup>a</sup>	0.09 <sup>b</sup>	0.10 <sup>b</sup>	0.11 <sup>b</sup>	0.10 <sup>b</sup>	0.007	0.028*

a, b: The differences between the group average values shown with different letters on the same column are important. SE: Standard error of the difference between the averages \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , ns: not significant

It was observed that the thigh tissue total lipid content and the saturated fatty acids are affected at a significant level by the addition of turmeric powder (Table 4). It was determined that adding 2 and 4 mg/kg turmeric to the ration increased the SFA and stearic acid (18:0) rate at a significant level. The lowest SFA values were determined at the higher levels of turmeric 8 and 10 g/kg.

Daneshyar et al. (2011) conducted a study and reported that adding turmeric to the broiler rations at high levels (0.75%) decreased the total SFA amount and plasma triglyceride rate at a significant level, which is similar to our

results. They claimed that the triglycerides that were produced with the hepatic lipogenes in the liver (Lanza-Jacoby, 1986; Herzberg and Rogerson, 1988) decreased with the effect of the turmeric, and depending on this, the SFA rate may have decreased in the thigh tissue. Salah et al. (2019) reported that supplementation of 100 mg curcumin /kg broiler diet significantly decreased SFA contents in the breast meat. However, Hang et al. (2018) reported that no significant changes in the SFA contents of breast and thigh meats of chickens were fed curcuminoids-supplemented diets.



The myristic acid (14:0) and arachidic acid (20:0) rates were not affected by the treatments; but the palmitic acid (16:0) rate was observed to reach the lowest level at 2 g/kg turmeric level, and to the highest level at 10 g/kg level (P<0.05).

Daneshyar et al. (2011) reported that the palmitic acid rate decreased with the addition of turmeric at a significant level.

Palmitic acid is the fatty acid that is responsible for the increase of LDL cholesterol that causes cardiovascular diseases (Rowe et al. 1999; Muchenje et al. 2009a; b). For this reason, the palmitic acid being lower in the tissues that are consumed is a positive development for health (Daneshyar et al. 2011). Asai and Miyazawa, (2001) reported that curcumin supplementation inhibited hepatic fatty acid synthase (FAS) activity and increased beta oxidation of fatty acids.

**Table 4.** Effect of dietary supplementation of turmeric powder (*Curcuma longa*) on fatty acid composition in thigh meats of broilers

Fatty acids	Groups	Control	Turmeric 2 g/kg	Turmeric 4 g/kg	Turmeric 6 g/kg	Turmeric 8 g/kg	Turmeric 10 g/kg	Antibiotics 10 mg/kg	SE	P value
Lipid contents		3.24 <sup>bc</sup>	3.33 <sup>bc</sup>	3.43 <sup>bc</sup>	3.22 <sup>c</sup>	3.26 <sup>c</sup>	3.00 <sup>d</sup>	3.65 <sup>a</sup>	0.04	0.05*
Myristic acid (14:0)		0.48	0.46	1.00	0.37	1.31	0.35	0.42	0.118	ns
Myristoleic acid (14:1n-5)		0.13 <sup>bc</sup>	0.05 <sup>c</sup>	0.18 <sup>ab</sup>	0.15 <sup>b</sup>	0.25 <sup>a</sup>	0.13 <sup>bc</sup>	0.12 <sup>bc</sup>	0.017	0.013*
Palmitic acid (16:0)		18.08 <sup>ab</sup>	19.21 <sup>a</sup>	18.39 <sup>ab</sup>	18.27 <sup>ab</sup>	17.00 <sup>bc</sup>	16.24 <sup>c</sup>	17.69 <sup>bc</sup>	0.276	0.022*
Palmitoleic acid (16:1n-7)		3.07	2.24	2.43	2.64	3.06	2.34	2.74	0.098	ns
Heptadecenoic acid (17:0)		0.19 <sup>bc</sup>	0.12 <sup>d</sup>	0.37 <sup>a</sup>	0.17 <sup>cd</sup>	0.24 <sup>b</sup>	0.18 <sup>bc</sup>	0.18 <sup>c</sup>	0.021	0.000**
Heptadecenoic acid (17:1n-7)		0.25 <sup>b</sup>	1.01 <sup>a</sup>	0.46 <sup>b</sup>	0.44 <sup>b</sup>	0.24 <sup>b</sup>	0.34 <sup>b</sup>	0.28 <sup>b</sup>	0.077	0.021*
Stearic acid (18:0)		5.79 <sup>b</sup>	7.62 <sup>a</sup>	7.57 <sup>a</sup>	5.69 <sup>b</sup>	5.26 <sup>b</sup>	6.86 <sup>ab</sup>	5.34 <sup>b</sup>	0.292	0.025*
Oleic acid (18:1n-9)		32.35	23.70	24.33	31.25	29.32	21.35	31.64	1.449	ns
Linoleic acid (18:2n-6)		31.48 <sup>ab</sup>	28.39 <sup>b</sup>	28.04 <sup>b</sup>	32.62 <sup>a</sup>	32.92 <sup>a</sup>	32.58 <sup>a</sup>	33.26 <sup>a</sup>	0.641	0.047*
Linolenic acid (18:3n-3)		2.83 <sup>a</sup>	1.90 <sup>bc</sup>	1.69 <sup>c</sup>	3.11 <sup>a</sup>	2.88 <sup>a</sup>	2.48 <sup>ab</sup>	2.83 <sup>a</sup>	0.148	0.006**
Arachidic acid (20:0)		0.19	0.21	0.18	0.25	0.15	0.12	0.12	0.024	ns
Eicosenoic acid (20:1n-9)		0.28	0.35	0.36	0.55	0.33	0.38	0.32	0.036	ns
Eicosadienoic acid (20:2n-9)		0.48 <sup>c</sup>	1.08 <sup>a</sup>	0.97 <sup>a</sup>	0.81 <sup>ab</sup>	0.43 <sup>c</sup>	0.54 <sup>bc</sup>	0.52 <sup>bc</sup>	0.071	0.005**
Eicosatrienoic acid (20:3n-6)		0.47	0.78	0.87	0.86	0.45	0.47	0.34	0.068	ns
Arachidonic acid (20:4n-6)		2.03	4.73	5.88	2.09	2.44	4.58	3.51	0.471	ns
EPA (20:5n-3)		0.23	0.70	0.39	0.37	0.24	0.14	0.26	0.056	ns
DHA (22:6n-3)		0.22 <sup>d</sup>	1.58 <sup>a</sup>	1.29 <sup>ab</sup>	1.43 <sup>ab</sup>	1.01 <sup>bc</sup>	0.75 <sup>c</sup>	0.30 <sup>d</sup>	0.143	0.001**
SFA		24.72 <sup>b</sup>	27.62 <sup>a</sup>	27.51 <sup>a</sup>	24.74 <sup>b</sup>	23.95 <sup>b</sup>	23.74 <sup>b</sup>	23.74 <sup>b</sup>	0.673	0.015*
MUFA		36.09	27.35	27.77	35.04	33.09	24.53	35.09	1.370	ns
PUFA		37.73	39.15	39.12	41.30	40.37	41.53	41.015	0.455	ns
n-3 (omega3)		3.28 <sup>b</sup>	4.17 <sup>ab</sup>	3.36 <sup>b</sup>	4.91 <sup>a</sup>	4.12 <sup>ab</sup>	3.37 <sup>b</sup>	3.38 <sup>b</sup>	0.178	0.033*
n-6 (omega6)		34.45	34.98	35.76	36.39	36.24	38.16	37.63	0.421	ns
n-3/n-6		0.10 <sup>bc</sup>	0.12 <sup>ab</sup>	0.09 <sup>bc</sup>	0.14 <sup>a</sup>	0.11 <sup>abc</sup>	0.09 <sup>c</sup>	0.09 <sup>c</sup>	0.005	0.025*

a, b: The differences between the group average values shown with different letters on the same column are important. SE: Standard error of the difference between the averages \*: P<0.05, \*\*:P<0.01, ns: not significant

It was determined that adding turmeric powder did not affect the MUFA, palmitoleic acid (16:1n-7), oleic acid (18:1n-9) and eichosenoic acid (20:1n-9) at a significant level. However, it was determined that the turmeric 2 g/kg level decreased the myristoleic acid (14:1n-5) rate at a significant level, and increased the heptadecenoic acid (17:1n-7) rate.

It was observed that adding turmeric powder to the ration had no influence on the ΣPUFA, eichosatrienoic acid (20:3n-6), arachidonic acid (C20:4n-6), EPA (20:5n-3), and n-6 (omega-6) fatty acids in the thigh tissues. The effect of turmeric powder on linoleic and eichosadienoic acids were significant statistically. Linoleic acid (C18:2n-6) rate in 2 and 4 g/kg turmeric was determined as the lowest, and the eichosadienoic acid (C20:2n-9) rate was observed as the highest. Linoleic acid (18:3n-3) rate was determined in the 4 g/kg turmeric group with the lowest level, 1.69%; and the 2 g/kg turmeric group followed this. It was observed that the DHA (22:6n-3) rate increased at a significant level with the addition of turmeric (P<0.01) when compared with the control group, and the highest value was determined at the 2 g/kg level. It was observed that the total omega-3 and omega-

3/omega-6 rates were affected by the addition of turmeric at a significant level (P<0.05) and these rates reached peak values at 6 g/kg level. Similarly, these findings, Hang et al. (2018) reported that supplementation of curcumin (20 mg/kg curcuminoids) to broiler diets significantly increased the linoleic acid and total n-6 PUFA contents in the breast meats. The enzyme Δ-6 desaturase promotes the desaturation of linoleic acid into arachidonic acid and α-linolenic into docosohexaenoic acid (DHA) and eicosapentaenoic acids (EPA) (Pereira et al. 2011). The main component of turmeric powder may promote the transformation of α-linolenic into its derivate DHA. We can associate the reason of the n3/n6 increase in total omega 3 fatty acids to this. Carrillo-Dominguez et al. (2005) conducted a study and determined that the increase in the long-chain n-3, and the DHA in the tissues might be because of the desaturation and the elongation of α-linoleic acid in the livers of hens. It was reported that DHA had a significant impact on brain and retinal neonatal development (Simopoulos, 2000). Humans can synthesize long-chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), from alpha linolenic acid, but this synthesis capability is limited. For this reason,

the meats of the animals that are fed with the turmeric-added feed have the quality of being an alternative dietary source for these fatty acids.

In conclusion, the results of our experiment show that the dietary supplementation of turmeric (2, 4, 6, 8, 10 g/kg) decreased the TBARS values in thigh tissues on the 5<sup>th</sup> day of the storage. This result shows that turmeric powder might have an effect that increases the shelf life in broiler carcass. It was determined that 4 g/kg turmeric in breast, and 2 g/kg turmeric in thigh tissues increased the DHA (22:6n3) rate at a significant level. 6 and 8 g/kg turmeric decreased the SFA rate both in thigh and in breast at a significant level. As a conclusion, it was determined that the 2, 4, 6 and 8 g/kg levels of turmeric powder, whose possible uses as an alternative to antibiotics is investigated, influenced the fatty acid composition and the TBARS of the meat except for the 10 g/kg level. Based on the results of this study, it can be recommended to supplement broiler feed with 4 g/kg turmeric.

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## RESEARCH ARTICLE

### The Effect of Rose Water (*Rosa damascena mill*) Supplementation in Broiler Rations on Growth Performance, Some Carcass Parameters and Intestinal Histomorphology

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#### ABSTRACT

The purpose of this study was to investigate the effect of added to different levels of rose water broiler diets on growth performance, some carcass parameters and intestinal histomorphology. In the literature on the conducted research, there are very few studies about the use of rose water in animal as feed additive. A total of 216 day-old chicks were randomly divided into 3 groups each containing 72 chicks. While the control group was fed with basal ration, the experimental groups were fed with rose water supplementation at 2% and 4% dose, respectively, in addition to the basic ration. At the end of the experiment, the use of rose water in the rations did not statistically affected live weight (LW), live weight gain (LWG) and feed intake (FI), but feed conversion ratio (FCR) was adversely affected. In the study, hot carcass weight was positively affected while some internal organ weights were not affected by the addition rose water. On the 21<sup>st</sup> and 35<sup>th</sup> days of the experiment, when histolomorphology of ileum and jejenum were examined, it was observed that villus height, crypt depth and villus height: crypt depth ratio were not affected by rose water addition. On the 21<sup>st</sup> day of the study, ileum and jejenum and on the 35<sup>th</sup> day of the study, ileum villus heights were positively affected by the addition of rose water numerically. In conclusion, hot carcass weight and intestinal health were positively affected by rose water. However, performance and some internal organ weights were not affected.

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#### Introduction

The use of antibiotics as a growth factor in farm animals provides significant economic benefits (Jetacar, 1999; Baurhoo et al., 2009). But the long-term use of antibiotics leads to an increase in resistance to antibiotics in bacteria (Smith et al., 2003). This situation concluded with it becoming more difficult to treat bacterial infections. For these negative reasons, the use of antibiotics as a growth factor in animal feed have

beenrohibited. As a result of the ban on the use of antibiotics and other growth factor chemical substances, the search began for other alternative feed additive substances (Baurhoo et al., 2009; Su et al., 2009). Prebiotics, probiotics, enzymes, organic acids, plant extracts, and humates have been used as feed additives. Because they are environmentally friendly, they do not adversely affect animal and human health, and they increase the quality and quantity of products obtained (Karademir and Karademir, 2003; Kutlu and Serbest, 2014).

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Aromatic plants and their extracts caught the attention of the science world in the search for new feed additive substances. Plant extracts come to the fore with their antifungal, antibacterial, antiviral, antioxidant and anti-lipidemic properties. (Svoboda and Hampson, 1999; Lambert et al., 2001). Because of this, in our study was used rose water acquired from roses belonging to the species *Rosa damascena Mill* found in Burdur (Lisinia) in Turkey.

*Rosa damascena Mill* is a plant native to Europe and Asia, particularly the Middle East, that belongs to the *Rosaceae* family (Ghahreman, 2001). *Rosa damascena Mill* is the most important species in the production of rose oil. The main rose oil producers in the world are Turkey and Bulgaria (Baydar et al., 2004). The main components of the essential oil belonging to the *Rosa damascena* plant are *B-citronellol* (23%), *nonadecane* (16%), *geraniol* (16%), and *heneicosane* (5%) (32). Various phytochemical contents were isolated from the leaves and petals of *Rosa damascena*, which contains flavonoids, glycosides, terpenes, and anthocyanins (Schiber et al., 2005). *Rosa damascena's* primary active components are *kaempferol*, *quercetin*, *gallic acid*, *cyranidin 3, 5, D-glycoside*. *B-citronellol*, *nonadecane*, *geraniol*, *nerol*, and *kaempferol* are the *Rosa damascena* flower's volatile fatty acid's primary chemical components (Loghmani-Khouzani et al., 2007).

Roses are antiseptic, antispasmodic, antiviral, and antibacterial (Boskabady et al., 2011). Rose water was used among the public for medical purposes. It is believed that rose water had healing effects in the instances of many diseases like chronic bronchitis, asthma, skin conditions, cancer, ulcers, and wrinkles (Sharafkandy, 1990; Mirheydar, 1993). Rose oil showed no toxicity or adverse effects when taken orally (Kirov and Bainova, 1999).

In recent years, several studies were conducted focused on the aromatic plants and their extracts. However, in the literature conducted research, there is little information about the performance and intestinal histomorphology parameters of the use rose water in broiler diets. Based on the previously reported favorable effects of plant and its extract, the current study was designed to investigate the effects of different levels of rose water on growth performance, some carcass parameters and intestinal histomorphology of broiler rations.

## Materials and Methods

This study was carried out with the permission of the Ankara University Animal Experiments Local Ethics Committee (Decision No: AU-HAYDEK /2016-15-151) report.

### Animals, Experimental Design and Feed

A total of 216 day-old chicks (Ross 308 male) were randomly divided into 3 groups of 72 chicks each regardless of gender. Each group was randomly divided into 6 subgroups of 12 chicks each. The rations were based on corn-soybean meal and were offered to the animals from during experimental period (Table 1). All diets were formulated to NRC (1994) nutrient recommendations. Ration nutrient analyses were performed according to AOAC (2000). The animals were fed with corn, soybean meal basal ration and trial continued for 35 days. Each

subgroups was equipped with manual feeders and automatic nipple drinkers. Water and feed (in mash form) were given *ad libitum*. The house temperature was monitored thermostatically throughout the study. The temperature, which was 32 °C -35 °C on the first day, was gradually lowered and maintained at 22 °C for the last two weeks. Artificial light program as carried out as in commercial conditions (23 h of light throughout the experiment for per day). While the control group (C) was fed with basic ration, the experimental groups were fed respectively with 2% rose water (RW1), and 4% rose water (RW2) added to the basal diet. Rose water is a by product of rose oil production (Lisinia Nature, Burdur, Turkey). Rose water taken from the production factory was added to the ration after dilution of 100 times. The volatile fatty acid profile of the rosewater used in the study is shown in Table 2.

**Table 1.** The composition of the rations used in the study (%)<sup>1</sup>

Ingredient	Broiler starter 0-14. days	Broiler grower 15-28. days	Broiler finisher 29-35. days
Corn	51.00	52.25	56.45
Soybean (Full fat), 38%	19.62	18.00	14.00
Vegetable oil	1.00	2.00	3.00
Soybean meal, 48%	24.00	24.00	23.00
DCP	2.40	2.00	2.00
Limestone	0.8	0.85	0.85
Bicarbonate	0.10	0.10	0.10
Salt	0.25	0.25	0.25
DL-Metiyonin	0.37	0.25	0.15
L-lizin	0.2	0.10	0
Vitamin premix <sup>2</sup>	0.10	0.10	0.10
Mineral premix <sup>3</sup>	0.10	0.10	0.10
Anticoccidial	0.06	-	-
Total	100.00	100.00	100.00
<b>Chemical composition, calculated</b>			
Crude protein, %	22.01	21.56	20.03
ME, kcal/kg	3099	3158	3219
Ca, %	1.01	0.92	0.91
Total P, %	0.5	0.44	0.44
Methionine + Cysteine, %	1.09	0.96	0.82
Lysine, %	1.44	1.33	1.14
<b>Analysis Values:</b>			
ME, kcal/kg	3131	3153	3200
Crude protein, %	23.45	21.70	19.60
Ca %	1.04	1.00	0.93
Total P %	0.53	0.50	0.48

<sup>1</sup>As-fed basis.

<sup>2</sup>Provided per kilogram of complete diet: vitamin A, 12,000 IU; vitamin D3, 2,500 IU; vitamin E, 40 IU; vitamin K3, 5 mg; thiamin, 2.5 mg; riboflavin, 6 mg; pyridoxine, 5 mg; pantothenic acid, 15 mg; niacin, 25 mg; folic acid, 1 mg; biotin, 50 µg; vitamin B12, 20 µg.

<sup>3</sup>Provided per kilogram of complete diet: Cu, 5 mg; I, 1 mg, Co, 200µg; Se, 150 µg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg. Folic Acid 1.000 mg kg<sup>-1</sup>, Biotin 50 mg kg<sup>-1</sup>, Copper 5.000 IU kg<sup>-1</sup>, Iodine 1.000 IU kg<sup>-1</sup>, Cobalt 200 mg kg<sup>-1</sup>, Selenium 150 mg kg<sup>-1</sup>, Iron 60.000 mg kg<sup>-1</sup>, Zinc 60.000 mg kg<sup>-1</sup>, Mangan 80.000 mg kg<sup>-1</sup>.

**Table 2.** Essential oil acid profile of rose water commercial product (the values are taken from the production facility)

Ingredient	%
<i>Linalool</i>	0.5
<i>β-citronellol</i>	34.9
<i>Nerol</i>	6.8
<i>Geraniol</i>	14.1
<i>Eugenol</i>	2.5
<i>Farnesol</i>	9.3

### Growth Performance

In study, live weights (LW) were recorded for each subgroup weekly. Live weight gain (LWG) was determined by the difference between these measurements. Each subgroup's feed intake (FI) of animals was recorded weekly and used for the calculation of the feed conversion ratio (FCR).

### Carcass Characteristics Parameters Analysis

On the 35<sup>th</sup> day of the study, one chick from each subgroup was randomly chosen. From each subgroup, one animal was cut off with a suitable method for the determination of the carcass parameters. Carcasses were weighed to determine hot carcass weights after cutting. Carcass composition data were expressed as a percentage of LW. The hot carcass weight is calculated by the following formula:

$$\text{Hot Carcass Yield, \%} = (\text{Hot Carcass Weight, g} / \text{Live, Weight g}) \times 100 \quad (1)$$

### Sampling Procedures

On the 21<sup>st</sup> and 35<sup>th</sup> days of the experiment, one bird from each subgroup was randomly selected. Birds were slaughtered cut off with a suitable method and the intestinal tract was removed immediately. The tissue samples for histomorphological analysis were taken from the jejunum and ileum. To ensure the uniformity of samples, approximately 2 cm length of the mucosal segments of jejunum and ileum was excised as follows: 8 cm proximal to Meckel's diverticulum (jejunum), and 8 cm proximal to the ileo-cecal junction (ileum), respectively.

### Histomorphologic Measurements

Tissue samples were fixed in 10% neutral buffered formaline for 24 h and washed with tap water subsequently dehydrated in graded ethanol solutions, cleared with xylol and embedded in paraffin, respectively. Intestinal segments were sectioned at the thickness of 5 µm with microtome. Cross sections were prepared and stained with Mallory's triple stain modified by Crossman in order to determine the intestinal morphometry (Culling et al., 1985). Villus height was measured from the top of the villus to the crypt mouth, and crypt depth was defined as the depth of the invagination between adjacent crypt mouths. Villus width was measured at the bottom of the villus (Sakamoto et al., 2000). Histological sections were examined under light microscope (Leica DM 2500, Leica Microsystems GmbH, Wetzlar, Germany) and photographed with Leica DFC450 (Leica Microsystems, Heerbrug, Germany) digital microscope camera. The images were evaluated using

ImageJ software (Image J, US National Institutes of Health, Bethesda, MD, USA).

### Statistical Analysis

The one-way analysis of variance (ANOVA) method was used for the statistical calculations of the groups and a suitable post hoc test (Duncan's test) was used for the determining the importance of the differences between the groups. The statistical analysis was done with the SPSS software package (SPSS, 2011).

### Results

The effect of using 2% and 4% doses of rose water use in broiler rations on the parameters of LW, LWG, FI and FCR is given in Table 3. On the 7<sup>th</sup> and 28<sup>th</sup> days of the experiment, the LW value among the groups was adversely significant ( $p < 0.05$ ). LWG values among the groups were adversely significant in the first and fourth weeks of the experiment ( $p < 0.05$ ). FI values between the groups were statistically significant in the third week of the experiment ( $p < 0.05$ ). In the mentioned week, the lowest feed intake value belongs to the RW1 group. The feed conversion ratio (FCR) among the groups at the 3<sup>rd</sup> week of the experiment was found to be adversely significant ( $p < 0.05$ ). In the mentioned weeks, the lowest feed conversion ratio value belongs to the control group. At the end of the experiment, the use of different doses of rose water in rations did not statistically affect the LW, LWG and FI ( $p > 0.05$ ). However, on the 35<sup>th</sup> day of the study, FCR value was found to be significant among the groups ( $p < 0.05$ ). Feed conversion ratio was the lowest in the control group and the highest in the RW2 group.

The effect of using 2% and 4% doses of rose water use in broiler rations on the parameters of carcass yield, internal organ weights, and 100 g live weight ratios is given in Table 4. In the study, the value of hot carcass weight was positively influenced by the increased doses of rose water ( $p < 0.05$ ). Some internal organ weights are not affected by the addition of 2% and 4% rosewater (*Rosa damascena mill*) while hot carcass weight was positively affected ( $p < 0.05$ ). The values among groups were not statistically significant in terms of organ the ratio of live weight for these per 100 grams ( $p > 0.05$ ).

Histomorphological measurements of jejunum and ileum at 21<sup>st</sup> and 35<sup>th</sup> day of the experiment are given in Table 5. On the 21<sup>st</sup> and 35<sup>th</sup> day of the experiment, when histology of ileum was examined, it was observed that villus height, crypt depth and villus height: crypt depth ratio were not affected by rose water addition ( $p > 0.05$ ). However, on the 21<sup>st</sup> day of the study, ileum and jejunum and on the 35<sup>th</sup> day of the study, ileum villus heights were positively affected by the addition of rose water numerically.

**Table 3.** The effect of dietary supplementation of rosewater on the LW, LWG, FI and FCR in the broiler chickens (g)

Performance Parameters	Control		RW1		RW2		Significance
	$\bar{x}$	S $\bar{x}$	$\bar{x}$	S $\bar{x}$	$\bar{x}$	S $\bar{x}$	P
<b>Live Weight, g Days</b>							
0	41.78 <sup>a</sup>	0.02	41.13 <sup>b</sup>	0.08	41.07 <sup>b</sup>	0.05	0.000***
7	152.49 <sup>a</sup>	1.78	141.69 <sup>b</sup>	2.38	142.60 <sup>b</sup>	1.39	0.002***
14	389.80	5.11	389.82	4.77	395.46	3.31	0.598
21	828.06	6.53	825.31	5.15	808.81	9.42	0.162
28	1451.39 <sup>a</sup>	16.71	1400.38 <sup>ab</sup>	20.60	1353.09 <sup>b</sup>	34.89	0.047*
35	2186.07	39.30	2112.91	23.11	2102.35	39.76	0.215
<b>Live Weight Gain, g, Days</b>							
0-7	110.71 <sup>a</sup>	1.77	100.56 <sup>b</sup>	2.39	101.53 <sup>b</sup>	1.38	0.003***
7-14	237.31	5.97	248.12	6.38	252.85	3.70	0.155
14-21	438.26	9.39	435.49	3.57	413.34	9.54	0.085
21-28	623.33 <sup>a</sup>	13.39	575.06 <sup>ab</sup>	16.81	544.28 <sup>b</sup>	28.30	0.047*
28-35	734.68	36.20	712.53	16.86	749.26	27.94	0.657
0-35	2144.29	39.31	2071.78	23.13	2061.28	39.72	0.220
<b>Feed Intake, g Days</b>							
0-7	142.25	3.69	138.66	4.27	130.58	2.96	0.105
7-14	387.16	5.83	394.11	14.57	418.33	3.9	0.073
14-21	605.25 <sup>b</sup>	18.99	710.02 <sup>a</sup>	16.69	687.38 <sup>a</sup>	19.58	0.004***
21-28	946.08	20.64	928.26	30.06	885.03	20.74	0.220
28-35	1286.99	45.27	1174.26	32.25	1230.27	38.81	0.160
0-35	3367.75	66.42	3332.17	49.08	3340.93	62.27	0.909
<b>Feed Conversion Ratio Days</b>							
0-7	1.28	0.01	1.38	0.04	1.28	0.03	0.130
7-14	1.63	0.04	1.54	0.05	1.61	0.01	0.237
14-21	1.38 <sup>b</sup>	0.04	1.62 <sup>a</sup>	0.03	1.66 <sup>a</sup>	0.05	0.001***
21-28	1.51	0.02	1.61	0.01	1.64	0.06	0.107
28-35	1.76	0.04	1.64	0.02	1.64	0.04	0.098
0-35	1.56 <sup>b</sup>	0.01	1.61 <sup>ab</sup>	0.01	1.62 <sup>a</sup>	0.01	0.041*

Statistically not significant ( $p > 0.05$ ). The mean ( $\bar{x}$ ) and standard error (S $\bar{x}$ ) values of 6 subgroups in each group. a, b, c: Differences between the mean values of different letters in the same row are statistically significant \* ( $P < 0.05$ ), \*\*\* ( $P < 0.01$ ) Groups; C: Control, RW1: 2% rose water added to basal ratio, RW2: 4% rose water added to basal ratio

**Table 4.** Effects of dietary supplementation of rosewater on the carcass yield, visceral organ weights and 100 g live weight in the broiler chickens

Groups	C		RW1		RW2		P
	$\bar{x}$	S $\bar{x}$	$\bar{x}$	S $\bar{x}$	$\bar{x}$	S $\bar{x}$	
Carcass parameters							
Carcass weight	1589.41 <sup>b</sup>	88.45	1812.33 <sup>a</sup>	55.14	1853.8 <sup>a</sup>	50.26	0.029*
Liver weight	50.54	4.29	49.99	1.02	51.45	2.61	0.941
Heart weight	12.64	1.06	12.62	0.71	12.49	0.79	0.991
Spleen weight	2.37	0.32	3.06	0.23	2.79	0.26	0.245
Bursa Fabricius weight	3.80	0.42	4.79	0.53	4.93	0.41	0.204
Abdominal fat weight	27.41	2.32	27.20	3.74	25.78	1.45	0.897
Carcass ratio g/ 100 g BW	68.09	4.37	69.97	0.69	70.24	0.40	0.812
Liver ratio g/ 100 g BW	2.16	0.19	1.93	1.93	0.05	1.94	0.06
Heart ratio g/ 100 g BW	0.54	0.05	0.48	0.01	0.47	0.03	0.442
Spleen ratio g/100 g BW	0.10	0.013	0.12	0.007	0.10	0.009	0.518
Bursa Fabricius ratio g/	0.16	0.01	0.18	0.01	0.18	0.01	0.605
Abdominal fat ratio g/ 100g CA	1.17	0.10	1.04	0.12	1.04	0.08	0.425

Statistically not significant ( $p > 0.05$ ). The mean ( $\bar{x}$ ) and standard error (S $\bar{x}$ ) values of 6 subgroups in each group. a, b, c: Differences between the mean values of different letters in the same row are statistically significant \* ( $P < 0.05$ ), \*\*\* ( $P < 0.01$ ) Groups; C: Control, RW1: 2% rose water added to basal ratio, RW2: 4% rose water added to basal ratio.

**Table 5.** Effect of rose water supplementation on histomorphology of ileum and jejunum on the 21st and 35th days of the trial ( $\mu\text{m}$ )

Histomorphology Parameters	C		RW1		RW2		P
	$\bar{x}$	S $\bar{x}$	$\bar{x}$	S $\bar{x}$	$\bar{x}$	S $\bar{x}$	
<b>Ileum 21</b>							
Ileum villus height ( $\mu\text{m}$ )	683.52	33.95	718.24	37.11	738.17	27.31	0.527
Ileum crypt depth ( $\mu\text{m}$ )	170.38	15.12	146.33	3.66	143.00	7.98	0.170
Ileum villus height/crypt depth	4.13	0.29	4.85	0.35	5.21	0.25	0.053
<b>Jejunum 21</b>							
Jejunum villus height ( $\mu\text{m}$ )	1017.28	77.20	978.06	61.50	1100.39	85.83	0.520
Jejunum crypt depth ( $\mu\text{m}$ )	196.28	12.71	163.39	12.80	175.17	3.86	0.120
Jejunum villus height/crypt depth	5.27	0.45	6.22	0.75	6.25	0.40	0.387
<b>Ileum 35</b>							
Ileum villus height ( $\mu\text{m}$ )	938.94	64.96	971.11	64.51	984.11	49.82	0.863
Ileum crypt depth ( $\mu\text{m}$ )	157.17	15.78	170.89	25.32	181.06	12.26	0.668
Ileum villus height/crypt depth	6.11	0.40	6.18	0.84	5.56	0.45	0.729
<b>Jejunum 35</b>							
Jejunum villus height ( $\mu\text{m}$ )	1378.89	38.15	1265.61	81.77	1302.17	34.19	0.366
Jejunum crypt depth ( $\mu\text{m}$ )	201.33	7.89	202.72	13.13	218.33	15.22	0.557
Jejunum villus height/crypt depth	6.90	0.32	6.44	0.69	6.08	0.40	0.522

Statistically not significant ( $p > 0.05$ ). The mean ( $\bar{x}$ ) and standard error (S $\bar{x}$ ) values of 6 subgroups in each group. Groups; C: Control, RW1: 2% rose water added to basal ratio, RW2: 4% rose water added to basal ratio

## Discussion

Medicinal and aromatic plants in broiler feeding, increased appetite, stimulation of digestion, daily live weight gain, improved feed use and against pathogenic microorganisms in the intestines of broilers by showing strong inhibitory effect, contribute greatly to the formation of a microflora suitable for digestion and health (Dalkılıç et al., 2005). The use of aromatic plants and extracts in animal diets has a positive effect on performance. Essential oils stimulate the digestive system of animals and increase the efficiency of digestive enzymes (Simsek et al., 2005). Due to these properties, essential oils have a positive effect on performance parameters. Further studies are needed to demonstrate the effects of the volatile fatty acids in *Rosa damascena Mill* and its extracts on the performance and intestinal health depending on their effective doses.

In our study in the period of 0-35 days, it was observed that the use of rosewater in broiler diets did not affect LW, LWG and FI, but FCR values increased values. In the study, rose water was added to the groups, LW values were close to each other on the 14<sup>th</sup>, 21<sup>st</sup> and 35<sup>th</sup> days of the study ( $p > 0.05$ ). However, the LW value of the study at day 28 was lower than the control group ( $p < 0.05$ ). In a study that used *Rosmarinus officinalis* in broiler diets of 100 mg kg<sup>-1</sup> and 200 mg kg<sup>-1</sup>, the results indicated that LW was lower than the control group (Yildirim et al., 2018). This study is the current study supporting the results of our study. However, there are findings about how different aromatic plants and extracts positively affect live weight in broiler rations (Zhang et al., 2013; Hasan et al., 2016). The difference in the results obtained from these studies can be explained by housing conditions, environmental factors, and plant-dependent factors like the type of plant extracts used, dose, containing volatile fatty acids, active substance rate and interaction.

In our study, the addition of different doses of rosewater to broiler rations was no have a positive effect on live weight

gain between 21-28 days ( $p < 0.05$ ). There was no significant difference between the groups in 0-35 days ( $p > 0.05$ ). Although there was no negative effect of the high dose throughout the total duration of the study in terms of LWG, it was found lower compared with the control group on the other days. At the beginning of the study, in terms of LWG, RW1 and RW2 groups were found to be lower in the 1.51% and 1.65% levels than the control group, respectively. LWG was low at levels of approximately 3.04% and 3.52%, respectively, at the end of the study. It was reported that there was no effect of any kind on live weight increase in studies where different plant extracts were used (Toghyani et al., 2010; Amad et al., 2011; Farahat et al., 2017). On the other hand, there are many studies that the use of herbal extracts as a feed additive substance in rations positively affects increases in live weight (Khattak et al., 2014; Wang et al., 2015). The difference between the results is assumed to depend on the effective dose and the active ingredient content of the rose water used and the care feeding conditions.

In our study, the addition of different levels of rosewater in broiler rations, on the 14<sup>th</sup>-21<sup>st</sup> days of study increased feed intake. However, there was no significant effect at the end of the experiment. The addition of aromatic plants in broiler rations did not statistically affect the intake of feed, and this find is consistent with the 35-day feed intake results from our study. (Khattak et al., 2014). In a study where *Camellia oleifera* seed extract was used at a level of 300 mg/kg that was not affected feed intake (Dong et al., 2016). On the other hand, there are studies in which the use of extracts increased (Wang et al., 2015) and decreased feed intake (Yildirim et al., 2018). This situation can be explained as the aromatic plant extract used stimulating feed intake in the animals.

On the 14<sup>th</sup>-21<sup>st</sup> and 0th-35<sup>th</sup> days of our study, in terms of feed conversion ratio, the RW1 and RW2 groups were higher than the control group. Studies using different plant extracts confirm that feed conversion rate is adversely affected by



increasing doses (Karangiya et al., 2016). These studies are in line with our findings. There are many studies, using in broiler diets of various aromatic plants and extracts, the feed conversion ratio did not affect (Franciosi et al., 2016; Yildirim et al., 2018) and were affected (Durrani et al., 2006; Kumari et al., 2007; Rezaei et al., 2015). The differences observed between the results are thought to depend on the conditions of the poultry, the moisture content of the feed, the environmental factors and the type of plant extracts used, the dose, the amount of volatile fatty acids.

In our study, the values among groups were not statistically significant in terms of organ weight and the ratio of live weight for these per 100 grams. Studies in which the *Rosmarinus officinalis* plant oil and essential oil are compared with probiotics support our findings (Bugdayci and Ergun, 2011; Ciftci et al., 2013). Contrary to these studies, it was reported in studies where (*Ocimum sanctum*) tulip petal extract was used that some organ weights increased (Hasan et al., 2016). Hot carcass weight is the highest in the groups to which additions of rosewater were made at different levels in the rations, and the control group had the lowest value. It was seen that the addition of rosewater positively affected hot carcass weight directly proportional to dosage increase. It was reported that hot carcass weight was positively affected in the study where *Tinospora cordifolia*, *Azadirachta indica* ve *Andrographis paniculata* were used as three different aromatic plants that exhibited similar effects as the findings of our study (Shraddha et al., 2017). Contrary to these results, there are other studies with regard to hot carcass weight not being affected by the addition of plant extracts (Ciftci et al., 2013; Wang et al., 2015). According to the results of the study, the heart, liver, and abdominal fat weight did not increase in the testing groups, despite the high carcass weight, and spleen weight and *bursa Fabricius* weight increased slightly. The differences observed between the results can be explained with considerable diversity that the plant type of the extracts, region where they were acquired, essential oils they contain, and biological active substance contents show.

Morphological changes in the small intestine, villus height, villus width and villus height crypt depth ratio (VH: CD) may improve poultry performance by improving nutrient digestion and absorption (Calik and Ergün, 2015). In our study, on the 21<sup>st</sup> and 35<sup>th</sup> day of the experiment, when histology of ileum was examined, it was observed that villus height, crypt depth and villus height: crypt depth ratio were not affected by rose water addition into broiler diet. However, on the 21<sup>st</sup> day of the study, ileum and jejunum and on the 35<sup>th</sup> day of the study, ileum villus heights were positively affected by the addition of rose water numerically. In literature review, no studies have been found which the effects use of rose water and its extract of in broiler diets on intestinal histomorphology. Therefore, it will be discussed with different studies that other aromatic plants and extracts are used as feed additive. In the study of Ghazanfari et al. (2015), they indicated that the use of aromatic plants and their extracts did not affect the mucosal morphology of the jejunum. The result is consistent with our study. However, in the study conducted by Boka et al. (2014), the investigators used 0%, 1%, 2% and 3% black

cumin in the laying hens and reported that 2% black cumin increased significantly the villus height, crypt depth, and VH/CD parameters in the jejunum. In a study in which cashew leaf extract was used in Jawa Super chicks, villus length and crypt depth were positively affected (Setiawan et al., 2018). The differences observed between the results are thought to be caused by differences in plant species, obtained regions, essential oils and biological active substance content. In order to explain the effect level of broiler feeding histomorphological parameters from rosewater, many studies should be performed in this field.

In conclusion, hot carcass weight and intestinal health were positively affected by the addition of 2% and 4% rosewater (*Rosa damascena mill*). However, performance and some internal organ weights were not affected. The use of rose water in broiler rations may increase profitability in the poultry industry by favorably affecting the hot carcass and intestinal health. Therefore, it was concluded that rose water can be used as feed additive in broiler rations. In the literature review, there is little information about the performance and intestinal histomorphology parameters of the use rose water in broiler feeding. New studies to be conducted with winged animals in different doses and species will shed light on the understanding of the potential of being able to use rosewater as a feed additive substance. Our study is a good literature for further studies.

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## RESEARCH ARTICLE

# The Potential of Orange Peel Oil as a Suppressor of Cell Proliferation in Animal Feed and Human Nutrition: An Experimental Study

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### ABSTRACT

This study aimed to investigate the in vitro cytotoxic activities of orange peel oil on HaCaT cell lines by using an MTT cytotoxicity assay after administering orange peel oil at different doses and time-points. Our objective was to assess the in vitro cytotoxic activities of orange peel oil on HaCaT cell lines. Cell viability was determined with the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] assays. The HaCaT cells (100  $\mu$ L) were cultured in plates and treated with different concentrations of orange peel oil (25  $\mu$ M, 50  $\mu$ M, 100  $\mu$ M, 150  $\mu$ M and 200  $\mu$ M) for durations of 24 and 48 hours. Cell death was determined by collecting and staining with 0.4% Trypan blue for 5 minutes at room temperature, followed by microscopic examination. There was a significant difference between the doses concerning both time zones ( $p < 0.05$ ). There was a significant ( $p < 0.05$ ) difference between the control group and all other doses, including 200  $\mu$ L/mL and 25  $\mu$ L/mL, 50  $\mu$ L/mL, 100  $\mu$ L/mL, and 20% DMSO. Orange peel oil showed toxic effects at all dose levels and time points when compared with the control group. According to the results of our research, and in light of previous investigations, it can be said that orange shell oil may have protective effects such as anti-cancer, anti-microbial, and antioxidant properties, and thus, may be used in human and animal nutrition.

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### Introduction

One of the features distinguishing humans from other creatures is the ability to learn and transfer knowledge from generation to generation. This ability has given us a significant advantage in the struggle for survival. People had to deal with plants for many years to meet their basic needs and had detailed information about plants. As a result of these efforts, a lot of information was gathered within thousands of years about the use of plants as food or medicinal products.

Orange is an important tree species belonging to the Rutaceae family. Orange peel oil obtained from the pulp of

oranges obtained from these trees has traditionally been widely used for different purposes, including treatment and nutrition.

With 115,650,545 tons, citrus is the most cultivated fruit group in the world. On the other hand, oranges constitute 55.26% of the citrus production in the world. Worldwide the highest orange producing countries are the United States, Brazil, Mexico, Spain, Italy, India, Israel, Egypt, Argentina, and Turkey. Extracts and essential oils derived from medicinal and aromatic plants are widely used in the food industry, cosmetic production, and medicine today.

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Citrus fruits are marketed mainly as fresh fruits or processed juice. Large amounts of shells generated during the processing of citrus fruits are discarded because they do not add value to the product as a by-product.

Citrus essential oils can contain many components (more than 200), including terpenes, sesquiterpenes, aldehydes, alcohols, esters, and are described as a mixture of terpene hydrocarbons, oxygenated compounds, and non-volatile residues.

Citrus avonoids have antioxidant, anticancer (Lai et al., 2013, Im et al., 2014), anti-mutagenic (Hosseinimehr and Karami, 2005; Demir et al., 2009), anti-allergic, anti-inflammatory, and antimicrobial (Hamdan et al., 2013) properties. Additionally, citrus shells were used as a source of animal feed, fiber production, and fuel production (Bampidis and Robinson, 2006; Lashkari and Taghizadeh, 2013).

While cancer cells are destroyed in chemotherapy, which is a conventional treatment method, controlling the disease may fail because it can cause adverse and toxic side effects on normal cells. The alternative solution for the detrimental effects of synthetic agents is the use of natural plants, which have the potential of making an outstanding contribution to modern therapeutics (Sultana et al., 2014).

Orange essential oil (*Citrus sinensis* L. Rutaceae) produced by the cells in the orange peel is extracted from the shell of the fruit. The main constituent of orange peel oil is D-limonene (more than 90%) (Bauer et al. 2001). In addition to its antioxidant properties, citrus essential oils provide physical and mental energy and help remove toxins and harmful substances from the cells. Adding citrus oils as a food and beverage aroma is a great idea (Fisher and Phillips 2008). In the pharmaceutical industry, citrus oils are used as spice ingredients to hide the unpleasant flavors of medicines. They are also used in the perfume and cosmetics industry (Steuer et al., 2001).

In studies carried out to determine the effects of aromatic plants on performance and other yields in animals, significant improvement in parameters such as feed consumption, feed utilization, live weight gain, and carcass yield were observed by using aromatic plants and essential oils obtained from these plants as growth factors (Güler et al., 2005).

Due to its antimicrobial and antioxidant properties, the use of citrus shell oils as feed additives has recently gained importance. Essential oils and flavonoids are intensely present in the shell of citrus fruits such as orange (*Citrus sinensis*), lemon (*Citrus lemon*) and bergamot (*Citrus bergamia*). These parts contain very high amounts of essential oils such as limonene and linalool (Min-Hsiung, 2009). The antioxidant activity of limonene, which is the most essential component of the orange peel oil, has been reported to be quite high (Roberto, 2010).

The aim of this study was to investigate the *in vitro* cytotoxic effects of orange peel oil on a non-tumor keratinocyte cell line (HaCaT) using an MTT cytotoxicity assay, after applying the orange peel oil at different doses and at different time intervals.

## Materials and Methods

### Herbal Extract

The orange peel oil used in this experiment was obtained commercially from Ege Lokman Plant Industry and Trade Co. The orange peel oil I bought commercially was obtained by the cold press method. The active ingredient levels of orange peel oil were investigated by GS-MS (Gas Chromatography and Mass Spectrometry) in Çukurova University Faculty of Fisheries Laboratory (Table 1).

**Table 1.** Orange essential oil chemical composition and active ingredient ratios (%)

Orange Peel Oil	(%)
Beta-Myrcene	1.987581
Limonene	97.46377
Camphene	0.018475
Linalool	0.222917
Trans-Limonene Oxide	0.005973
Citronella	0.017979
Decanal	0.188161
Alpha-Terpinene	0.012065
Alpha-Copaene	0.010914
Germacrene-D	0.005194
Germacrene-B	0.006875
Trans-Caryophyllene	0.010227
Beta-Cubebene	0.014546
Valencene	0.028449
Delta-Cadinene	0.006875

### Cell Culture

The immortalized human non-tumorigenic keratinocyte cell line (HaCaT) was acquired from Cell Culture and Biological Resources Unit at Yeditepe University. These cells were seeded at a concentration of 5.000 cell/well on a 96-well plate (BIOFIL, TPC, Switzerland) and conserved in RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO), which includes HEPES (Sigma) buffer along with 10% heat-inactivated fetal bovine serum (FCS) (Hyclone Lab., Logan, UT), 100 µg/mL streptomycin (Sigma), and 100 U/mL penicillin (Sigma). The materials were incubated in disposable plastic tissue culture flasks in a 5% CO<sub>2</sub>/95% air incubator at 37 °C. After incubation, the culture media were removed, the cells were washed with PBS, and MTT cell proliferation assay was performed.

### Cell Viability (MTT) Assay

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays were used to determine cell viability. The HaCaT cells (100 µL) were cultured in 96-well plates at 2 x 10<sup>4</sup> cells per well and treated with 25 µM, 50 µM, 100 µM, 150 µM, and 200 µM concentrations of citrus peel oil for durations of 24 and 48 hours. Following treatment, each well was filled with 10 µL of MTT reagent (5 mg/mL) and incubated for 4 hours at 37 °C. Then, the medium was eliminated, and 150 µL of DMSO was added to make the MTT formazan soluble. The absorbance of solubilized MTT formazan products was measured by an ELISA plate reader (Biotek, USA) at 590 nm at 24, 48, and 72 hours.

Cell death was determined by collecting and staining with 0.4% of Trypan blue for 5 minutes at room temperature before microscopic examination. Viable cells were counted via Trypan blue exclusion. Dead cells that stained blue were deemed positive and summed against the total.

### Statistical Analysis

The Statistical Package for Social Sciences (SPSS, version 22, IBM, Armonk, New York 10504, NY, USA) was used for data analysis. As descriptive statistics, numerical variables were summarized as mean ( $\pm$  standard deviation). Comparisons between the groups were made with the one-way ANOVA or Kruskal Wallis test. A p-value below 0.05 was considered statistically significant.

### Results and Discussion

In this study, we investigated the effect of orange peel oil on the cell viability of human HaCaT keratinocytes. When the literature on the effects of orange peel oil on HaCaT keratinocyte cells is examined, it is seen that there is no scientific study on HaCaT cells. When Table 1 and Figure 1 were examined, it was found that there were significant differences between the two time zones and doses concerning cell viability between the groups ( $p < 0.05$ ).

The Duncan post hoc multiple comparison test was applied to determine the doses between which the differences

occurred during the 24-hours. As a result, a significant ( $p < 0.05$ ) difference was found between the control group and all other doses and between 100  $\mu\text{l}/\text{mL}$  and 20% DMSO. As a result of multiple comparisons to determine the differences at 48 hours, there were significant differences between the control group and all other doses, as well as between the 200  $\mu\text{l}/\text{mL}$  dose and 25  $\mu\text{l}/\text{mL}$ , 50  $\mu\text{l}/\text{mL}$ , 100  $\mu\text{l}/\text{mL}$ , and DMSO 20% ( $p < 0.05$ ). Data in Table 1 reveals that each concentration of orange peel oil shows toxic effects in the HaCaT cell line. Orange peel oil appears to have cancer therapeutic effects.

Citrus essential oils and their components also receive attention from the point of chemoprevention agents in cancer treatment. For example, the Palestine sweet lime essential oil was shown to inhibit inflammation and activate apoptosis of human SW480 colon cancer cells by suppressing the expression of both COX-2 and IL-6 (Jayaprakasha et al., 2012). d-Limonene, a major constituent of citrus essential oil, is recognized as a potential chemotherapeutic agent because it can induce human colon cancer cell apoptosis via the mitochondrial death pathway and suppress the PI3K/Akt pathway (Jia et al., 2013). Perillyl alcohol, an oxygenated monoterpene constituent of citrus essential oil, is effective in the clinical treatment of patients with malignant brain tumors (Chen et al., 2015). The essential oil of blood orange can inhibit angiogenesis, metastasis, and cell death in human colon cancer cells (Murthy et al., 2012).

**Table 2.** Comparison of cell viability between the different groups and time points

Time	Group	n	Mean $\pm$ SD	SE	95 % CI		Min.	Max.
Hour 24	DMSO%20	4	0.00825 $\pm$ 0.0005 <sup>c</sup>	0.00025	0.00745	0.00905	0.008	0.009
	Kontrol	4	0.172 $\pm$ 0.019511 <sup>a</sup>	0.009755	0.14095	0.20305	0.153	0.193
	25ul/mL	4	0.01075 $\pm$ 0.0005 <sup>bc</sup>	0.00025	0.00995	0.01155	0.01	0.011
	50ul/mL	4	0.0135 $\pm$ 0.001291 <sup>bc</sup>	0.000645	0.01145	0.01555	0.012	0.015
	100ul/mL	4	0.02075 $\pm$ 0.0025 <sup>b</sup>	0.00125	0.01677	0.02473	0.018	0.024
	150ul/mL	4	0.022 $\pm$ 0.000816 <sup>b</sup>	0.000408	0.0207	0.0233	0.021	0.023
	200ul/mL	4	0.018 $\pm$ 0.004243 <sup>bc</sup>	0.002121	0.01125	0.02475	0.013	0.022
	P			0.000				
Hour 48	DMSO%20	4	0.00775 $\pm$ 0.0005 <sup>d</sup>	0.00025	0.00695	0.00855	0.007	0.008
	Kontrol	4	0.266 $\pm$ 0.029098 <sup>a</sup>	0.014549	0.2197	0.3123	0.229	0.293
	25ul/mL	4	0.015 $\pm$ 0.004243 <sup>d</sup>	0.002121	0.00825	0.02175	0.012	0.021
	50ul/mL	4	0.02075 $\pm$ 0.003096 <sup>cd</sup>	0.001548	0.01582	0.02568	0.018	0.025
	100ul/mL	4	0.02825 $\pm$ 0.007719 <sup>cd</sup>	0.00386	0.01597	0.04053	0.021	0.039
	150ul/mL	4	0.03825 $\pm$ 0.00789 <sup>bc</sup>	0.003945	0.0257	0.0508	0.029	0.047
	200ul/mL	4	0.058 $\pm$ 0.018493 <sup>b</sup>	0.009247	0.02857	0.08743	0.034	0.074
	P			<0.001				

SD: Standard deviation. SE: Standard error. CI: Confidence interval. \*cells with the same letters denote non-significant differences.

Many studies have reported that citrus shell oils have antioxidant effects (Turhan et al., 2006; Wilkins et al., 2007; Wang et al., 2008; Al-Saadi et al., 2009; Yapo, 2009; Janati et al., 2012; Oboh and Ademosun, 2012; Fidrianny et al., 2014; Canan et al., 2016). Different kinds of oxidants are present in human food. The oxidants in our diet can induce diseases, such as hypertension, diabetes, arteriosclerosis, cancer, and senescence. Although synthetic antioxidants can be of help, they bear some risks and side effects. Hence, antioxidants originating from plants are attracting attention (Suttirak and Manurakchinakorn, 2014; Stone et al., 2014)

Some studies have demonstrated that orange peel oil can be used as a medical agent that protects against cancer and prevents the growth and proliferation of cancer cells by d-Limonene. The orange essential oil contains large quantities of d-Limonene is present, which has anti-proliferative and apoptosis-inducing effects (Mauro et al., 2013; Crowell and Gould, 1994). Thus, it is used as a chemopreventive and chemotherapeutic agent against multiple types of tumors (Vigushin et al., 1998; Chaudhary et al., 2012).

Essential oils are among the most valuable plant products used in contemporary medicine and complementary therapies.

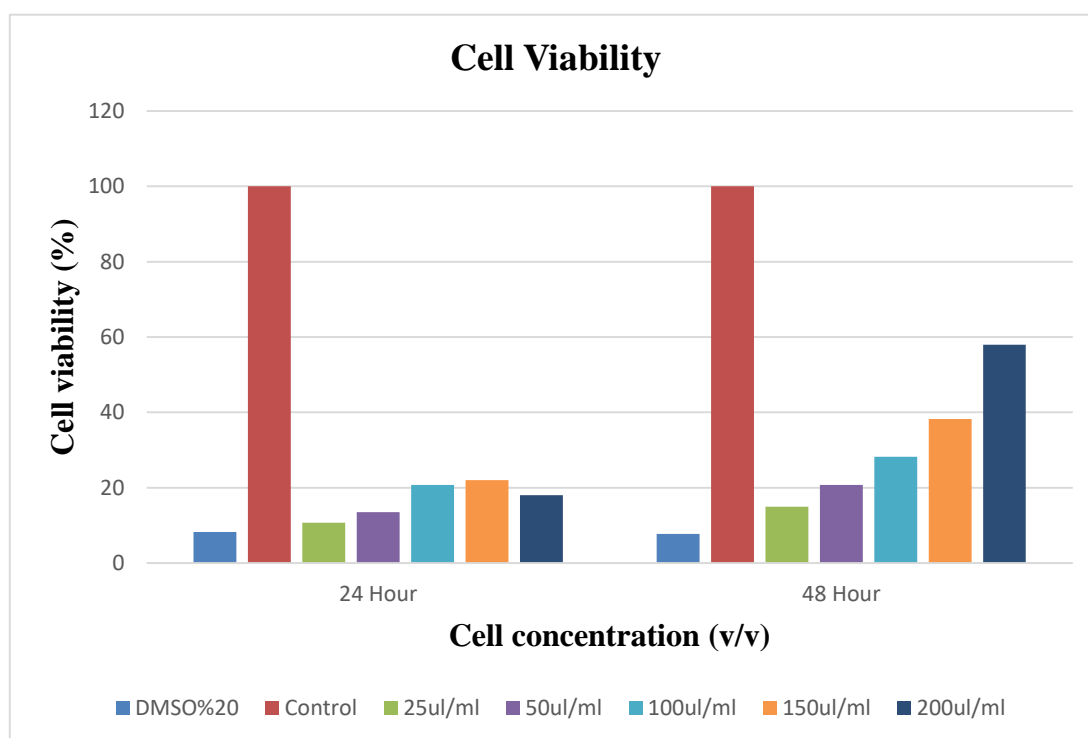
However, the beneficial role of orange peel oil and its constituents in cancer treatment needs further elucidation (Lesgards et al., 2014).

Many studies are claiming that essential oils might inhibit pathogenic bacteria in the small intestine. Herbal extracts have an antimicrobial effect against *E. coli* in poultry and pigs (Bölükbaşı et al., 2007, 2009; Bruggeman, et al., 2002; Kamel, 2001; Mitsch, et al., 2004).

In a study conducted by Erhan and Bölükbaşı in 2017, they looked at the effect of citrus shell oils added to broiler feeds on the numeric density of blood and lymphatic capillaries, and the length and density of jejunal villi. The density score of the blood and lymphatic capillaries of the broilers fed with a diet containing 3 mL/kg orange peel oil was very dense. The blood capillary and lymphatic capillary density scores of the groups consuming 1 mL/kg citrus peel oil (bergamot, lemon, and orange) were low. The increase of the nutrient absorbing surface and the amount of nutrient absorption are related to the rise in the density of blood capillaries. However, they reported that increasing the concentration of lymphatic capillaries would result in an increase in both the absorption surface and the amount of fat absorption.

In animal feed, plant extracts are used as yield enhancers. Yield enhancers serve two purposes. The first objective is to prevent the growth of some pathogenic microorganisms in the digestive system of animals such as *Salmonella* and the Coliform group, which are the source of diseases in the digestive system and threaten people with the food chain. The second objective is to convert the gastrointestinal tract of animals in favor of positive microorganisms and to ensure that the host can benefit from the nutrients in the feed at the highest level (Nir and Şenköylü, 2000; Yavuz, 2001).

Addition of herbal extracts to poultry feeds provides benefits such as weight gain, higher egg yield and better feed conversion efficiency, killing of pathogenic microorganisms in the digestive system starting in the mouth, increasing the flavor of feed, increasing the secretion of digestive juices, increasing effectiveness of digestive enzymes, promoting the immune system, providing low-cholesterol animal products, increasing protein synthesis by stimulating the production of higher quality and lean meat, and establishing a cleaner and healthier environment by binding ammonia (Kutlu and Görgülü, 2001; Gill, 1999).



**Figure 1.** Distributions of mean cell vitality (%) between the different experimental groups

## Conclusion

We had previously examined the density of blood and lymphatic capillaries, the length and density of jejunum villi, and the effect of tissue fatty acid composition and shelf life by adding orange shell oil to broiler feed at different levels (Erhan and Bölükbaşı, 2017; Erhan and Bölükbaşı Aktaş, 2017). In this study, we wanted to move the topic one step further and examine whether or not orange peel oil has antiproliferative

effects on the immortalized human keratinocyte non-tumorigenic cell line (HaCaT).

When the results of this study (Table 1 and Figure 1) are examined, it is seen that orange peel oil has a toxic effect on the non-tumorigenic cell line (HaCaT). When compared with the control group, it appears that all doses have a lethal impact on the cells. However, in our previous studies (Erhan and Bölükbaşı, 2017) we found that orange shell oil increased

the number of villi in the jejunum of the small intestine and the number of goblet cells where absorption was achieved.

In this cell culture study, we observed the killing effect of orange shell oil in the cells. However, in the previous study, adding it to the ration and allowing it to enter the metabolism increased the number of villus and goblet cells. Thus, I assume that orange shell oil has different effects in the metabolism. I recommend this subject to be studied by comparing it in both live animals and cell-culture studies.

Combining the findings of this study with the data of previous studies, the areas where orange peel oil can be used may be as follows:

- i- As an auxiliary agent in cancer prevention and cancer treatments due to its anti-cancer effects,
- ii- Natural anti-oxidant effect,
- iii- Anti-allergic, anti-inflammatory, and antimicrobial effects,
- iv- The impact of controlling pathogen microorganisms,
- v- Use as food and beverage aroma,
- vi- The use of medicines as spice ingredients in the pharmaceutical industry to hide unpleasant flavors, its use in perfumes and cosmetics,
- vii- Finally, based on the results of this study, it is estimated that orange peel oil can be used as a preservative to prevent bacterial growth in animal feed and human food. To determine its protective effect in feeds, it is recommended to perform feed toxicity studies after adding orange shell oil.

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## RESEARCH ARTICLE

### The Investigation of the Biological Control of *Icerya purchasi* Maskell, 1878 (Hemiptera: Margarodidae) with Entomopathogenic Fungi and Bacteria

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#### ABSTRACT

Cottony cushion scale *Icerya purchasi* Maskell, 1878 (Hemiptera: Margarodidae) is an important pest that inhibits the plant growth and development by sap sucking of the plants, and causes sooty mold in more than 200 plant species, especially in citrus plantation. The present study investigated the biological control of the nymphs and adult *I. purchasi*, which densely populates the mimosa plants (*Acacia dealbata*) in Artvin, Turkey. For this purpose, one fungal isolate [*Beauveria bassiana* (ET 10)] and eight bacterial strains [*Brevibacillus brevis* (CP-1), *Bacillus thuringiensis* subsp. *kenyae* (FDP-8, FDP-42), *B. thuringiensis* (FDP-1), *B. sphaericus* (FD-49), *B. pumilus* (TV-67C), *Pseudomonas fluorescens* (RK-1773) and *B. atrophaeus* (RK-1774)] were assessed against the nymphs and adult of *I. purchasi* under controlled conditions. Fungal and bacterial suspensions were sprayed onto 20 nymphs and 20 adults of *I. purchasi* in plastic boxes. The death rates of the nymphs and adults were recorded. The *B. bassiana* (ET 10) caused a death up to 100% and 80% in nymphs and adults, respectively. Moreover, *P. fluorescens* (RK 1773) caused 90.5% death of nymphs and *B. thuringiensis* subsp. *kenyae* (FDP-42) presented 88.5% death to the nymphs of *I. purchasi*. However, the use of the bacterial strains was not much successful against the adults, as compared to the nymphs.

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#### Introduction

*Icerya purchasi* Maskell, 1878 (Hemiptera: Margarodidae) is a cosmopolitan plant pest native to Australia that causes harm to more than 200 plant species including especially citrus fruits, other subtropical fruits, ornamental plants and weeds (Kollar et al., 2016). It was first identified in New Zealand and spread to the other regions of the world through global trade and continued to spread to the northern regions due to global warming. In Turkey, it is spread along the entire coastline and

passages. Different from other *Icerya* species, its tolerance to climatic factors allows its residence in Northern Europe (Salisbury and Booth, 2004). Due to the damage caused by the species, the offshoots and branches of forest trees and ornamental plants dry and the trees and plants lose their leaves. Furthermore, its inhibition of photosynthesis by causing sooty mold in leaves is another important damage caused by the pest. The uncontrolled infestation of cottony cushion scale has a severe effect on the pomiculture and horticulture industries and the endemic fauna of small islands.

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Moreover, the damage it causes to plants leads to the extinction of the hosts of other species from the Lepidoptera order that feed on these plants and their natural enemies are also negatively affected by this issue (Hoddle, 2011). Organophosphates and petroleum oils are used to control the pest, and although buprofezin is effective on young nymphs, it fails to affect the adult pests. On the other hand, predator, *Rodolia cardinalis*, had shown considerable potential to control the population of cottony cushion scale (*Anonymus*, 2008, 2015).

However, the activity of natural enemies diminishes due to blind use of broad-spectrum insecticides by farmers that have adverse effects on the environment (Carruthers and Hural, 1990; Inglis et al., 2001).

The inclusion of effective biopesticides that do not have the risk of resistance and any toxic effects on the environment and human health especially in areas where the activity of its natural enemies is low is of great importance for the control of the pest whose chemical control is not recommended. Many entomopathogens such as *Bacillus thuringiensis*, *Beauveria bassiana* and *Metarhizium anisopliae* can be mass produced, formulated, and applied to pest populations in a manner analogous to chemical pesticides, i.e. as nonpersistent remedial treatments that are released inundatively (Bhattarai et al., 2016).

This study investigated the insecticidal effects of eight bacterial strains and one fungal isolate against *I. purchasi*. The review of the literature has not revealed any survey on the

control of *I. purchasi* by using fungal and bacterial microorganisms.

## Materials and Methods

### Host Plant, Pest, Bioagent Fungal Isolate and Bacteria Strains

The materials of the study consist of the mimosa (*Acacia dealbata* Willd. var. *dealbata* (Link)) plants naturally infected with *I. purchasi* and adult *I. purchasi* and its nymphs in the Artvin Çoruh University Seyitler Campus in Artvin, Turkey (Figure 1).



Figure 1. *Acacia dealbata* infected with *Icerya purchasi* adults

Table 1. Identification and similarity indices of the bacterial strains and fungal isolate used in the study

Isolate and Strains Number	Fungal Isolate				
	Isolated from	ITS	S*	AN**	Literature
ET 10	<i>Sphenoptera antiqua</i>	<i>Beauveria bassiana</i>	0.99	GB  KY806126	Tozlu et al., 2017
	Bacterial Strains				
	Isolated from	MIS Identification Results	S	HR***	Literature
CP-1	<i>Ricania simulans</i>	<i>Brevibacillus brevis</i>	0.65	-	Göktürk et al., 2018
TV-67C	Raspberry	<i>Bacillus pumilus</i>	0.63	-	Erman et al., 2010
FDP-1	<i>Malacosoma neustria</i>	<i>Bacillus thuringiensis</i>	0.64	-	Göktürk et al., 2018
FDP-8	<i>Hypera postica</i>	<i>Bacillus thuringiensis</i> subsp. <i>kenyae</i>	0.45	-	Tozlu et al., 2011
FDP-42	<i>Apion</i> spp.	<i>Bacillus thuringiensis</i> subsp. <i>kenyae</i>	0.47	-	Tozlu et al., 2011
FD-49	<i>Culex</i> sp.	<i>Bacillus sphaericus</i>	0.71	-	Dadaşoğlu, 2013
RK-1773	<i>Pseudaulacaspis pentagona</i>	<i>Pseudomonas fluorescens</i>	0.59	-	In this study
RK-1774	<i>Pseudaulacaspis pentagona</i>	<i>Bacillus atrophaeus</i>	0.63	-	In this study

\*S: Similarity; \*\*AN; Accession number (GenBank); \*\*\*HR: Hypersensitivity -: Negative Reaction

The bacterial strains and fungal biocontrol isolate the insecticidal effects of which were investigated in the study were obtained from the Atatürk University Faculty of Agriculture Plant Protection Department Culture Collection. Previous studies have determined the effectiveness of a portion of these agents on different plant pests and pathogens (Göktürk et al., 2018; Erman et al., 2010; Tozlu et al., 2011, Dadaşoğlu, 2013), while others were first tested in this study (Table 1). The bacterial biocontrol strains cultured in the

Nutrient Agar (NA; Difco) medium were kept in 30% glycerol-containing Nutrient Broth (NB; Difco) at -80 °C, while the fungal isolate was kept in slant Potato Dextrose Agar (PDA, Difco) at 4 °C in the Atatürk University Faculty of Agriculture Plant Protection Department Culture Collection.

### Identification of the Bacterial Species by MIS

Preparation and analysis of FAME from whole cell fatty acids of bacterial strains were performed according to the

method described by the manufacturer's manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA) (Miller and Berger, 1985; Roy, 1988). FAMES were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25m×0.2mm×0.33µm) with cross-linked 5% phenylmethyl silicone. FAME profiles of each bacterial strain were identified by comparing the commercial databases (TSBA 40) with the MIS software package. The identity of bacterial strains was revealed by computer comparison of FAME profiles of the unknown test strains with those in the library. For MIS, Biolog, ELISA, hypersensitivity, pathogenicity and biochemical tests *E. amylovora* strain PD-761 was used as a positive control.

### **Hypersensitivity to Tobacco Test of the Bacterial Biocontrol Agents (HR)**

The fresh leaves of the tobacco, *Nicotiana tabacum* L. var. Samsun were grown in pots were used in the hypersensitivity to tobacco test. The suspensions ( $10^8$  cells/mL) that were prepared, using the bacterial cultures grown in the NA medium for 24h-48h, were injected between two adjacent trachea and the leaves were inspected for signs of symptoms. Those that didn't show symptoms in tobacco leaves were regarded as unfavorable, while those that show symptoms in tobacco leaves were regarded as positive (Klement, 1964).

### **Preparation of the Bioagent Fungal Isolate and Bacterial Strains**

The bacteria inoculated onto NA media were incubated at 28 °C for 48h and, then, transferred to NB media and incubated in a horizontal shaker. Then, the concentration of the bacterial suspension was adjusted to  $1 \times 10^8$  cfu/mL. For this purpose, the absorbance of the suspension was spectrophotometrically adjusted to 0.1 at 600 nm.

The potential bioagent fungus was cultured in PDA for about 15 days to allow spore formation. Then, sterile water was poured into the Petri dishes containing the PDA in which spore-forming fungus was grown. The spores were transferred into the baker with a glass pipette to make the suspension stirred homogeneously with a micropipette up to clear suspension. Finally, the spore concentration was adjusted to  $1 \times 10^6$  conidia/mL with a hemocytometer.

### **Testing the Fungal Isolate and Bacteria Strains Against the Pest Under Controlled Conditions**

The effectiveness of the bacterial strains and fungal isolate against *I. purchasi* was tested under controlled conditions. The mimosa branches naturally infected with *I. purchasi*, were brought to the Plant Clinical Laboratory of the Atatürk University, Faculty of Agriculture, Plant Protection Department. The branches were cut into small pieces, with 20 insects on each piece and placed in plastic boxes (19×12.5×7 cm). All suspensions were separately sprayed onto the branches. After the application, the plastic boxes were kept at 25 °C and 80% humidity and a photoperiod of 12h:12h (light:darkness). Only the NB medium in which bacteria were grown was applied to the control group. The study was carried

out in accordance with the randomized block design in three repetitions.

The number of dead insects was recorded daily and the death rates in percentages (%) was calculated using the formula below

$$\text{Death rate (\%)} = 100 \times \frac{\text{Number of dead insects}}{\text{Total number of dead insects}} \quad (1)$$

The potential bioagent bacterial strains and fungal isolate were re-isolated from the dead insects and the Koch's postulates were fulfilled.

### **Analysis of the Results**

Arcsine transformation was applied to the data and, then, one-way variance analysis was applied and the differences between the mean values were compared using the LS Means Student test at a significance level of  $P < 0.01$ . The data analysis was carried out using the JMP IN statistical software (SAS Institute, Cary, NC, 0% PC version).

### **Results and Discussion**

Table 2 shows the insecticidal effects of the bacterial strains and fungal isolate on the nymphs and adults of *I. purchasi*.

The results revealed the bioagent fungal isolate and bacterial strains had varying levels of insecticidal effect on the nymphs ( $49.50 \pm 27.79\%$  and  $100 \pm 0.00\%$ ) and adult pests ( $0.00 \pm 0.00\%$ - $80.00 \pm 37.75\%$ ) (Table 2). The highest death rate was determined in the nymphs. A death rate of 100% was obtained in the application in which the ET 10 isolate of *B. bassiana* was applied to the nymphs, followed by the application of *P. fluorescens* that was isolated from *Pseudaulacaspis pentagona* (mulberry scale) (RK 1773) (90.50%). The FDP-42 (*B. thuringiensis* subsp. *kenyae*) (88.50%) and FDP-8 (*B. thuringiensis* subsp. *kenyae*) (82.00%) applications were in different groups, while the CP-1 (*B. brevis*) (58.00%), FDP-1 (*B. thuringiensis*) (58.00%), TV-67C (*B. pumilus*) (55.00%), RK 1774 (*B. atrophaeus*) (54.50%) and FD-49 (*B. sphaericus*) (49.50%) applications were in the same group and yielded different results from the control group (0.00%).

In the applications to adult pests, the highest death rate was obtained with the ET 10 isolate of *B. bassiana* (80.00%), as was the case in the nymphs, followed by the TV-67C (20.00%) strain of *B. pumilus* and FDP-1 (18.06%) strain of *B. thuringiensis*. RK 1774 (*B. atrophaeus*) (7.22%), RK 1773 (*P. fluorescens*) (6.94%), FDP-8 (*B. thuringiensis* subsp. *kenyae*) (5.56%), FDP-42 (*B. thuringiensis* subsp. *kenyae*) (5.28%), FDP-49 (*B. sphaericus*) (1.11%) were in the same group, while no deaths were observed in the CP-1 (*B. brevis*) and control applications (0.00%) (Table 2).

Table 2. Efficacy of some entomopathogen bacterial strains and fungal isolate on *Icerya purchasi*

Treatment	Percentage death ratio (%)*	
	Nymph	Adult
ET 10 ( <i>Beauveria bassiana</i> )	100.00±0.00 A	80.00±37.75 A
RK 1773 ( <i>Pseudomonas fluorescens</i> )	90.50±9.65 AB	6.94±6.67 C
FDP-42 ( <i>Bacillus thuringiensis</i> subsp. <i>kenyae</i> )	88.50±6.25 B	5.28±4.99 C
FDP-8 ( <i>Bacillus thuringiensis</i> subsp. <i>kenyae</i> )	82.00±17.73 B	5.56±8.20 C
CP-1 ( <i>Brevibacillus brevis</i> )	58.00±38.07 C	0.00±0.00 C
FDP-1 ( <i>Bacillus thuringiensis</i> )	58.00±37.85 C	18.06±12.30 B
TV-67C ( <i>Bacillus pumilus</i> )	55.00±18.44 C	20.00±15.05 B
RK 1774 ( <i>Bacillus atrophaeus</i> )	54.50±16.83 C	7.22±7.71 C
FD-49 ( <i>Bacillus sphaericus</i> )	49.50±27.79 C	1.11±2.30 C
Control	0.00±0.00 D	0.00±0.00 C
<b>CV</b>	<b>24.28</b>	<b>78.19</b>
<b>LSD</b>	<b>11.15</b>	<b>7.42</b>

\*Mean values in the same column by the same letter are not significantly different to the test of LS Means Differences Student's ( $p < 0.01$ )

Figure 2 shows the effects of the ET 10 isolate, which was determined to be the most effective application, on the nymphs and adult of *I. purchasi* and the effects of RK 1773, FDP-42 and FDP-8 on the nymphs.

Entomopathogens are among important factors suppressing pest populations. Various pesticides are commonly used worldwide in the biological control of pests in greenhouse products, ornamental plants, stored products, forest products and products from vegetable and fruit gardens (Lacey et al., 2001).

There are many studies on *B. bassiana* (Kumar and Suktana, 2017), which is frequently included in Integrated Pest Management (IPM) programs due to its environmental-friendly structure, biological retention and ability to kill pests at various developmental periods in their life cycles (Diehl-Fleig, 1986; Adane et al., 1996, Loureiro and Monteiro, 2005; Marannino et al., 2006, Sabour et al., 2007; Castilho, 2010; Zibae et al., 2013, Tangtrakulwanich et al., 2014; Swiergiel et al., 2015). The studies have revealed that the fungal infection begins with the attachment of the spore cuticle. The fungus penetrates through the thin regions of the cuticula or mouthparts and the host become dead by feeding the inoculated food. The death of the host is caused due to the production of fungal toxins e.g. beauvericin, bassianin, bassianolide, beauverolide, tenellin, oosporein, oxalic acid, bassiacridine, cyclosporin A and hydrophilic chitinase (Yıldız, 2015). Today, there are many commercial products of *B. bassiana* and they are effectively used against different insect pests. The results of the present study about the effectiveness of *B. bassiana* represented significant potential against the nymphs and adult of *I. purchasi* and the ET 10 isolate caused a 100% death rate in the nymphs and 80% death rate in adults.

Facultative aerobic and spore-forming bacteria that have crystal proteins were commonly used for eco-friendly biological control of pests (Katı, 2008). Insecticidal crystal proteins cause a death against numerous insect pests and vector pests (Jackson et al., 2000; Katı, 2008; Azizoğlu et al., 2012). Crystal proteins are taken from the water during feeding, dissolves in the intestines of the pest, a high-alkali environment, and the toxin activated by protease goes through the cell wall after binding to specific parts of middle intestinal

cells and perforates the cell wall. This disturbs the ion balance of intestinal epithelial cells and kills the pest (Nielson-LeRoux et al., 2001; Smith et al., 2005; El-Bendary, 2006).

Among these bacteria, the most important group is the *Bacillus* species (Gray et al., 2001; Alper et al., 2013) and the most investigated bacteria from the *Bacillus* genus is *B. thuringiensis*, which constitutes 2% of world insecticide market (Bravo et al., 2007) and known as a pesticide with a lower risk than chemicals (Ertürk and Yaman, 2019). The bacteria have many varieties and each variety could kill a specific insect and produce a different toxin. Accordingly, in this study, *B. thuringiensis* subsp. *kenyae* was the most effective bacterial agent against the nymphs while *B. thuringiensis* was on adults. However, Hajaij et al. (2005) reported that there were significant decreases in the number of spores immediately after the application and bacteria spores could not reproduce during the dead stages of the pest in areas where *B. thuringiensis* was applied and emphasized that the *B. thuringiensis* applications should be periodically repeated.

In the study, *P. fluorescens* was the another bacterial species that was effective on the nymphs. Suganthi et al. (2017) reported that the MP-13 isolate of *P. fluorescens* resulted in a death rate of 100% in tea mosquito bug (*Helopeltis* spp. (Hemiptera: Miridae)) under *in vitro* conditions and recorded that *P. fluorescens* affected the pest by enzymatically hydrolyzing the chitin in the exoskeleton of the insect. Moreover, the researchers also reported that the enzyme affected the digestion of the insect and caused death by directly inhibiting the growth and development of the insect.

Another important species used in the biological control of pests is *B. sphaericus*. In general, after the application of *B. sphaericus* to the pests, the feeding of the insect stepped, the insect activity decreased within two hours and insect was paralyzed after around six hours. Furthermore, the reproduction of the *B. sphaericus* spores on dead larvae has been reported to be important in the control of pests (Boonserm et al., 2006). In this study, the FD-49 strain of *B. sphaericus* whose insecticidal effect on *I. purchasi* was investigated caused a death rate of 49.40% in the nymphs and

1.11% in the adults and, thus, was less effective compared with other bioagents.

In conclusion, the fungal bioagent *B. bassiana* ET 10 isolate was found to be effective on the nymphs and adult *I. purchasi*

under controlled conditions. In addition, *P. fluorescens* RK 1773 and *B. thuringiensis* subsp. *kenyae* FDP-42 were effective on the nymphs and *B. pumilus* TV-67C and the FDP-1 strain of *B. thuringiensis* were effective on the adults.

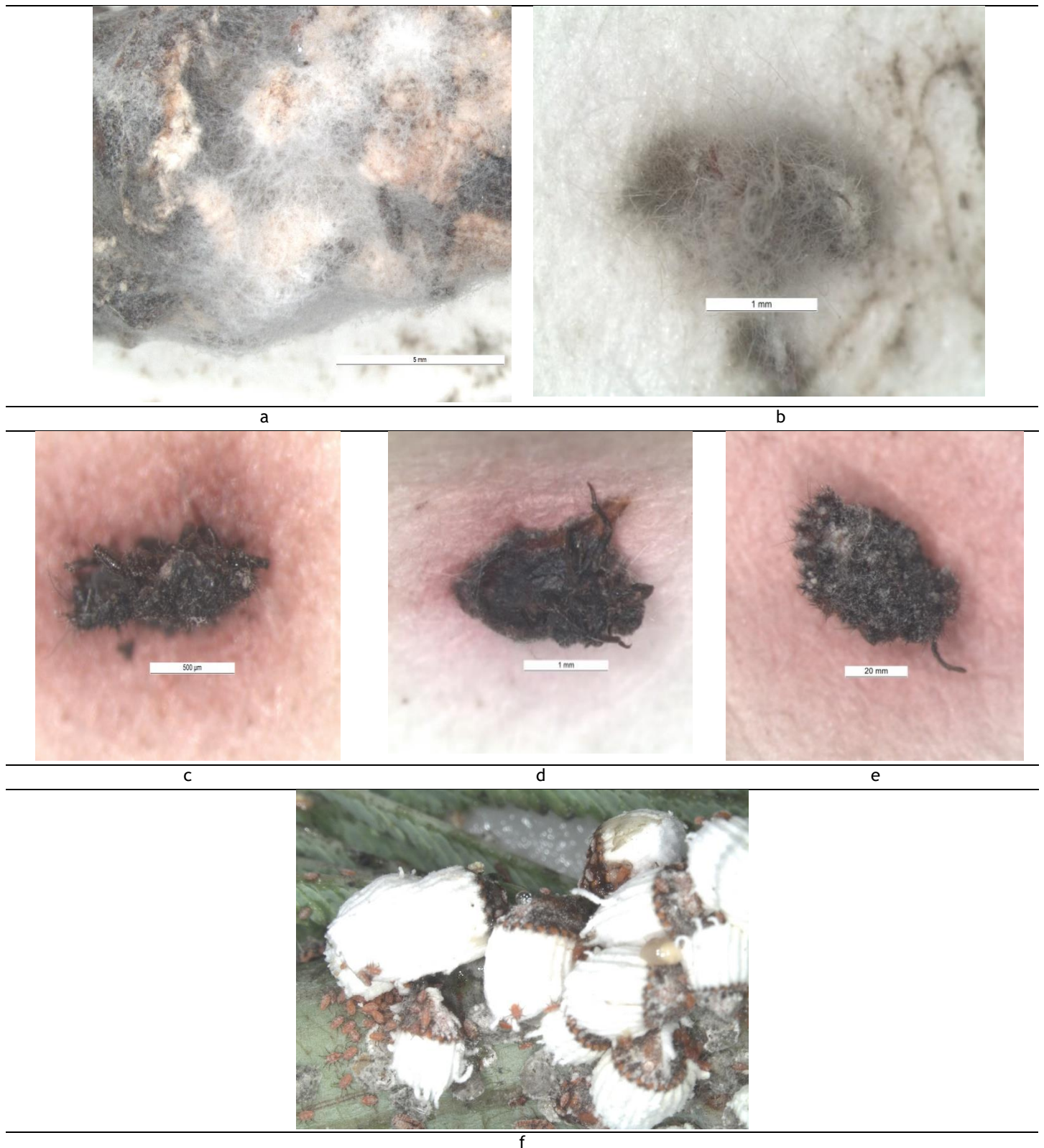


Figure 2. ET 10 on *Icerya purchasi* adults (a), ET 10 (b), RK 1773 (c), FDP-42 (d), FDP-8 (e) on *Icerya purchasi* nymphs and control (f)

## Conclusion

The study emphasizes the importance of the inclusion of biopesticides that do not have the risk of resistance and any toxic effects on the environment and human health and are useful in the areas where the effectiveness of the natural enemy is low into the control systems. The effectiveness of the bioagents used in the study may vary under the field conditions at different temperatures and humidity levels. Thus, carrying out field studies involving the bacteria strains and fungus isolate that are effective on the pest in the future is of great importance.

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## RESEARCH ARTICLE

### Quest for the Profitability and Sustainability of Tea And Pine Apple Plantation in Unorganized Sectors of Assam, India

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#### ABSTRACT

Country like India is mostly dependent upon the agriculture produce as even in the twenty-first-century agriculture is the backbone of Indian economy. The main challenge for any activity is to know whether it is going to survive. This study focuses on determining the profitability and sustainability of tea and pineapple growers. Both the plantation grows well in the studied region. The researcher used a structured questionnaire for collecting data from the growers. Cost-Benefit Ratio, Profit volume, break-even point, Margin of safety, and profit is ascertained in the study. In both the plantation the results show the CBR is greater than one, which implies it is economically feasible. The total cost of planting tea in one acre of land is Rs 189505. Whereas the total cost pineapple plantation is Rs 49600. The average total revenue that planters generate from sales proceeds of tea is Rs 400925 and for pineapple is Rs 287000 from one acre of land. The tea planters are earning a net profit of Rs 211420 and pineapple planters are earning a net profit of Rs 237400. It can be said that the growers earn a fairly good sum of money. None of the pineapple growers are using any kind of fertilizer or chemicals which make them organic and healthy to consume. Both tea and pineapple Plantation can generate good revenue and are feasible investment decisions to take on, among the two pineapples is generating more revenue than tea.

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#### Introduction

India from its history is known for its rural economy, most of the people are dependent upon the agriculture produce directly. In the 21<sup>st</sup> century the economy of India has moved from an agrarian economy to a service-based economy. The most common reason given for this shift in the economy is due to non-profitability in their crops. It is reported that most farmers in India don't get a fair price for their crops. That's the reason why people are shifting from agriculture to better job opportunities. In the northeastern part of India, especially in the state of Assam where there are plenty of hills which are more congenial for plantation of Tea and Pine Apple is witnessing a downfall in the plantation. Tea is a perennial

shrub, whose bud and two leaves are used in making tea. In India it is commonly known as "chai" or "cha".

The pineapple (*Ananas comosus*) is a tropical plant that bears an edible fruit; also called pineapples are the most economically significant plant in the plant family Bromeliaceae. India ranks 5<sup>th</sup> in terms of total cultivation output in the year 2017 as per UN reports of the Food and Agriculture Organization Corporate Statistical Data. Pineapple is cultivated in an area of 89 thousand hectares hilly land and total production is 1415.00 thousand tons per annum. It is abundantly grown in almost the entire North East region of India, other states like West Bengal, Kerala, Karnataka, Bihar, Goa, and Maharashtra. The major pineapple growing countries in the world are Brazil, followed by Thailand, Philippines,

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Costa Rica, China, and India. The total area under pineapple cultivation in the world is 909.84 thousand hectares with production around 19412.91 thousand tons.

(Bakhsh et al., 2006) studied Profitability and Cost in Growing Mango Orchards finds that cost and benefit ratio doesn't serve a true measurement for making decisions. The benefit-cost ratio is positive in his study. It shows the growing mango is profitable in the selected region of study. (Uzunoz & Akcay, 2016) analyzed the Profitability Analysis of Investment of Peach and Apple Growing in Turkey by using Cost-benefit Ratio, Net Present Value, and Internal rate of return. The researcher concludes that in the selected region peach and apple farming is economically viable and can be an important source of income for the farmers. (John et al., 2017) finds the benefit-Cost Analysis of Apiculture Enterprise in District Pulwama and Srinagar as per the researcher's findings the business was profitable. It has become a source of small scale employment opportunities and creating jobs for rural people to reduce poverty in the rural economy. (Sharma, 2019) conducted a Cost-Benefit Analysis of Small Tea Growers in Padumani Development Block of Golaghat District of Assam finds the CBR greater than one. Which mean plantation decision will be a profitable one. He also finds the sector is very promising and can generate a quite number of Employments.

### **The rationale of the Study**

The study will be useful for the unemployed youth, farmers thinking of shifting to other crops, government agencies so that they can form new policies to make it more profitable. It will also help the people to those who are in a dilemma to choose one option between tea and pineapple. The study will help understand the economic feasibility of plantation. The objectives set in the next section will help them to determine the actual amount they need to invest at the initial stage on per hectare basis and how much they can expect in return, thus it will help them in decision making.

### **Objectives of the Study**

- To determine the Cost-benefit Ratio of Tea and Pine Apple plantation.
- To determine the Fixed cost, Variable cost, and Total cost for Tea and Pine Apple plantation.
- To determine the revenue generated per acre for Tea and Pine Apple.
- To determine the Profit on sale of Pine Apple per piece.
- To determine the profit on the sale of Tea leaves per Kilogram.

## **Materials and Methods**

### **Area and Population**

The study is conducted in the Morongi developmental Block of Golaghat, the upper Assam districts along with Karbi Anglong districts of Assam. The population of the study is roughly known, as the study is conducted in the unorganized sector and the planters are scattered. As the number of tea and pineapple growers data in the targeted region is known but the actual location of the growers are not known. They are doing plantation on their level. All the growers of the studied region have been taken as the population of the study.

### **Sample Size**

The size of the sample for the study is determined after the pilot survey. The pilot survey schedule uses interval scale and nominal and in the form of 5 points Likert scale. 15 statements are used on a Likert scale. The variance calculated after the pilot survey for tea plantation is 0.195 and for pineapple plantation is 0.241. The variation implies that larger the variance; larger will be the sample size and smaller the variance smaller will be sample size. The formula to be used in determining the sample size is as follows:

Sample size determination for Tea Plantation (Kothari. C.R., Garg.G 2019)

$$n = \frac{Z_{\alpha/2}^2 N \delta^2}{(N-1)e^2 + Z_{\alpha/2}^2} \quad (1)$$

This formula is used in the calculation because the population is finite. The calculation is done at a 5% level of significance and a 95% level of confidence.

$$n = \frac{(1.96)^2 * 233 * .195}{(233-1)(0.05)^2 + (1.96)^2}$$

$$n = \frac{3.84 * 45.435}{232 * 0.0025 + 3.84}$$

$$n = \frac{174.47}{0.58 + 3.84}$$

$$n = 39.4$$

$$n \approx 40$$

After the calculation the sample size turns out to be 39.4. So, 40 respondents were taken as sample size.

Sample size determination for Pine Apple Plantation

$$n = \frac{Z_{\alpha/2}^2 N \delta^2}{(N-1)e^2 + Z_{\alpha/2}^2} \quad (2)$$

The calculation is done at a 5% level of significance and a 95% level of confidence.

$$n = \frac{(1.96)^2 * 207 * .241}{(241-1)(0.05)^2 + (1.96)^2}$$

$$n = \frac{3.84 * 49.88}{240 * 0.0025 + 3.84}$$

$$n = \frac{191.53}{0.6 + 3.84}$$

$$n = 43.13$$

$$n \approx 43$$

The calculation of the sample size turns out to be 43.13. So, 43 respondents were selected as sample size.

## Method

The convenience and Snowball sampling method are used for the study. As the growers of tea and pineapple are scattered in various places in the studied region and unavailability of any reliable data about growers of unorganized sector. The researcher has chosen this method to collect data from the growers as it suits the purpose of the study. All the data collected is in INR.

## Data Collection

Keeping objective in mind data has been collected from both primary and secondary sources. Primary data is collected with the help of a structured schedule keeping in mind the objectives of the study. Utmost care has been taken while collecting data, only reliable and authentic information for the study is included in the study. Secondary sources of data collected from books, journals, periodicals, government websites magazines.

## Tools for Analysis

**Cost-Benefit Analysis:** It is a process for estimating the cost involved and probable profit to be derived from a business opportunity. It is used to ascertain the soundness of any business investment opportunity and provide a basis for making decisions. Those projects are taken into consideration whose outcome is more than one. To determine the cost of cultivating green tea leaves and the revenue earned from per acre of land. It will be determined by the following:

$$\text{Cost-Benefit Analysis (CBA)} = \frac{TR}{TC} \quad (3)$$

Where, TR = Total Revenue, TC = Total Cost.

**Profit Volume ratio:** PV ratio also called a contribution margin ratio. It is a measurement of the rate of change of profit due to a change in volume of sales. It expresses the gross profit made on one unit of production as a fraction of the percentage of its selling price. It shows the relationship between contribution and the value of sales. The contribution is derived after deduction Variable cost from Sales and Variable cost are those cost which increases or decreases as the output increases or decrease. It is used to measure efficiency. It is determined by the following formula

$$\text{P/V ratio} = \frac{\text{Contribution}}{\text{Sales}} \times 100 \quad (4)$$

Where, Contribution = Sales- Variable Cost

**Break-even point:** BEP is that point where there is no profit no loss situation prevails. Or in other words we can say BEP as a point of intersection where total Expenses (cost) and total Revenue (sales) curve cut each other. Graphically, it is a point where total cost and total revenue curve meet. BEP can be shown with the help of the following diagram.

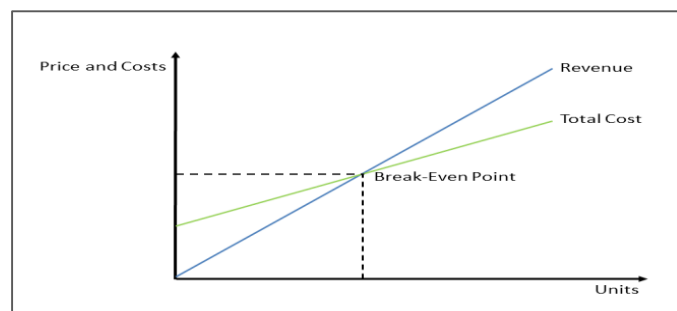


Figure 1. Break-even curve

Figure 1 showing Break Even point. It can be calculated by the following formula:

$$\text{BEP} = \frac{\text{Total fixed cost}}{\text{Contribution}} \quad (5)$$

Contribution = selling price - variable cost

Or; Fixed cost + profit.

**The margin of Safety:** The margin of safety is a measure of risk. The excess of sales over the Break-Even of sales is known as the Margin of Safety. With the high Margin of Safety businesses have a low risk of Shut down and with low margin businesses have a high rate of Shut down. Higher the margin the better for the business. It is represented as:

$$\text{Actual sales} - \text{BEP} \quad (6)$$

Where, BEP = Break-Even Point

**Profit on sale:** It is used to determine the gross profit on the sale, Symbolically it is written as

$$\text{Profit on Sale} = \text{Sales} - \text{TC} \quad (7)$$

Where, TC = Fixed Cost + Variable Cost

The cost of producing per kilogram of the raw leaf will be studied per strata. Along with the return, the cultivators will be getting after selling the leaf to the middleman or directly to the nearby factory

## Results and Discussion

### Cost Components along with Analysis for Tea Plantation

The tea plant & plantation cost include the cost of preparing land for planting, the cost of the tea plant, and planting of each tea plant. Fertilizer and insecticide in fixed cost indicate for those chemicals used once during plantation to protect the tea plant from insects and other diseases. The wage for plucking of leaves is paid Rs. 4 per kg of leaves.

The total output in a year is 26068 kilograms. The average rate as responded by the grower is taken at Rs. 15.38/kg. The price of raw leaves ranges from Rs 23 to 9 Rs per kg. The growers get an amount of Rs 400926 per acre from the sale proceeds.

**Table 1.** Fixed and variable cost components

Fixed Cost per Acre/ Year		Variable Cost per Acre/ Year	
Particulars	Amount in Rs. (INR)	Particulars	Amount in Rs. (INR)
Land Revenue	700	Wage for Spraying chemical	3834
Sprayer	1237	Wage for leaf plucking	104272
Tea Plant & plantation	2025	Fertilizers/Insecticide	52500
Fertilizers/Insecticide	122	Wage for pruning tea bushes	4725
Handheld cart	1400	Bamboo Fences	14350
Weighting Scale	830	Bamboo Basket	490
Spade	260	Tarpaulin	760
		Nylon Bag	750
		Pruning Knife	1250
<b>Total</b>	<b>6574</b>	<b>Total</b>	<b>182931</b>

Source: Field Survey

**Table 2.** Output, rate and amount derived from the sale of leaf

Fixed cost in Rs.	Fixed cost/ kg in Rs.	Variable cost in Rs.	Variable cost/ kg in Rs.	Total cost in Rs.	Total cost / kg in Rs.	Total Output in Kg	Rate/ Kg	Amount in Rs
6574	0.25	182931	7.01	189505	7.26	26068	15.38	400926

Source: Field Survey

The fixed cost per kg is derived by dividing the fixed cost from the total output and variable cost is derived by dividing variable cost by total output. Similarly total cost per unit or kg is derived after dividing total cost by total output. The variable cost per kg is Rs 7.01 and the fixed cost is Re 0.25. The total cost per kg is Rs 7.26.

**Table 3.** Fixed cost, variable cost, total cost, output and selling price

Fixed Cost	Variable Cost	Total Cost	Output in Kg	Selling Price in Rs.
6.574	182931	189505	26068	15.38

Source: Field Survey

The exhibits show fixed cost incurred in one acre of land is Rs 6574 variable cost Rs 182931 while the total cost is Rs 189505. The output per acre during per harvesting season is 26068 kg having an average selling price of Rs 15.38.

**Table 5.** Cost components per acre

Particulars	Units	Rate per Unit cost in Rs.	Amount in Rs.(INR)
Plantation Seed (F)	(15,000 seeds)	@.80	12000
Spade(F)	2	@200	400
Knife (F)	10 pcs	@150	1500
Land Revenue (F)			700
Labour charges for cleaning for plantation (V)	20 man hour	@200	4000
Labour charges for plantation (V)	45 man hour	@200	9000
Cleaning of bushes half yearly (V)	60 man hour	@200	12000
Harvesting (V)	10,000	@ 1	10000
<b>Total</b>			<b>49600</b>

Source: Field Survey. (F)= Fixed Cost, (V) = Variable Cost

**Table 6.** Fixed cost, variable cost and total cost

	Fixed Cost in Rs	Variable Cost in Rs	Variable Cost per Unit	Total Cost in Rs
Small Fruit	1459	3501	3.50	4960
Medium Fruit	4380	10500	3.50	14880
Big Fruit	8759	21001	3.34	29760
<b>Total</b>	<b>14600</b>	<b>35000</b>		<b>49600</b>

Source: Field Survey

**Table 4.** Results of financial tools for tea plantation

Cost Benefit Ratio	Contribution	Profit Volume Ratio	Break Even Point	Margin of Safety	Profit on sale/Kg
2.11	8.37	54.42	785.42	388847	8.12

Source: Field Survey

#### **Cost Components along with Analysis for Pine Apple Plantation**

Per acre the seed required is around 15000 which costs around Rs 12000. The first thing which the growers required is the cleaning of the land where pineapple is to be planted. For cleaning the area where the plantation is to be done it required 20 man-hour days per day costing @200 amounting to Rs 4000. Once the seeds were planted it becomes ready to be harvested within 2 years. It requires cleaning of grasses in six months. For 15000 seeds planted it will bear fruit in around 10000. Harvesting per fruit cost Re 1. The total cost for one acre of land is Rs 49600.

**Table 7.** Fixed cost, variable cost and total cost

Fruit Grade	Production in pieces	Average Selling price	Revenue	Proportionate Total Cost	Cost per piece
Small Fruit	1000	8	8000	4960	4.96
Medium Fruit	3000	23	69000	14880	4.96
Big Fruit	6000	35	210000	29760	4.96
<b>Total</b>	<b>10000</b>		<b>287000</b>	<b>49600</b>	

Source: Field Survey

**Table 8.** Results of financial tools for pine apple plantation

Cost Benefit Ratio	Fruit Type	Contribution	Profit Volume Ratio	Break Even Point	Margin of Safety	Profit on sale/Piece
<b>5.79</b>	Small	4.5	56.25	324.22	5406.24	3.04
	Medium	19.5	84.78	224.61	63833.97	18.04
	Big	31.65	90.42	276.74	200314.1	30.04

Source: Field Survey

The cost-benefit ratio for the plantation of tea is 2.11 and for Pine Apple the ratio is 5.79. The implication for the cost-benefit ratio is that if it is greater than 1 then it implies that it is profitable. In both the plantation the CBR is favorable for the growers which means it is economically feasible. The total cost of planting tea in one acre of land is Rs 189505. Whereas the total cost pineapple plantation is Rs 49600. The average total revenue that planters generate from sales proceeds of tea is Rs 400925 and for pineapple is Rs 287000 from one acre of land. The tea planters are earning a net profit of Rs 211420 and pineapple planters are earning a net profit of Rs 237400. In percent terms tea planters earn about 211 percent return per annum from per acre of land while pineapple planters earn 578 percent returns which is quite high, so in comparative terms pineapple is more profitable. From selling per kilogram of tea leaves the planters earn Rs 8.12. From sale proceeds of small fruit, medium fruit, and big fruit of pineapple the growers can earn Rs 3.04, 18.04, and 30.04 respectively. It can be said that the growers earn a fairly good sum of money. None of the pineapple growers are using any kind of fertilizer or chemicals which make them organic and healthy to eat.

## Conclusion

The study finds many answers to cost-related questions regarding plantation. In both the plantation it was found that it is economically feasible. The farmers along with the government should work together for the benefit of the planters. Overall the profitability is good. As Assam is producing more than half of the tea production in India, it continues to have profitability. The youth who were looking for jobs can take up entrepreneur activity and starts generating employment. The government has launched many schemes for the tea planters. Pineapple being a perishable commodity if the government sets up some food processing industry in pineapple farming areas the farmers can earn more money. The government is targeting to double the incomes of the farmers by 2022. The targeted region is near the international border so it makes it more advantageous for farmers, they can read the benefit of export if they continue to produce good quality products. The Act East policy of the government is also in effect which will help the farmers to export. To conclude it can be said that both the plantation is having good economically and financially viability.

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## RESEARCH ARTICLE

### Assessment of Phenolic Content, Antioxidant Properties and Antimicrobial Activity of Flower and Leaf Extracts from Some *Hypericum* Species Affected by Wild Habitat Altitude

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#### ABSTRACT

In this study, determination of habitat altitude effect on the total phenolic contents, antioxidant and antimicrobial activities of flower and leaf extracts from *Hypericum montbretii*, *H. orientale* and *H. perforatum* species was aimed. The plants were collected randomly from forages (altitudes were ranged from 430 to 1105 m a.s.l.) located in Western Black Sea Region, Turkey. Antioxidant properties of ethanolic extracts were determined with DPPH and ABTS assay and antimicrobial activities of the extracts on *Bacillus pumilis* NRRL BD-142, *B. subtilis* NRRL B-209, *B. licheniformis* NRRL-B-1001, *B. cereus* NRRL B-3711, *Staphylococcus aureus* ATCC 33862, *Pseudomonas aeruginosa* ATCC 27853, *Listeria innocua* ATCC 33090, *L. monocytogenes* ATCC 7644 and *Escherichia coli* ATCC 25922 were examined. Total phenolic contents and antioxidant activities of *Hypericum* species changed depending on the habitat altitude. The antimicrobial activity of the ethanolic extracts was evaluated by minimal inhibitory concentrations (MIC) method. Flower and leaf extracts exhibited a broad antibacterial spectrum, but they were not effective against *Escherichia coli* (ATCC 25922). Phenolic contents of all *Hypericum* species and antimicrobial activity of only *H. perforatum* extracts were significantly increased by altitude rising, but no positive correlation was detected in antioxidant activity of extracts due to habitat altitude.

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#### Introduction

In recent years, consumer demand to natural products has increased in the sense of medicine and food all over the world. This current of thought has brought up the use of some natural plant species for the prevention of disease, natural living, and natural feeding. It is determined that some plant extracts have effects such as antimicrobial, antispasmodic, antimutagenic, antioxidant and antiviral according to many active ingredient

which they contain in the recent studies (Sudharameshwari and Radhika 2007). Therefore, studies related to the determination of the characteristics of plants and plant active ingredients have become more important. The genus of *Hypericum* is one of the grouped of these plants. *Hypericum* L. is a genus of flowering plants in the family *Hypericaceae* (a subfamily of *Clusiaceae*). This family consists of 46 genera and 1000 species, distributed across tropic and subtropics regions, as

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well as Europe, West Asia, North Africa and North America, and particularly Anatolia (Saroglou et al. 2007; Dadkhah et al. 2014) and it is stated that 46 endemic totally 96 species present in Turkey flora (Çirak et al. 2016). In recent years, *Hypericum* species have gained popularity due to their antidepressant effects (Çirak et al. 2006). According to Bingol et al. (2011) all *Hypericum* species used as sedative, antiseptic and antispasmodic in Turkish folk medicine and have different names like kantaron, peygamber çiçeği, kılıçotu, kuzukıran and binbirdelik otu. Moreover, it is known that some *Hypericum* species have been consumed as tea.

It is known that several environmental factors such as temperature, wind velocity, precipitation, duration of snowpack, soil, temperature extremes, radiation intensities, and length of the vegetation period vary with the altitude of the natural growth locality (Camas et al. 2014). Thus, the chemical composition of *Hypericum* species may significantly alter depending on variation among *Hypericum* species as well as these factors (Xenophontos et al. 2008). Several studies have been conducted to the effects of the habitat altitude of the plants on the chemical content (Xenophontos et al. 2008; Camas et al. 2014) but there is no previous report about the functional properties of *H. perforatum*, *H. orientale* and *H. montbretii*. Therefore, the main aim of this research was to

determine the effect of the habitat altitude of the plants on the antimicrobial activity, total phenolic content and antioxidant activity of the ethanol extracts of three *Hypericum* species.

## Materials and Methods

### Collection and Identification of Plant Materials

Three wild species of *Hypericum* (*H. montbretii* (3), *H. orientale* (4) and *H. perforatum* (5)) were collected randomly from forages located (altitudes ranged from 430 to 1105 m a.s.l.) in the Western Black Sea Region, Turkey. Details about the locations where the plants collected were presented in Table 1. The distance between the two collection locations was minimum 15 km. The plant materials were collected in June 2015 during flowering stage. In general, the climate of the collecting area is classified as warm and temperate. The Köppen-Geiger climate classification is Cfb. At first, the plants were identified taxonomically by the Department of Biology, Ondokuz Mayıs University, Samsun, Turkey. The aerial parts were air dried in shadow and fractionized. The leaf and flower parts of the plants were separated and powdered using a laboratory mill. Powdered materials were protected from light until analyzes.

Table 1. Details about the locations of the *Hypericum* species

Species	Location Code	Locality	Altitude (m)	Coordinates
<i>H. montbretii</i>	A1	Between Taşköprü and Boyabat	430	41°48' N, 34°11' E
	A2	Between Hanönü and Sinop	496	41°63' N, 34°43' E
	A3	Between Kastamonu and Taşköprü	700	41°62' N, 33°72' E
<i>H. orientale</i>	B1	Between Kastomonu and Daday	774	41°43' N, 33°75' E
	B2	Between Ağlı and Seydiler	1012	41°64' N, 33°65' E
	B3	Between Ağlı and Seydiler	1039	41°66' N, 33°71' E
	B4	Seydiler, Tokazlar village	1050	41°62' N, 33°72' E
<i>H. perforatum</i>	C1	Between Boyabat and Sinop	339	41°54' N, 33° 47' E
	C2	Hanönü, Çayköy vilage	497	41°63' N, 34°43' E
	C3	Between Ağlı and Kastamonu	803	41°48' N, 33°76' E
	C4	Seydiler, Selmanlı village	994	41°65' N, 33°60' E
	C5	Daday, Karacaören village	1105	41°47' N, 33°21' E

### Extraction Procedure of Plant Materials

The maceration technique was used for extraction of plant materials. Five grams of the ground sample was extracted with 200 mL of ethanol (Merck, 99.5%, v/v) at room temperature in the dark with shaking (220 rpm) for 2 days. After maceration, the liquid extract was separated from the solid residue by filtering through Whatman No. 4 filter paper. The solvent was removed with a rotary evaporator at 40°C, at 45 mbar to obtain a dry extract. The crude extracts were dissolved in 10 mL of ethanol and the amount of crude extract in mL was calculated. All the extracts were placed in a glass bottle and stored in the dark at -20°C until use. All assays were made three times and the results were presented as the average of triplicate analyses.

### Test Microorganisms

The *in vitro* antimicrobial activities of the plant extracts were analyzed for antimicrobial activity against following of 9 microorganisms: *Staphylococcus aureus* (ATCC 33862), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus pumilis* (NRRL BD-142), *Bacillus subtilis* (NRRL B-209), *Bacillus licheniformis* (NRRL-B-1001), *Listeria innocua* (ATCC 33090), *Listeria monocytogenes* (ATCC 7644), *Escherichia coli* (ATCC 25922), and *Bacillus cereus* (NRRL B-3711), obtained from Food Engineering Department, Ondokuz Mayıs University (Samsun-Turkey). Microorganisms were maintained in glycerol broth at -80°C. These microorganisms were activated 2 times in Mueller Hilton broth (Merck, Darmstadt, Germany) at 30°C overnight before use.

### Minimum Inhibitory Concentration (MIC) of Extracts

The minimum inhibitory concentration (MIC) of the extracts was determined according to Agar diffusion assay described by CLSI (2006). Serial dilutions of the plant extracts (1024, 512, 256, 128, 64, 32, 16, 8, 4, 2 µL/mL) were prepared in Muller Hilton Agar medium (Merck, Darmstadt, Germany) according to the standard procedure. After solidification, the plates were incubated at room temperature (22-23°C) for 6 hours to obtain dry the agar surface. Suspensions of the test microorganisms were prepared by matching a McFarland 0.5 turbidity standard. Inoculations were applied to agar surfaces in 1 µL spots, giving approximately  $1.5 \times 10^5$  CFU per spot. Plates without added extract were inoculated as positive controls. All plates were incubated at 30°C for 24-48 hours. The MIC was considered as the lowest concentration of extract which caused a marked inhibition in growth as compared to control and expressed in µg/mL. All data represent at least three replicated experiments per microorganism.

### Total Phenolic Content of Extracts

The total phenolic content of the extracts was determined by using the Folin-Ciocalteu (Sigma Aldrich, Steinheim, Germany) phenol reagent method. Gallic acid (Sigma Aldrich, Steinheim, Germany) was used as a standard. The concentration of total phenolic contents in the plants was determined as µg of Gallic acid equivalents (GAE) per 1 mg of extract using the following equation obtained from a standard Gallic acid graph ( $R^2=0.9973$ ). Briefly, 50 µL (two replicates) of the filtered extracts were mixed with 450 µL of distilled water and 2.5 mL of 0.2 N Folin-Ciocalteu reagents. After 5 min, 2 mL of saturated sodium carbonate (75 g/L) were added on it. The absorbance was measured at 765 nm using a spectrophotometer (Shimadzu Scientific Instruments, Japan) after incubation at 30°C for 1.5 h with discontinuous shaking. Quantitative measurements were performed, based on a standard calibration curve of Gallic acid. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligram per gram of dry material.

### Antioxidant Activities of Extracts

Free radical scavenging activity of ethanolic plant extracts was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma Aldrich, Germany) by using the method of Brand-Williams et al. (1995) with some modifications. Briefly, 0.06 mM solution of DPPH in ethanol was prepared. Then, 1 mL of this solution was incubated with varying concentrations of ethanolic plant extracts. After that, the mixtures were shaken well and incubated for 30 min in dark at room temperature. The absorbance of the resulting solution was measured at 515 nm by a spectrophotometer against a blank. The DPPH radical scavenging activity of extracts was expressed as mg of Trolox equivalents/per gram of sample (mg Trolox equivalent/g dry weight).

The improved ABTS assay, described by Thaipong et al. (2006), was used to determine the antioxidant activity of the extracts. 2,2'-azinobis (3-ethylbenzthiazolin-6-sulfonic acid) diammonium salt (ABTS) radical cation was prepared by

reacting 7 mmol ABTS stock solution with 2.45 mmol potassium persulfate. ABTS inhibition against Trolox was measured spectrophotometrically. The absorbance was measured at 734 nm by spectrophotometer. Trolox equivalent antioxidant capacity (TEAC) values of samples were calculated from the Trolox standard curve and expressed as Trolox equivalents (mg Trolox equivalent /g dry weight).

### Statistical Analyses

Statistical analyses were performed by using SPSS 20.0 software and all values were presented as mean  $\pm$  standard deviation. To determine the statistical significance between samples, a one-way analysis of variance (ANOVA) was applied then, multiple comparisons was carried out by Scheffe's multiple comparison test. Pearson's bivariate correlation test was also carried out to calculate correlation coefficients ( $r$ ) among antioxidant activity, reducing power and total phenolic content.

## Results and Discussion

### Antimicrobial Activities of Extracts

In the current study, minimal inhibitory concentration (MIC) of the flowers and leaves of 12 plants extracts were established for a lot of microorganisms and the results were shown in Table 2. This analysis was also applied to determine whether plant extract samples have antimicrobial activity or not. As clearly seen in Table 2, MIC values of the flower and leaf extracts varied in the range of 4 to 1024 µg/mL and 16 to 1024 µg/mL, respectively. Furthermore, the flower extracts exhibited higher antimicrobial activity than the leaf extracts. Being active at dilution level of 100 µg/mL, plant extracts could be considered to have a promising antimicrobial activity. Moreover, MIC values lower than 30µg/mL has higher antimicrobial activity compared to those usual antibiotics (Dall'Agnol et al. 2003). The results of extract samples were individually evaluated according to this information. Firstly, when considering in terms of flower extracts, it is observed that extract samples of *H. montbretii* (A1, A2 and A3) had the most effective antimicrobial activity against *B. pumilus*, *B. licheniformis* and *Ps. aeruginosa* (MIC values 16 to 32 µg/mL). Antimicrobial activity of extracts (A1, A2 and A3) against *B. cereus* and *B. subtilis* (MIC values 16 to 64 µg/mL) followed this assessment. Antimicrobial activity values shown against *S. aureus* and *Listeria* ssp. were less than the others. Also, the lowest antimicrobial activity of *H. montbretii* samples was shown against *E. coli*. Besides, it was determined that the activity of A3-sample collected from higher locations was lower than the other samples. If the flower extract samples of *H. orientale* were examined, it was seen that they had a similar antimicrobial effect to the flower extract samples of *H. montbretii* except for B3-sample.

Where the antimicrobial activity against *Ps. aeruginosa* and *Bacillus* species was found as high (MIC values 16 to 32 µg/mL), but for *E. coli* it was insufficient. The flower extract of the B3-sample showed a low antimicrobial activity (1024 or >1024 µg/mL) against all tested microorganisms. The flower extracts of *H. perforatum* samples showed higher antimicrobial activity



than the other tested *Hypericum* species. Especially, *Ps. aeruginosa*, *Bacillus* and *Listeria* species were found as significantly sensitive (MIC values, 4 to 64 µg/mL) to the flower extract samples of this specie. However, like the other *Hypericum* species, the antimicrobial effect of the *H. perforatum* flower extract samples against *E. coli* was insufficient. It was determined that there was no significant

relationship between antimicrobial activity of these species and location altitudes. Additionally, it was emphasized that the other microorganisms except for *S. aureus* and *E. coli* showed a quite high susceptibility against the flower extract of C5-sample (MIC values, 4 to 8 µg/mL) and C2-sample (MIC values, 8 to 16 µg/mL), respectively.

**Table 2.** Minimal inhibitory concentrations (MIC; µg/mL) of ethanolic *Hypericum* extracts

Species	Plant Tissue	Location Code	Indicator Microorganisms Code*								
			1	2	3	4	5	6	7	8	9
<i>H. montbretii</i>	Flower	A1	16	16	16	16	32	16	64	32	1024
		A2	16	16	16	32	32	16	16	16	1024
		A3	32	64	32	32	128	32	128	128	1024
	Leaf	A1	32	64	32	32	256	32	256	64	1024
		A2	128	128	128	128	512	128	128	128	1024
		A3	1024	1024	512	1024	1024	256	512	>1024	>1024
<i>H. orientale</i>	Flower	B1	16	16	16	16	32	16	16	16	1024
		B2	32	32	32	32	64	32	128	128	1024
		B3	1024	1024	>1024	>1024	>1024	1024	>1024	>1024	>1024
		B4	16	16	32	16	256	32	256	256	1024
	Leaf	B1	16	16	16	16	64	16	32	32	1024
		B2	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
		B3	>1024	1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
		B4	128	128	128	128	512	128	256	256	1024
<i>H. perforatum</i>	Flower	C1	16	16	16	16	64	16	16	16	1024
		C2	16	8	8	8	64	8	16	16	1024
		C3	16	16	16	16	64	16	32	32	1024
		C4	64	64	64	64	64	32	64	64	1024
		C5	4	8	4	4	16	8	8	4	1024
	Leaf	C1	128	32	32	128	256	32	128	128	1024
		C2	16	16	16	16	256	16	16	16	1024
		C3	256	256	256	256	512	256	512	512	1024
		C4	512	512	256	256	256	64	64	256	1024
		C5	128	128	64	512	256	128	256	256	1024

\*1: *B. pumilus*, 2: *B. subtilis*, 3: *B. licheniformis*, 4: *B. cereus*, 5: *S. aureus*, 6: *Ps. aeruginosa*, 7: *L. innocua*, 8: *L. monocytogenes*, 9: *E. coli*

When viewing all the *Hypericum* species tested, generally, it was observed that flower extracts showed higher antimicrobial activity than the leaf extracts. On the other hand, both flower and leaf extract samples showed an insufficient antimicrobial activity against *E. coli*. Except for *E. coli*, *L. innocua* and *S. aureus*, a significant difference was not determined among the antimicrobial activities of A1-leaf extract against other microorganisms. Besides, the antimicrobial activity of A1-leaf extract was found as greater than the other samples of *H. montbretii*. For *H. montbretii*, it was seen that there was an inverse relationship between location altitudes and antimicrobial activity. Likewise, the leaf extracts of *H. orientale* and *H. perforatum* showed a significant difference against the microorganisms except *E. coli* and *S. aureus*. However, more effective antimicrobial activity was determined against *Bacillus* species and *Ps. aeruginosa* by the leaf extracts of *H. orientale* and *H. perforatum* species, respectively. It was determined that their leaf extracts had less effective than the flower extracts.

According to the results, it was observed that the antimicrobial activity of both flower and leaf extract samples of *H. montbretii* and *H. perforatum* were higher than *H.*

*orientale* species. *H. perforatum* species showed highest antimicrobial activity among the species. Especially, effect of the C5 and C2-flower extract samples were highlighted by MIC values ranging from 4 to 8 µg/mL and from 8 to 16 µg/mL, respectively, except for *E. coli* and *S. aureus*. Besides, it was determined that the leaf extract of C2-sample had a significant activity with a MIC value of 16 µg/mL, and it was determined that the flower extract of A1, A2, B1, C1 and C3-samples had MIC value ≤ 30 µg/mL for most of the tested indicator microorganisms.

Previous reports showed that some *Hypericum* species growing in various regions of the world have remarkably broad spectrum of antimicrobial activities (Rabanal et al. 2002; Dulger et al. 2005). Rabanal et al. (2002) investigated antimicrobial activities on these species of *Hypericum* from the Canary Islands and MIC values were found between 30 and 290 µg/mL. In this study, the most significant activity was observed on the chloroform fraction of *H. canariense*, showing the lower MIC values against *Micrococcus luteus*, *S. aureus* and *S. epidermidis* (with the same MIC value of 30 µg/mL), followed by *Bordetella bronchiseptica* (70 µg/mL) and *B. cereus* (the least affected with a MIC of 290 µg/mL). In a study

by Reichling et al. (2001), hydrous solutions of *H. perforatum* teas were found to be effective against gram-positive bacteria, especially toward methicillin-resistant strains of *S. aureus* (MIC values, 1300 to 2500 µg/mL). Unal et al. (2008) studied on antimicrobial activities of some plants used as remedies in Turkish Medicine. They were stated that the chloroform, acetone, ethanol and water extracts of three *Hypericum* species (*H. heterophyllum*, *H. hyssopifolium* ssp. *Elogatum* var. *elongatum* and *H. scabrum*) showed antimicrobial activity against 10 pathogenic bacteria with MIC values ranging between 62.5 and 250 µg/mL. Milosevic et al. (2007) reported that *Ps. glycinea* and *Azotobacter chroococcum* showed extreme sensitivity to the extract of *H. perforatum*, while no effect was observed on *Klebsiella pneumoniae*.

In general, antimicrobial activity of ethanolic *Hypericum* species extracts varied significantly depending on the altitudinal gradient. In both leaf and flower extracts, antimicrobial activity of *H. perforatum* increased with altitude. Contrarily, *H. montbretii* and *H. orientale* exhibited lower activity with altitude. It was considered that the reason of this result was the effect of the relationship between the amounts of the compounds which exhibit antimicrobial properties in plants and the altitude. The naphthodianthrones, flavonoids, xanthonenes, tannins, essential oils, phloroglucinols and chlorogenic acid are the major compounds studied of *Hypericum* species as phenolic acid so far and antimicrobial activity has been found to be closely related to them (Radulovic et al. 2007; Çirak et al. 2012). Moreover, some compounds like flavonoids, phenolic acids, proanthocyanidins are overproduced depending on the altitude due to the degree of environmental stress factors like UV-B radiation and temperature etc. (Zidorn et al. 2005; Rieger et al. 2008; Xenophontos et al. 2008; Camas et al. 2014). Thus, we expected increasing of the antimicrobial activity of *Hypericum*

extracts in proportion to the altitude of plant growth locations. But, the findings for *H. montbretii* and *H. orientale* did not correlate with the mentioned assume. However, Martz et al. (2010), one of the study not related to the plant species, reported that the compounds like chlorogenic acid derivatives were produced with lower contents depending on the altitude. Therefore a clear relationship could not be established between the antimicrobial activities of the samples and altitude of the plant growth locations.

### Total Phenolic Content of Extracts

The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties. Plant phenols constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers (Bernardi et al. 2008). In this study, it was determined that there is large variation, ranging from 75.22 to 140.72 mg GAE/g dry weight for flower extracts and from 94.89 to 212.49 mg GAE/g dry weight for leaf extract, in total phenolic content of the plant species (Table 3). For flower extracts, while B1-sample showed highest total phenolic content with value of 140.72 GAE/g dry weight (P<0.05), leaf extracts C4-sample showed the highest total phenolic content with value of 212.49 mg GAE/g dry weight (P<0.05). These results are similar to the amounts (104 to 451 mg GAE/g dry weight) of the total phenolic compound of *H. montbrettii*, *H. origanifolium* and *H. perforatum* species determined by Öztürk et al. (2009) and, likewise, the amounts (125 to 257 mg GAE/g dry weight) of the total phenolic compound in the aerial parts of *H. perforatum* L. determined by Gioti et al (2009). According to literature, total phenolic content of the plant species having GAE >20 mg/g dry weight are remarkably high (Türkan and Demiral 2009). Therefore, it was concluded that the plant species in this study showed as a rich total phenolic source.

**Table 3.** Bioactive properties of ethanolic *Hypericum* extracts

Species	Location Code	Total Phenolic Content*		ABTS Assay**		DPPH Assay**	
		Flower	Leaf	Flower	Leaf	Flower	Leaf
<i>H. montbretii</i>	A1	122.02±1.50 <sup>d</sup>	94.89±1.01 <sup>h</sup>	304.58±1.03 <sup>b</sup>	291.45±0.52 <sup>h</sup>	21.22±0.77 <sup>cd</sup>	19.92±0.54 <sup>gh</sup>
	A2	118.40±1.11 <sup>e</sup>	158.21±1.25 <sup>de</sup>	304.36±0.79 <sup>e</sup>	416.43±0.42 <sup>d</sup>	17.65±1.37 <sup>def</sup>	27.99±0.44 <sup>cd</sup>
	A3	100.37±1.09 <sup>g</sup>	166.56±0.76 <sup>cde</sup>	310.18±0.88 <sup>d</sup>	377.78±0.42 <sup>f</sup>	18.96±0.43 <sup>de</sup>	26.58±0.54 <sup>f</sup>
<i>H. orientale</i>	B1	140.72±0.44 <sup>a</sup>	141.01±0.62 <sup>f</sup>	324.10±0.85 <sup>a</sup>	337.41±0.42 <sup>g</sup>	27.64±1.07 <sup>a</sup>	32.10±0.52 <sup>abc</sup>
	B2	113.43±0.70 <sup>e</sup>	151.54±0.48 <sup>de</sup>	344.99±1.63 <sup>c</sup>	480.02±0.30 <sup>a</sup>	23.44±0.25 <sup>cd</sup>	35.64±0.62 <sup>a</sup>
	B3	80.66±1.13 <sup>h</sup>	140.03±0.52 <sup>f</sup>	279.11±0.96 <sup>f</sup>	403.29±0.48 <sup>e</sup>	19.76±0.78 <sup>de</sup>	26.99±1.88 <sup>de</sup>
	B4	126.61±1.40 <sup>bc</sup>	149.45±2.00 <sup>e</sup>	371.46±0.88 <sup>b</sup>	430.44±0.44 <sup>c</sup>	24.97±1.79 <sup>ab</sup>	27.92±0.57 <sup>d</sup>
<i>H. perforatum</i>	C1	75.22±0.69 <sup>hi</sup>	128.72±0.96 <sup>g</sup>	254.14±1.13 <sup>g</sup>	387.69±0.62 <sup>f</sup>	16.27±1.73 <sup>def</sup>	30.01±0.37 <sup>bc</sup>
	C2	100.63±1.83 <sup>g</sup>	93.21±1.89 <sup>h</sup>	285.49±0.55 <sup>f</sup>	338.46±0.27 <sup>i</sup>	12.61±2.94 <sup>g</sup>	15.07±0.82 <sup>j</sup>
	C3	104.79±1.35 <sup>g</sup>	163.24±0.88 <sup>cde</sup>	334.37±1.89 <sup>c</sup>	356.81±0.94 <sup>j</sup>	19.24±1.76 <sup>de</sup>	21.66±0.89 <sup>g</sup>
	C4	121.43±0.39 <sup>d</sup>	312.49±0.47 <sup>a</sup>	411.86±0.45 <sup>a</sup>	457.28±0.23 <sup>b</sup>	24.36±0.99 <sup>bcd</sup>	24.67±0.74 <sup>ef</sup>
	C5	123.42±0.77 <sup>cd</sup>	172.31±1.34 <sup>b</sup>	353.86±1.38 <sup>bc</sup>	255.07±0.74 <sup>j</sup>	28.98±1.61 <sup>a</sup>	22.97±1.24 <sup>hi</sup>

\*: mg gallic acid equivalent /g dry weight, \*\*: mg trolox equivalent /g dry weight, \*\*\*: All values are presented as mean ± S.D and different letters within columns for each sample differ significantly at the level of P < 0.05.

The altitude of the *Hypericum* growth locations significantly affected the total phenolic content of the ethanolic extract samples (P<0.05). Total phenolic contents of flower extracts of *H. montbretii* and *H. orientale* were decreased with increasing altitude; however a positive correlation was determined between the altitude and total

phenolic contents of *H. perforatum*. For leaf extracts of *Hypericum* species except B3 and C5-samples, the higher total phenolic contents accumulation was observed in the higher growing sites. Recent papers reported that several environmental stresses like solar radiation and temperature had proven effect on plant metabolites and generally

increasing solar radiation and reducing temperature at higher altitude resulted in higher phenolic contents of plant (Rieger et al. 2008; Türkan and Demiral 2009; Camas et al. 2014).

### Antioxidant Activities of Extracts

Antioxidant activities of the *Hypericum* species, were evaluated by using two methods based on the free radical scavenging capacity (namely the DPPH radical scavenging assay) and the ABTS radical cation decolorization assay, were shown in Table 3. It can be concluded that the plant extracts free radical scavenging activities towards the ABTS assay were quite high antioxidant activity with the values in the range of 255.07-480.02 mg trolox equivalent /g dry weight and free radical scavenging activities towards the DPPH assay were found between 12.61 and 35.64 mg trolox equivalent /g dry weight. Leaf extracts have higher antioxidant activity than the flower extracts. Generally, leaves of the plants carry higher antioxidant activity with regards to the phenolic compounds compared to flowers (Güzey et al. 2011) and there are many studies which reported a positive correlation between total phenolic content and antioxidant activity (Tawaha et al. 2007; Şerbetçi et al. 2012). In spite of that, no correlation was found between the antioxidant activity and phenolic content of leaf extracts. But, especially, the free radical scavenging activities of the flower extract samples towards the ABTS assay are in a very good correlation with the phenolic contents ( $r = 0.8874$ ). As similar to this result, a moderate correlation with together Pearson's correlation coefficient ( $r = 0.5718$ ) were determined between free radical scavenging activities towards the DPPH assay of the flower extracts and the phenolic compound values of the same samples. It is considered to be reasons for the difference between the basic principles of the antioxidant activity methods to the difference between correlation forces. Besides, it is known that although the ABTS assay is convenient to determine the antioxidant activity of both hydrophilic and lipophilic compounds (Somogyi et al. 2007), the DPPH assay is convenient to determine that of hydrophilic compounds, since DPPH radical react slowly with peroxy radicals (Magalhaes et al. 2008). This situation show that chemical structure of the plant extracts is different from each other. In a previous study, determining the antioxidant activity of *Hypericum hircinum* ssp. *Majus* essential oil according to the ABTS and the DPPH assay, it was observed that the oil possessed a more remarkable antioxidant activity in the ABTS assay (90.30 mg trolox equivalent /g dry weight), about 2-fold higher than the activity (47.80 mg trolox equivalent /g dry weight) shown in the DPPH assay (Qassinti et al. 2013). The results of this study are in accordance with our findings.

Antioxidant activity of leaf and flower extracts of the *Hypericum* species studied significantly varied depending on altitudinal variations but there is no correlation with the altitude of the habitat. Marrelli et al. (2014) found that DPPH assay of *H. perforatum* extract no. 1 collected from 370 m altitude showed the best radical scavenging activity but, sample no. 3 collected from 1320 m altitude showed the lowest activity due to containing a minor amount of phenolic. Similarly, Rieger et al. (2008) reported that the plants from higher altitudes cannot contain higher amounts of radical

scavenging compounds as a result of their exposure to more climatic conditions.

### Conclusion

Total phenolic contents in flowers and leaf extracts of plants increased considerably with the growth altitude and extracts exhibited the best antimicrobial activity together with rising altitude. However, though there is a correlation between the total phenolic contents and antioxidant activity of plant extracts, antioxidant activity is not a positive influence of increasing altitude. *Hypericum* species are used as medicinal plants and today there is growing interest in these plants which are rich in secondary compounds. So that, further studies are necessary to determine the effect of altitudinal changes on secondary compounds of *Hypericum* species and investigate the relationship with antimicrobial/antifungal activity. Additionally, the usage of these plant species as food product, such as tea or food additive, like food coloring, antioxidant etc. should be expanded.

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## RESEARCH ARTICLE

### Effects of Seed Drop Height and Tillage System on the Emergence Time and Rate in the Single Seed Planters

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#### ABSTRACT

This study was conducted to determine the effects of seed drop height and tractor forward speed in different tillage systems on the mean emergence time, emergence rate index, and percentage emergence in single seed planters. The experiments were carried out in the conventional and reduced tillage systems, at seed drop heights of 120, 180, and 240 mm and tractor forward speeds of 1.1, 1.5, and 2.2 m s<sup>-1</sup>. Sunflower and maize seeds were used in the study. The results of the study showed that seed drop height significantly affected the mean emergence time, emergence rate index, and percent emergence in both experiments (P<0.05). Also, the tillage system significantly affected the mean emergence time and emergence rate index. The lowest mean emergence time and the highest emergence rate index were 10 d and 0.19 seedlings d<sup>-1</sup> m<sup>-1</sup> in the reduced tillage system for sunflower, respectively. The same parameters were 12 d and 0.37 seedlings d<sup>-1</sup> m<sup>-1</sup> in the reduced tillage system for maize, respectively. As a result, the seeds of sunflower and maize should be sowed in reduced tillage system, 180 mm seed drop height, and 1.1 - 1.5 m s<sup>-1</sup> tractor forward speeds for rapid germination and high percentage emergence.

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#### Introduction

Single seed planters have been developed for seeds such as sunflower, maize, cotton, and sugar beet. Sowing uniformity of these planters is an important factor that influences the germination rate (percent emergence), development of the plant, and consequently the yield. Staggenborg et al. (2004) reported that the most common characteristics used by producers to evaluate sowing performance are plant spacing and field emergence rate. In sowing, there is a significant relationship between germination rate and vertical seed distribution uniformity (Önal, 2011). Therefore, an important benchmark that can be used to control the precision of the planter is whether if the seeds are sown in the correct sowing depth. The uniform germination rate can be achieved when

using a consistent sowing depth (Chen et al., 2004). Unevenness in vertical seed distribution (sowing depth) leads to reductions in the percentage emergences. Sunderman (1964) reported that the mean emergence rate dropped from 74% to 23% as sowing depth changed from 7.6 to 12.7 cm in wheat sowing. Moreover, Krall et al. (1979) found that, while the maize sowing depth increased from 2.5 cm to 7.5 cm, the mean emergence time increased about 3 days and the final yield decreased.

In single seed planters, the part which allows seed-soil contact is furrow openers. Furrow openers can vary depending on the type of seed to be sowed. During sowing, the seeds are under the influence of many factors until placed in a row. These factors may directly affect both sowing quality and yield. The design of furrow openers is one of these factors.

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Karayel and Özmerzi (2008) reported that the design of the furrow opener is also a factor affecting the seed distribution in the soil. Even in a planter with the most current equipment and high-quality metering device, sowing may fail due to the fact that the furrow openers do not function properly (Önal, 2011). Therefore, the furrow opener is one of the most important parts of a planter. Furrow openers on planters have an important effect on seed distribution, especially in the vertical plane. Many studies have been conducted on furrow openers (Hayden and Bowers 1974; Morrison et al. 1988; Gebresenbet and Jönsson 1992; Chaudhuri 2001; Raoufat and Matbooei, 2007; Karayel ve Özmerzi 2008). In general, these studies were about opener types (Choudhary et al., 1985; Raoufat and Mahmoodieh, 2005; Karayel and Özmerzi, 2005), and opener design (Tessier et al., 1991; Karayel and Özmerzi, 2003).

In order to obtain a furrow profile that is suitable for different seed varieties, different types of furrow openers have been developed. One of them is the commonly used shoe type furrow opener. In prior market research, it was determined that generally, three types (small, standard or medium, and large) of shoe furrow openers used at the single seed planters. The height of these furrow openers generally ranges from 12 to 24 cm. However, heights of standard furrow openers may differ according to the manufacturing company. This difference also causes significant differences in the seed distribution pattern as it changes the drop height of seed. In addition, there are few studies investigating the effect of seed drop height can vary depending on the size of the furrow opener. Wanjura and Hudspeth (1969) used a vacuum wheel designed to perform the single seed sowing of the cottonseed in the stick tape experiment. The researchers reported that the seed spacing uniformity was disrupted by increase of seed drop height. Parish and Bracy (2003) modified vacuum planter by adding a slide or an enclosed tube to the metering unit to

reduce the effect of seed drop height on the seed distribution uniformity. The seed tube or slide was about 15 cm long. As a result of the study, they determined that although the seed drop height disrupted the seed spacing, the slide and tube did not have a positive contribution.

Commercial single seed planters usually consist of shoe type furrow openers which are designed according to physical properties such as dimension and size of seeds. The nature and behavior of furrow openers with different heights are likely to produce depth fluctuations resulting in variations in plant emergence time, plant growth, percent emergence, and yield. The specific objective of this research was to evaluate the field performance of single seed planter with three different heights of furrow openers for emergence time and percent emergence of plants in different tractor forward speeds and tillage systems.

### Materials and Methods

The study was carried out on a research field at the Erzurum province of Turkey in the growth seasons of 2011 and 2012. The experiments were conducted in 2011 for sunflower and 2012 for maize. The sunflower experiment was carried out in a field of 18 decares with a width of 100 m and a length of 180 m, and the maize experiment in a field of 13.2 decares with a width of 55 m and a length of 240 m. The soil properties related to the experiment fields are given in Table 1. The average temperature at the trial site is around 20 ° C in the warmest month. The average annual rainfall is below 500 mm and the majority of rainfall takes place in April, May, and June. After sowing, no irrigation was done during the period of measurements, so that they were not affected by soil moisture, seed germination, and percentage of emergence. In addition, there was no rainfall in the period between the start of the germination process and the completion of the experiments.

**Table 1.** Some of the important properties for experiment fields and the seeds

The soil property	Some soil properties of experiment fields				Physical properties of the seeds		
	Sunflower planting field		Maize planting field		Seed property	Sunflower	Maize
	CT*	RT	CT	RT			
Bulk density, g cm <sup>-3</sup>	1.31	1.18	1.16	1.14	Bulk density, g cm <sup>-3</sup>	0.26	0.91
MCAS, %	37.20	37.46	22.81	26.12	TGW, g 1000 <sup>-1</sup> grain	140	326
MCAE, %	26.48	26.32	14.82	14.78	Repose angle, °	30	22
Penetration resistance, MPa*	1.16	0.97	0.85	0.43	Length, mm	19.52	10.02
MWD, mm	33.05	23.95	23.81	9.96	Width, mm	8.77	6.95
pH, %	7.26	7.26	7.62	7.62	Thickness, mm	4.64	5.97
Organic matter, %	0.73	0.73	1.01	1.01	Sphericity, %	47	74
Sand, %	39.23	39.23	39.11	39.11	Geometric mean diameter	9.26	7.46
Clay, %	35.36	35.36	37.80	37.80	Variety	Confeta	Bora
Silt, %	25.41	25.41	23.09	23.09	TGW: thousand grain weight,		
Texture class	Loamy	Loamy	Loamy	Loamy			

CT: conventional tillage, RT: reduced tillage, MCAE: moisture content after emergence, MCAS: moisture content after sowing, MWD: mean weight diameter, \*: for depth 10 cm

Sunflower and maize seeds were used for the experiments. The physical properties of these seeds are displayed in Table 1. Sowing rates for sunflower and maize were 35 714 and 69 348 seeds/ha, respectively. Depending on the physical

properties of the seeds, the recommended practical spacing between plants within a row ranges between 200-500 mm for sunflower and 100-300 mm for maize. In accordance with the values used in practice, the seed metering unit was adjusted

to the target seed spacing of 400 mm for sunflower and 206 mm for maize. The spacing between the rows was 700 mm. Both types of seeds were sowed at 60 mm nominal sowing depth by means of single seed planter with air suction and four rows. The planter was calibrated in the laboratory before field operation.

The seed spacing adjustment of the planter can be done by changing the number of seed plate holes or the transmission ratio. The air suction required for hold of seeds to the vacuum plate was provided by the fan unit of the planter. The fan was driven by tractor PTO (Power Take Off). The negative air pressure generated by the fan was 7.5 kPa for sunflower and 8.8 kPa for maize (Önal 2011). The hole diameters of the metering unit plate were selected as 3 mm for sunflower and 5 mm for maize.

In the experiments, each field was divided into 54 plots. The plots were 40 m in length and 3 m in width. 27 plots treated by conventional tillage and the remaining 27 plots were treated by reduced tillage. The experimental setup was a randomized factorial design with three repetitions. The main treatments of the study included conventional and reduced tillage systems. Sub treatments were sowing speeds and seed drop heights. In each repetition, four rows were sowed with a single pass of the planter. All plots were sowed inter-row of 700 mm. To measure the impact of parameters, the sub-plots were established for both of the experiments. These sub-plots were established in the center four rows of each sub-treatment (Heege 1993; Staggenborg et al. 2004). The lengths of sub-plots were 25 m for sunflower and 15 m for maize. Seedling

counts were performed on three rows randomly selected from each sub-treatment. As a result of these counts, mean emergence time (MET), emergence rate index (ERI), and percent emergence (PE) were calculated to determine factors indicative of sowing success.

In the conventional tillage system, the soil was tilled by moldboard plow, disc harrow twice, and using a roller twice. The soil was tilled by a power harrow, which was followed by a roller in reduced tillage. Tillage depth was set at 250 mm for conventional tillage, 120 mm for reduced tillage (Peterson et al. 1983; Raoufat and Mahmoodieh 2005; Stipesevic et al. 2009; Topakci et al. 2011). The experiment fields have not been processed within the previous year.

The nominal sowing speeds were selected as 1.0, 1.5, and 2.0 m s<sup>-1</sup>. The closest values to these speeds determined as 1.1, 1.5, and 2.2 m s<sup>-1</sup> in the field conditions depending on tractor gear stages.

The seed drop heights obtained by designing shoe furrow openers of different sizes. The seed drop height values determined by taking into consideration the smallest and largest dimensions of the shoe type furrow openers in market research conducted in Turkey. In this way, three different shoe furrow opener sizes, small, medium (standard), and large were obtained. The small, medium and large shoe furrow opener heights were 120 mm, 180 mm and 240 mm, respectively (Figure 1). In the manufacturing of shoe furrow openers, 60 mm high cast material on the bottom side of the furrow opener and 8 mm thick sheet material on the upper side were used.

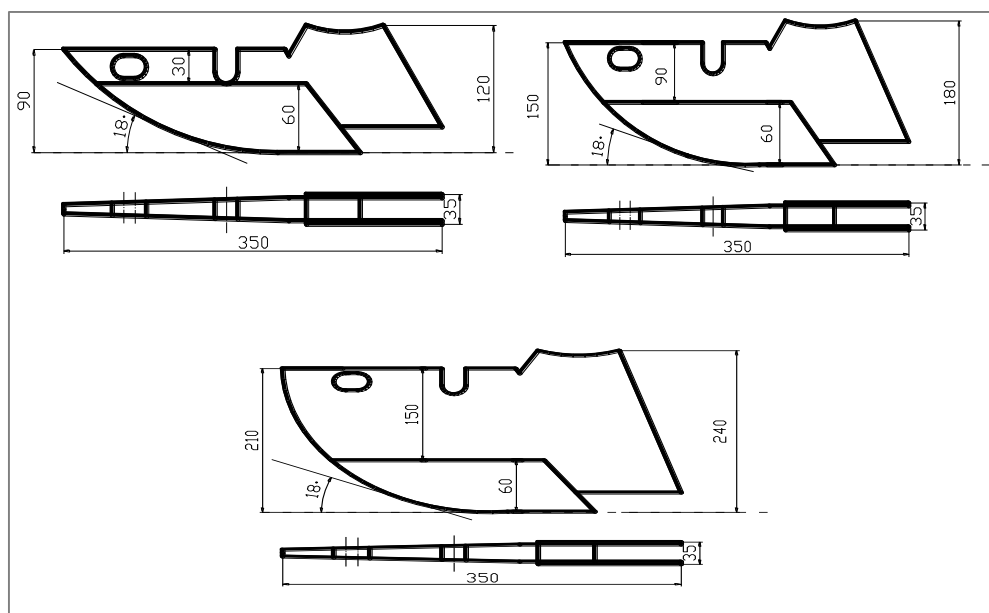


Figure 1. The shoe furrow openers

The plants were counted every day and 16 days throughout, after the first plant emergence (Bilbro and Wanjura, 1982). In order to ensure that the plant emergences are fixed, the stand counts were taken at least four times until emergence was deemed complete (Staggenborg et al. 2004). Mean emergence time (MET), emergence rate index (ERI), and percent

emergence (PE) were calculated related to the number of plants on the 16<sup>th</sup> day. MET, ERI, and PE were determined using the following equations (Bilbro and Wanjura, 1982; Altıkat and Çelik, 2011).

$$MET = \frac{N_1 T_1 + N_2 T_2 + N_3 T_3 + \dots + N_n T_n}{N_1 + N_2 + N_3 + \dots + N_n} \quad (1)$$

$$ERI = \frac{S_{te}}{MET} \quad (2)$$

$$PE = \left( \frac{S_{te}}{n} \right) * 100 \quad (3)$$

where  $N_{1, \dots, n}$  is the number of emerged seedlings since the time of the previous count;  $T_{1, \dots, n}$  is the number of days after sowing;  $S_{te}$  is the total number of emerged seedlings per meter;  $n$  is the number of seeds sown per meter;  $MET$  is the mean emergence time (day),  $ERI$  is the emergence rate index (seedling day<sup>-1</sup> m<sup>-1</sup>) and  $PE$  is the percentage of emergence (%).

SPSS package program was used for statistical analysis of the data. The data were evaluated by analysis of variance (ANOVA) to determine the effect of the parameters on  $MET$ ,  $ERI$ , and  $PE$ . In addition, the multiple comparison (Post-Hoc) test was used to determine significant differences and similarities between groups in the experiment at 0.01 and 0.05 significance levels. The results of the analyses were evaluated separately for each seed variety.

### Results and Discussion

Sowing success of sunflower and maize sowed by the single seed planter was analyzed first.  $MET$ ,  $ERI$ , and  $PE$  were combined for analysis of variance to determine significant differences in the variability among the parameters. The results of the analysis show that there were no significant differences in  $PE$  between two tillage systems, while the effect of tillage system on  $MET$  and  $ERI$  was statistically significant ( $P < 0.05$ ) for sunflower and maize (Tables 2 and 3). Depending

on the tillage systems, the highest  $PE$  was 87.52% in the reduced tillage system for maize, while the lowest  $PE$  occurred as 76.09% in the conventional tillage system for sunflower (Figures 2 and 3). The average sunflower plant population for  $CT$  and  $RT$  were 2.70 and 2.80 plants m<sup>-2</sup> respectively, which are 21.5 and 28.0% lower than the nominal sowing rate (3.57 plants m<sup>-2</sup>). The number of plants that emerged in the unit area in the maize experiment was 5.90 and 6.00 plant m<sup>-2</sup> respectively, for conventional and reduced tillage. These values were 14.86% and 13.41% lower than the nominal sowing rate (6.93 plants m<sup>-2</sup>). In general,  $PE$  values were higher in the maize experiment, although the moisture values measured in furrow after sowing were higher in the sunflower experiment. However, penetration resistance values measured in the sunflower experiment field were higher than those of the maize experiment field (Table 1). Therefore, higher values of  $PE$  obtained in the maize experiment are thought to a consequence of a lower penetration resistance measured in the furrow.

Tillage system significantly affected both  $MET$  and  $ERI$ .  $MET$  was higher in the conventional tillage system for both experiments (Figures 2 and 3). In connection to  $MET$ ,  $ERI$  was lower. Higher  $MET$  values in the conventional tillage system suggest that such higher values are due to higher penetration resistance. Bilbro and Wanjura (1982) reported that the penetration resistance of the soil was a critical factor affecting mean emergence time.

**Table 2.** The sunflower experiment analysis results

Parameters		MET, day	ERI, seedlings day <sup>-1</sup> m <sup>-1</sup>	PE, %	NSD*, mm	MSD, mm
Tillage system	CT	11.62 <sup>a</sup>	0.167 <sup>b</sup>	76.09 <sup>a</sup>	60	48.30
	RT	10.36 <sup>b</sup>	0.194 <sup>a</sup>	78.97 <sup>a</sup>	60	51.60
	<i>P</i>	0.033	0.000	0.186		
Seed drop height, mm	120	9.63 <sup>b</sup>	0.214 <sup>a</sup>	81.39 <sup>a</sup>	60	45.64
	180	11.12 <sup>a</sup>	0.177 <sup>b</sup>	81.60 <sup>a</sup>	60	51.25
	240	12.21 <sup>a</sup>	0.151 <sup>c</sup>	72.13 <sup>b</sup>	60	52.98
	<i>P</i>	0.003	0.000	0.003		
Tractor forward speed, m s <sup>-1</sup>	1.1	10.88 <sup>a</sup>	0.186 <sup>a</sup>	78.73 <sup>a</sup>	60	51.19
	1.5	10.86 <sup>a</sup>	0.186 <sup>a</sup>	76.64 <sup>a</sup>	60	50.36
	2.2	11.22 <sup>a</sup>	0.171 <sup>b</sup>	77.20 <sup>a</sup>	60	48.33
	<i>P</i>	0.846	0.046	0.714		

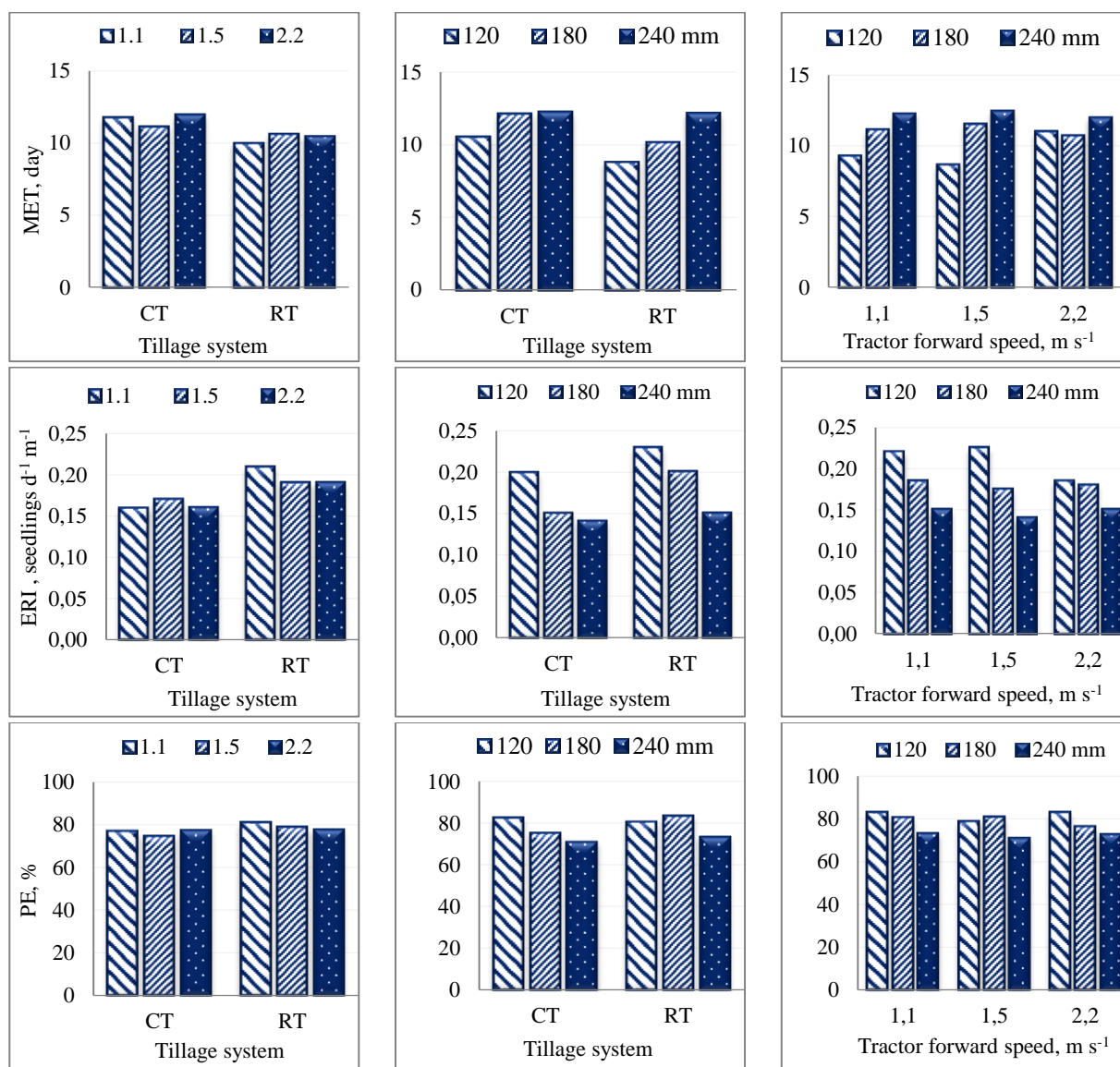
\*: NSD; nominal sowing depth, MSD; measured sowing depth

**Table 3.** The maize experiment analysis results

Parameters		MET, day	ERI, seedlings day <sup>-1</sup> m <sup>-1</sup>	PE, %	NSD*, mm	MSD, mm
Tillage system	CT	14.66 <sup>a</sup>	0.299 <sup>b</sup>	86.10 <sup>a</sup>	60	51.30
	RT	11.92 <sup>b</sup>	0.368 <sup>a</sup>	87.52 <sup>a</sup>	60	49.10
	<i>P</i>	0.004	0.002	0.374		
Seed drop height, mm	120	11.67 <sup>b</sup>	0.375 <sup>a</sup>	85.03 <sup>b</sup>	60	40.71
	180	13.87 <sup>ab</sup>	0.333 <sup>ab</sup>	90.92 <sup>a</sup>	60	53.10
	240	14.32 <sup>a</sup>	0.292 <sup>b</sup>	84.47 <sup>b</sup>	60	56.83
	<i>P</i>	0.046	0.011	0.003		
Tractor forward speed, m s <sup>-1</sup>	1.1	13.66 <sup>a</sup>	0.323 <sup>a</sup>	86.60 <sup>a</sup>	60	52.20
	1.5	13.27 <sup>a</sup>	0.336 <sup>a</sup>	87.37 <sup>a</sup>	60	51.26
	2.2	12.93 <sup>a</sup>	0.341 <sup>a</sup>	86.45 <sup>a</sup>	60	47.17
	<i>P</i>	0.804	0.771	0.878		

\*: NSD; nominal sowing depth, MSD; measured sowing depth



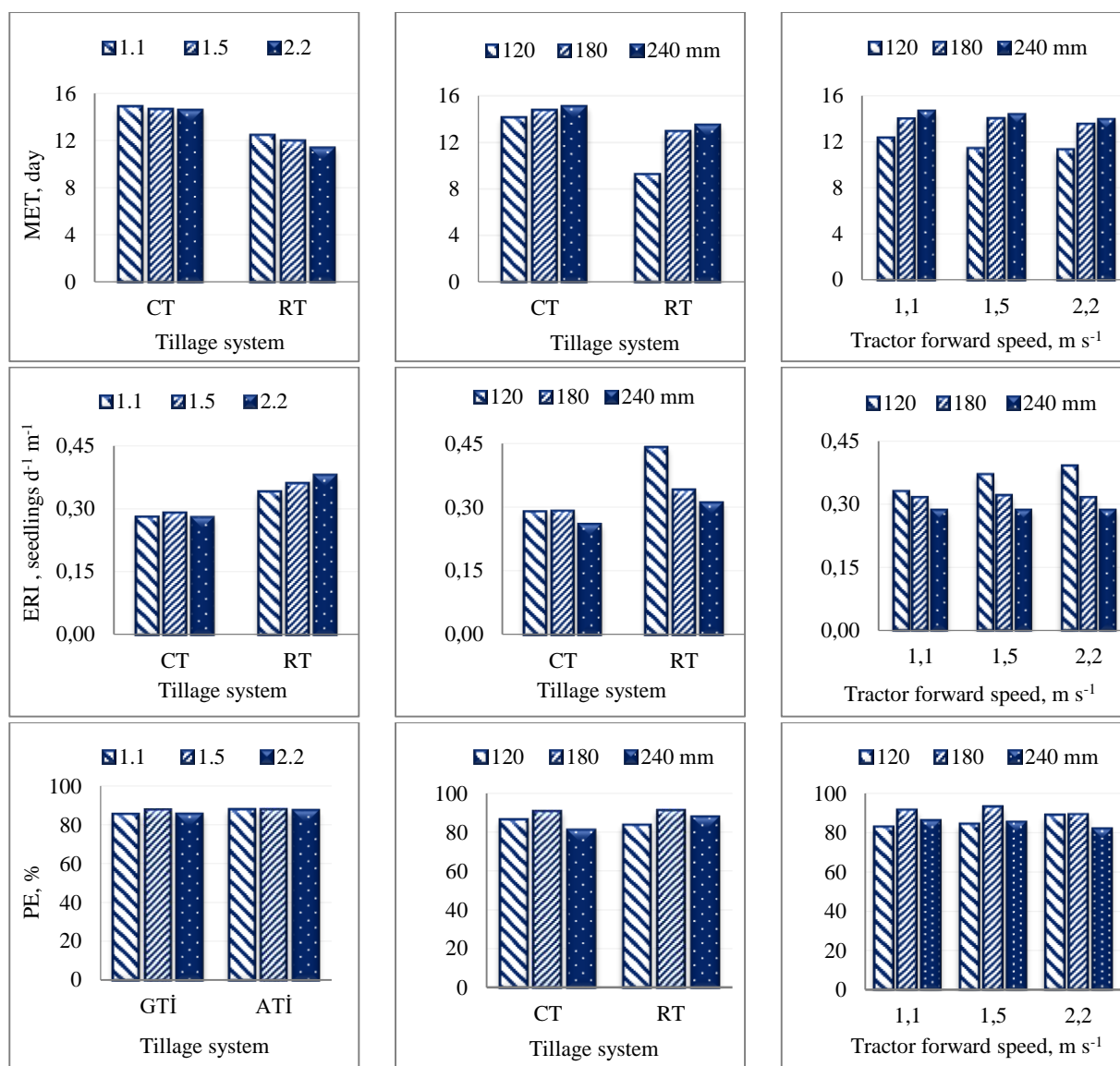


**Figure 2.** The change of MET, ERI, and PE in sunflower experiment

According to the results of the multiple comparison test, the effect of seed drop height on MET, ERI, and PE was generally significant. PE and ERI decreased and MET increased in the sunflower experiment as seed drop height increased (Table 2 and Figure 2). However, in the maize experiment, due to the increase in the seed drop height, MET increased while only ERI decreased (Table 3 and Figure 3). The largest MET values were obtained from a 240 mm seed drop height as 12.21 days for sunflower and 14.32 days for maize. On the other hand, the lowest MET values were obtained from 120 mm seed drop height as 9.63 and 11.67 days for sunflower and maize experiments, respectively. Depending on the seed drop height, the highest values of the percentage emergence were determined in seed drop height of 180 mm as 81.60% and 90.92% for sunflower and maize experiments, respectively. In both experiments, MET increased by about three days as a result of the increase of seed drop height from 120 mm to 240 mm. In addition, the measured sowing depth approached to

the target sowing depth 7 mm and 16 mm for sunflower and maize, respectively.

The results of statistical analysis showed that the effect of tractor forward speeds was insignificant on MET, ERI, and PE in both of the experiments. However, the effect on ERI of 1.5 and 2.2 m s<sup>-1</sup> forward speeds in sunflower experiment were statistically different from each other (Table 2). Depending on the forward speed, germinations in the sunflower experiment were completed in a shorter time than the maize experiment. While the shortest MET and the largest ERI were obtained at forward speed of 1.5 m s<sup>-1</sup> in the sunflower experiment, they were obtained at forward speed of 2.2 m s<sup>-1</sup> in the maize experiment. However, the highest percentage emergences in fields determined as 78.73% at 1.1 m s<sup>-1</sup> forward speed for sunflower experiment and 87.37% at forward speed of 1.5 m s<sup>-1</sup> for maize experiment (Tables 2 and 3).



**Figure 3.** The change of MET, ERI, and PE in maize experiment

Displacement of seeds from the intended position in furrow can occur by bouncing and rolling due to the velocity and time of fall of seeds and by soil movement during the sowing. Karayel and Özmerzi (2008) reported that soil bulk density and seed drop height of furrow openers were effective factors on soil movement and velocity of the fall of seeds. They reported that lower soil bulk density and higher seed drop height increased the displacement of seeds from the intended position. In the present study, the increase in seed drop height increased the displacement of seeds in furrow. This was understood from the different sowing depths obtained in experiments (Tables 2 and 3). The displacement is thought to occur as a result of bouncing at the seed drop height. This is more pronounced in conventional tillage. A relatively lower stubble and hard field surface were obtained in the conventional tillage system while a softer and stubbly a field surface was obtained in the reduced tillage system. Shoe furrow opener with the smallest seed drop height was insufficient in terms of the working performance. This furrow opener was affected more by field conditions. The planter

used in the operation has a single connection of the setting of the furrow opener with the presser wheel. In order to obtain the nominal sowing depth (60 mm), the press wheel was set to the highest level. Therefore, the smaller shoe furrow opener was penetrated deeper into the soil. With the effect of the clod and stubble in the field, blockages occurred at this furrow opener. This situation caused the seed to either rolling in furrow or remaining on the edge without falling to the nominal depth. Seeds that did not fall to the nominal depth caused disruption of seed distribution in sowing depth. Therefore, seeds falling to different depths were assumed to affect MET and PE.

Displacement of seeds from the intended position in furrow may also occur due to thousand grain weight. Thousand grain weight of corn seeds is about 2.5 times that of sunflower seeds. The heavy seeds were dropped better to the targeted position while the light seeds were sown shallower. It can stay on the edge without falling on the bottom of furrow the seeds that have lower thousand grain weight due to an increase of the seed drop height. In the maize experiment, therefore, it is

assumed that the drop to the base of the furrow was better due to the fact that higher thousand grain weight of the maize seeds are effective in increasing MET. The results of this study have supported the results of the study conducted by Krall et al. (1979) examined effects on MET of sowing depth in wheat.

### Conclusion

Tillage system affected the mean emergence time and emergence rate index. However, the percentage emergence was not affected by the tillage system. The highest percentages were obtained from the reduced tillage system. While MET, ERI, and PE affected by the seed drop height, they were not affected by tractor forward speed. In general, better results have been achieved in the reduced tillage system and lower seed drop height. In addition to the penetration resistance and bulk density of the soil, it will be useful to take into account the physical properties of the seeds to be sown, such as the sphericity and thousand grain weight. Moreover, it should not be neglected that the drop height of the seeds can negatively affect the uniformity of sowing depth. It can be concluded that the position of seed in the soil effects MET, ERI, and PE of plants. As a result of the deteriorated sowing depth, it should be noted that MET, ERI, and PE can be adversely affected.

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## RESEARCH ARTICLE

### Cost Analysis of Different Mixture Rates and Sowing Methods of Anatolian Clover (*Trifolium resupinatum* L.) and Italian Ryegrass (*Lolium multiflorum* Lam.)

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#### ABSTRACT

The study was carried out between 2015 and 2016 in accordance with the split plots in randomized blocks research design with 3 replications, which aimed to determine the optimal mixture rate for the production design. The results of the research were made into agricultural implementation and cost analysis was performed. Relative profit margin was used in determining the most favorable mixture rate that will be included in the production design. According to the application results, the production threshold was exceeded in all mixtures. The highest gross production value (95.90 USD da<sup>-1</sup>) and production cost (59.05 USD da<sup>-1</sup>) was obtained from the sole Anatolian Clover among the forage crop mixtures. Whereas; the lowest gross production value (71.32 USD da<sup>-1</sup>) and production cost (58.60 USD) was detected in the sole Italian ryegrass application. In addition to this, it was found that the highest relative profit margin (1.62) had been achieved in sole Anatolian clover. 50% Anatolian clover + 50% Italian ryegrass (K<sub>2</sub>) (1.40), 25% Anatolian clover + 75% Italian ryegrass (K<sub>3</sub>) (1.38) were followed to sole Anatolian clover, in terms of mixture rates. Perpendicular row sowing (E<sub>3</sub>) had the highest relative profit margin (1.45) in this study according to the sowing methods. As a consequently, perpendicular rows (E<sub>3</sub>) and sole Anatolian clover (T), 25A.C + 75 I.R (K<sub>3</sub>) were the most suitable sowing method and mixture ratios under Anatolian conditions, respectively.

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#### Introduction

The agricultural product cost results are utilized in formulating agricultural product price policies. Agricultural product costs are also commonly used by establishments in determining the usage levels of physical production inputs, planning of financing facilities and drawing up product budgets (Anonymous, 2001). The main purpose of economic analyses in agricultural establishments is to gain maximum benefit from production activities. Increasing yields in such establishments can be achieved through accurate and proper utilization of

new technologies in production activities. Increasing production and reducing production cost depend on the properties of the product to be grown as well as the technology to be used. In line with these principles, sustainability of agricultural production activities can be achieved by adherence to economic principles. And this, in turn, can be ensured by choosing the optimum production method to be used by the producer in the establishment. However, the economic condition of the establishment and many factors involved in the production design should be properly evaluated when deciding on the production design. These factors can be

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listed as climate conditions and soil structure of the region in which the establishment is located, as well as the geographical location, equipment availability and labor capacity of the establishment.

Producers have to consider financial profit of the product will generate when deciding on which products to be grown in the establishment. Minimizing the costs in agricultural production enables establishments to tolerate low prices in the market by creating a strong competition environment (Bayramoglu et al., 2005). Agricultural product costs need to be consistently analyzed for the evaluation of agricultural production policies, effective use of resources and monitoring of technical developments in the field of agriculture. Cost is defined as the rate of agricultural production costs to the produced yield. As with all other economic sectors, this cost rate also affects the business operation results in agricultural sectors (Karadas, 2016). Agricultural production factors cannot be used at optimum level due to the lack of capital and technical information in the agricultural establishments. This, in turn, adversely affects the product yield and farmer income (Gundogmus, 1998). It is often difficult to employ a standard method for the calculation of agricultural product costs in Turkey. Therefore, care should be taken in accurate calculation of production expenses and costs in the agricultural establishments. Because, costs vary considerably between countries and even establishment. In this respect, one can conclude that there is no single cost price in agriculture and each establishment (Kiral et al., 1999). Calculating agricultural product costs on regional and national basis is vital in respect to input utilization and the production planning. In order to achieve the objectives of cost and income studies, what is needed is to develop a sufficient, viable and up-to-date cost calculation model, which is developed by taking into consideration the current state of domestic agricultural production and the globalizing world economy and its nation-wide application (Ozkan and Yilmaz, 1999). Product costs in agricultural production keep rising with each passing year, thus a conducted cost study may lose its validity next year. Consequently, cost calculations should be conducted periodically to monitor the applicable technologies, evaluate the results of agricultural policy and keep track of the changes in resource utilization (Kızıloglu, 1995). Economic analysis can be used as a tool for making profitability comparisons for the production of different plants and prioritizing the production of the those that prove more profitable (Ziaei, 2015).

According to the data from Turkish Statistical Institute; the national forage crop production in Turkey is about 55.5 million tons. While grassland- and pasture-based production is about 11.7 million tons (Anonymous, 2019). Improving sowing conditions or increasing the number of cultivation areas alone may fall short in boosting forage crop production. Such measures also need to be backed by increasing the yield per unit area. In plant production, one of the solutions proposed for increasing yield is the adoption of the intercropping system for plant growth (Acar et al., 2006). Intercropping can also make it possible to meet the diverse nutrient needs of animals. Intercropping of poaceae and leguminous forage crops prove more advantageous compared to sole stand cropping. The

advantages of intercropping includes increased dry matter yield, higher protein content and forage quality and reduced fertilizer consumption need for plants. Providing necessary grazing conditions, high rate of forage production, prevention of soil erosion are the qualities that particularly distinguish different plant mixtures from others. Also, inclusion of legumes in intercropping enhances the palatability and digestibility of the forage. Providing economic benefits in regard to nutrient use of plants and soil moisture and preventing soil exhaustion are the distinct major benefits of intercropping. The greater part of the Turkish forage crop agriculture is provided by the production from alfalfa, silage corn, vetch, sainfoin farming. In addition to this production, high-quality, high-yield, and high-profit alternative forage crops should be adapted into forage crop farming. No studies on the mixture of Anatolian clover and Italian ryegrass have been conducted for different regions of Turkey. This study aims to increase the production of Anatolian clover and Italian ryegrass through the assessment of the cost analysis for different mixture rates and sowing methods of these crops under Ankara conditions. This research will also enable the forage crops producers to compare the production activities of Anatolian clover and Italian ryegrass with the main products in terms of cost and profitability.

### Materials and Methods

The research was conducted in between 2015 and 2016 in Ankara University, Faculty of Agriculture, Field Crops Research and Application area. The soil of the experimentation field has an argillaceous-loamy structure, is alkaline (7.37), calcareous (5.66%), harmless total salt level (0.042%), moderate in phosphorus (5.52 kg da<sup>-1</sup>), rich in potassium (192 kg da<sup>-1</sup>) and insufficient in organic matter (1.05%). The experimentation field also has good drainage and does not have any groundwater problems. Ankara province is one of the leading provinces of our country in terms of plant production. Total agricultural area on Ankara is 1.233.043 ha and constitutes 48% of the its total area. The total land area in the total field agricultural area is 842.659 ha and it has a share of 68%. 8.6% of its agricultural land can be irrigated. The most important plant produced in field agriculture is wheat. The most important wheat market in Turkey is Ankara. Ankara is one of the provinces with the highest uses of certified seeds in Turkey. Approximately 25% of the its population is living in rural areas to deal with agricultural activity in Turkey, where the share of agriculture in gross value added of Ankara's level is 8.5%. The location of Ankara in Turkey is presented in the figure 1.

The materials used in the study were 'Demet-82' Anatolian clover and "Hellen" Italian ryegrass varieties. The research was carried out in Ankara University, Faculty of Agriculture, Field Crops Research and Application area. Also, it was based on the split plots in randomized blocks experimental design with 3 replications in between the years of 2015 and 2016. Different sowing methods [same rows (E<sub>1</sub>), alternate rows (E<sub>2</sub>), perpendicular rows (E<sub>3</sub>) and broadcast (E<sub>4</sub>) sowing method] were employed for the main plots; different mixture rates [Anatolian clover (T) and Italian ryegrass (L) as sole, and 75%

Anatolian clover + 25% Italian ryegrass (K<sub>1</sub>), 50% Anatolian clover + 50% Italian ryegrass (K<sub>2</sub>) and 25% Anatolian clover + 75% Italian ryegrass (K<sub>3</sub>) were employed for the sub plots as mixture. The amount of seeds per decare were 2 kg for both Anatolian clover and Italian ryegrass and the seeds used were estimated based on the sowing method and the mixture ratio for each plot (Gençkan, 1995). The sowing and harvest times were April 12-July 15 in 2015 and April 15-July 17 in 2016, respectively. The results obtained from the research were

calculated by using the average values of 2015 and 2016. The cultivated plots had an area of 2m × 2m = 4m<sup>2</sup> and the row spacing was 20 cm in 10 rows. For both years of the experiment, 20 kg of diammonium phosphate (20:20) fertilizer had been applied per decare one week prior to sowing. Both experimentation years involved three irrigations each. In both experimentation years, hoeing was done for weed control purposes where necessary.



Figure 1. Research area

Method applied in data analysis used in the study represent 2015-2016 production period. The unit product costs were estimated using budget approach based on the physical and financial data obtained from the study. The production cost calculation for the crops were based on the data related to the input utilization levels for the production activities, product, input prices and production amount. The expense and income for the forage crop mixture under Ankara province conditions in terms of employed cultivation techniques are given in Table 1. 3% of the total cost under Ankara conditions was established as overall administrative expenditures (Erkus and Demirci, 1996). In Table 1, the use of seed plant is a significant variable and the fact that the sowing cost of perpendicular row sowing is two times higher than other sowing methods. The cost analysis of the forage mixtures used in the experiment was conducted using Relative Profit margin. The optimum mixture amount to be included in the forage crop production under Ankara conditions was identified through comparison of Relative Profit margin. In plant production activities, costs were regulated in a way to show the utilization level of average production inputs per decare and net profit levels per unit area were provided according to products. These following formulas were used in the calculation of gross and net profits.

$$\begin{aligned} \text{Gross profit} &= \text{Gross production value} - \text{varying costs} & 1 \\ \text{Net profit} &= \text{Gross production value} - \text{production costs} & 2 \\ \text{Relative profit margin} &= \text{Gross production value} / \text{production costs} & 3 \end{aligned}$$

The amounts used in intercropping by the producers for input utilization were taken as basis. The calculation of machinery costs were based on the local unit machinery rental fees. The general administrative expenses were calculated as 3% of the total varying costs. In fodder crop production, the government supports provided to the producers were not included in calculation. In this study, the production costs and incomes are calculated in Turkish Lira (TL) and converted to US Dollar (USD) (1 Dollar= 5.3 Turkish Liras). Some values in this study are used as abbreviation (decare=da, kilogram=kg, Turkish Lira=TL, US Dollar=USD).

The agricultural application method been used by the producers in forage crop production under Ankara conditions was identified. The data obtained from the study were evaluated in consideration of the agricultural application and the cost items were created. In the research, the data obtained from the plot area of 4 m<sup>2</sup> was converted to decare (4 m<sup>2</sup> × 250). This allowed for more comprehensible

parameters for the agricultural application. The amounts in other cost items were also converted to decare for the

calculations. The expense and income table was created according to these calculations.

**Table 1.** Expenses and incomes relating the forage crop mixture

Serial No	Expenses	Unit	Amount	Unit Price (USD)	Amount (USD)
1	First Release Fee (Plow)	da	1	6.15	6.15
2	Doubling Fee (Sweep)	da	1	3.07	3.07
3	Sowing Cost (Grain Drill)	da	1	1.55	1.55
4	Seed Plant	kg da <sup>-1</sup>	2	6.64	13.28
5	Bottom Fertilizer (DAP)	kg da <sup>-1</sup>	20	0.35	6.90
7	Fertilization Workmanship	da	1	1.31	1.31
8	Fighting Amount (Herbicide)	da	1	0.92	0.92
9	Fighting Workmanship	da	1	0.92	0.92
10	Woodsman Wage	da	1	0.92	0.92
11	Harvest (Mowing)	da	1	6.19	6.19
12	Transport	da	1	0.80	0.80
13	Bailing Fee	da	1	4.15	4.15
14	Exchange-Portage-Loading-Stoppage	da	1	1.29	1.29
15	Irrigation	da	3	2.04	6.13
<b>Total</b>					<b>53.99</b>
16	Other Expenses	3%			1.61
<b>Total</b>					<b>55.60</b>
17	General Administrative Expenses	3%			1.65
18	Interest on Capital	4%			2.21
<b>PRODUCTION COSTS</b>					<b>59.05</b>
<b>GROSS PRODUCTION VALUES (Mixture Amount kg da<sup>-1</sup>)</b>			Yield (kg da <sup>-1</sup> )	Price (USD da <sup>-1</sup> )	Amount (USD)
<b>NET PROFIT</b>			781.95	0.13	95.90
					36.85

## Results and Discussion

In agricultural production activities, making use of the scarce resources available to the establishment is vital for sustainable agricultural production. The study aimed to determine the optimum forage crop mixture amount to be included in the production design in the establishment under Ankara conditions by using relative profit margins. The expense and income, which was listed to establish the Relative Profit margins for the forage crop mixture under Ankara conditions in terms of in the study area cultivation techniques, are given in Table 1.

The data given in Table 1 was obtained by adapting the study results to the agricultural conditions. The calculations in Table 1 were based on the forage crop production design under Ankara conditions. The total production cost for forage crop mixture per decare was estimated as 53.99 USD da<sup>-1</sup> in the establishments whose unit product costs were evaluated. This value constitutes 90.7% of the total production cost. Among the production costs in forage crop cultivation, the largest share was claimed by machinery pulling power costs (plowing and sowing) with 20.2%. Similar previous studies showed that almost half of wheat production costs comprise of machinery pulling power and fertilizer costs and diesel fuel constitutes a significant part of machinery pulling power costs (Alemdar et al., 2014).

In the calculations, the yield of forage crop mixture rate was determined as 781.95 kg da<sup>-1</sup> and total production expense per decare was found to be 59.05 USD da<sup>-1</sup>. In forage mixture

production, net profit per decare was estimated to be 36.85 USD da<sup>-1</sup>. Accordingly, profit thresholds were exceeded, the varying and total costs of production activities are met. Under these circumstances, the production of forage crop mixture will be sustainable and economically consistent. The gross production values and the production costs of other mixture rates and sowing methods were calculated separately. The results of calculations are given in the Figure 2.

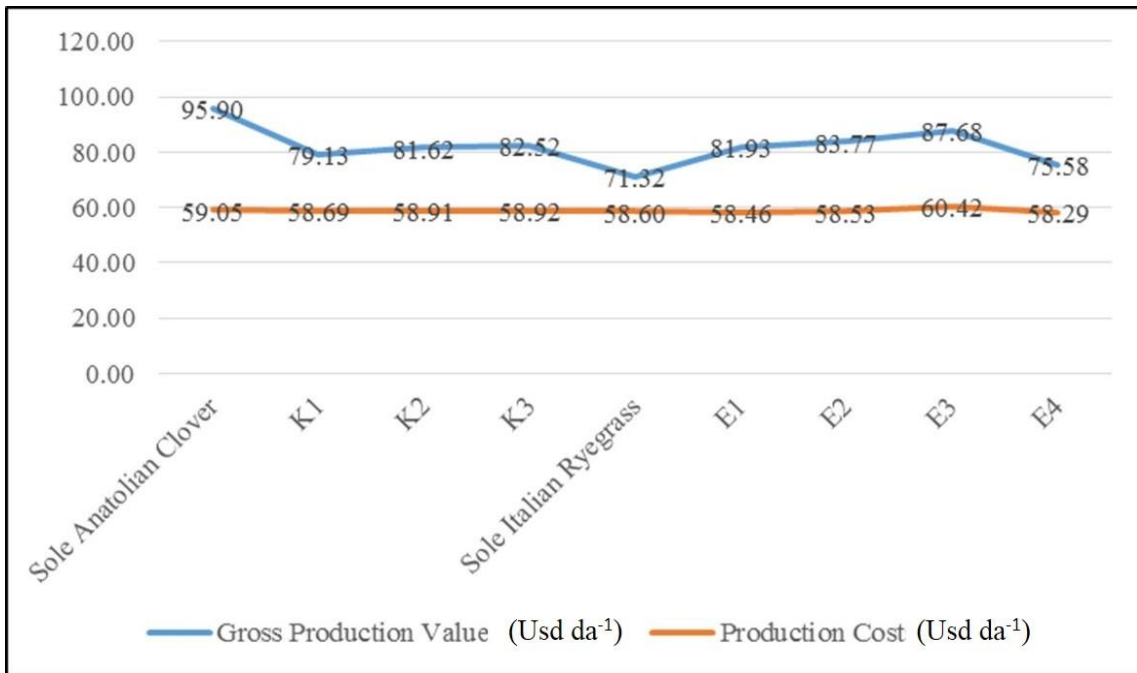
The highest gross production value (95.90 USD da<sup>-1</sup>) and the production cost (59.05 USD da<sup>-1</sup>) was obtained from the sole Anatolian Clover among the forage crop mixtures (Figure 2). Whereas; the lowest gross production value (71.32 USD da<sup>-1</sup>) and the production cost (58.60 USD) was detected in the sole Italian ryegrass application. In terms of sowing applications, the highest gross production value (87.68 USD da<sup>-1</sup>) and the production cost (60.42 USD da<sup>-1</sup>) was obtained in perpendicular row sowing application (E<sub>3</sub>). The lowest gross production value (58.29 USD da<sup>-1</sup>) was detected in broadcast sowing application (E<sub>4</sub>). As a result of the evaluation of the gross production values and the production costs obtained in the research, the relative profit margins were calculated and given in the Figure 3.

The relative profit margins in the the production values obtained from the implementation of mixture and sowing methods were all positive (Figure 3). This shows that the profit threshold is exceeded regardless of the method employed by the producer. Pursuant to the economic principles in the production, the producer needs to choose the method to provide the highest level of relative profit in these applications. In the study, the highest profit (1.62) was

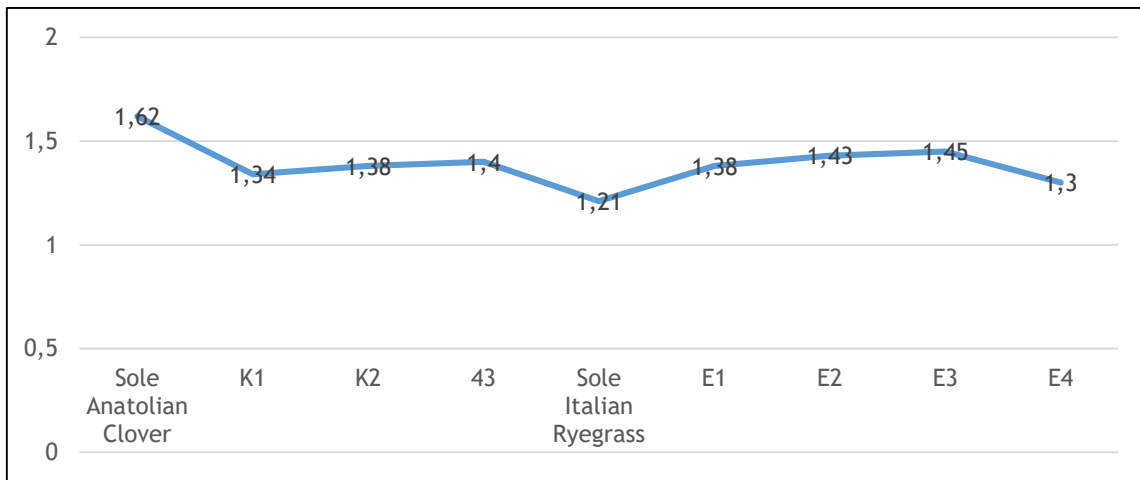


generated by sole Anatolian clover in forage crop mixture applications. It shows that when the application is chosen by the producer, an income of 1.62 USD is generated per 1 USD cost. This coefficient also shows that a profit of 0.62 USD can be generated per 1 USD cost in the production of mixture application. In mixture applications, the lowest profit margin

(1.34) was found in 75% Anatolian clover + 25% Italian ryegrass application ( $K_1$ ). It shows that 75% Anatolian clover + 25% Italian ryegrass application ( $K_1$ ) provides an income of 1.34 USD per 1 USD cost when the application is chosen by the producer. According to these results, this application should be preferred as a last resort by the producer.



**Figure 2.** Distribution of gross production values and production costs according to the different mixture rates and sowing methods (K1: 75 Anatolian clover: 25 Italian ryegrass, K2: 50 Anatolian clover:50 Italian ryegrass, K3: 25 Anatolian clover:75 Italian ryegrass, E1: Same rows, E2: Alternate rows, E3: Perpendicular rows, E4: Broadcast)



**Figure 3.** Distribution of relative profit according to different mixture rates and sowing methods (K1: 75 Anatolian clover: 25 Italian ryegrass, K2: 50 Anatolian clover:50 Italian ryegrass, K3: 25 Anatolian clover:75 Italian ryegrass, E1: Same rows, E2: Alternate rows, E3: Perpendicular rows, E4: Broadcast)

In the study, the highest income (1.45 USD) was obtained in perpendicular row sowing application ( $E_3$ ) in terms of sowing applications. It shows that when the application is chosen by the producer, an income of 1.45 USD is generated per 1 USD cost. This coefficient also shows that an income of 0.45 USD will be generated per 1 USD cost in forage crop sowing

applications. Sole Italian ryegrass was found to be the method with the lowest level of income in sowing applications. In this application, the profit to be generated per 1 USD cost was found as 0.21 USD.

An examination of the studies on the economic analysis of forage crop mixture rates revealed a lack of adequate analysis on the subject. Therefore, our findings were compared to the economic analysis findings from other plant products employing the same sowing method and mixture rates, and the comparison results were discussed in the study. A study showed that, in Tokat province, a profit of 0.29 USD is generated per 1 USD cost in wheat production (Bayramoglu et al., 2005). This finding was quite lower than the profit (0.62 USD) generated from the forage crop mixture rates. Accordingly, from business administration point of view, the producer is suggested to prefer sole Anatolian clover forage crop mixture over wheat production. Another study found that a wheat producer in Agri province spends 0.83 USD for 1 kg wheat and sell it at price of 0.58 USD, incurring a loss of -0.26 USD per kg (profit threshold not exceeded). Under these circumstances, it can be argued that wheat farming is not financially feasible (Karadas, 2016). There is no difference between the findings among present studies and this. Because, the profit threshold obtained from the findings of this study was positive in all mixture and sowing methods. The net income was found to be negative (-20.72 USD da<sup>-1</sup>) for the study conducted for silage corn production costs in Pasinler district of Erzurum province. It is argued that producers continuing their farming activities through such method may financially cripple the establishment (Akay Tuvanc and Dagdemir, 2009). The net profit from this study findings was found to be positive, which revealed a difference between our findings and that of the researchers. A study conducted in Ardabil, Iran showed that 20% of the the production costs comprise of machinery pulling power, and that a profit of 0.88 dollar is generated per decare (Mohammedi et al., 2009).

## Conclusion

This study compared the average values of different mixture rates and sowing methods of Anatolian clover and Italian ryegrass under Ankara conditions. According to the comparison, perpendicular rows (E<sub>3</sub>) sowing application stood out among others in terms of relative profit. In terms of relative profits of mixture rate, sole stand Anatolian clover ranked number one. It was followed by 25% Anatolian clover + 75% Italian ryegrass (K<sub>3</sub>), 50% Anatolian clover + 50% Italian ryegrass (K<sub>2</sub>), 75% Anatolian clover + 25% Italian ryegrass (K<sub>1</sub>) and sole Italian ryegrass, respectively. In sole Anatolian clover, the gross profit per decare was estimated as 95.90 USD da<sup>-1</sup>, gross profit as 36.85 USD da<sup>-1</sup> and relative profit as 1.62. The above-mentioned sowing methods and mixture rates should be preferred in terms of sustainable production. No loss was incurred from all these mixture rates and sowing methods, and the study results were satisfactory. Also, the level of gross profit per decare was found to be high in forage crop mixture rates used by establishments. Accordingly, the production threshold is exceeded and the varying costs of production activity are met. It can be argued that using the five forage crop mixture rates in the production activities in the experiment area is effective in terms of business management principles. This study finding is crucial and exemplary for the establishments that carry out mixed forage crop production

activities. Because in this way, the forage crop producer will decide on which mixture rate to be preferred, how much expense will be made for this mixture, how much profit will be generated for this cost, and how the establishment revenue will be increased. Under these circumstances, the production of forage crop mixture will be sustainable and financially feasible. It can also be argued that this will prove useful in making the production decision by making a comparison with other vegetable products in the establishment. This study finding is crucial and exemplary for agricultural establishments that carry out agricultural activities.

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## RESEARCH ARTICLE

### Effects of Chemical Fertilizer and Some Bacterial Formulations on Growing Medium and Plant Heavy Metal Content in Poinsettia Cultivation

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#### ABSTRACT

High amount of chemical fertilizers and various pesticides are used in the cultivation of poinsettia. This study was conducted to compare the amount of heavy metals (Zn, Cu, Pb and Cd (mg kg<sup>-1</sup>) and B (mg kg<sup>-1</sup>) metalloid) accumulated by the use of different bacterial formulations and chemical fertilizers in the cultivation of poinsettia. The research was conducted in climate controlled research greenhouse conditions between July 2015 and July 2017. In the study, rooted cuttings of poinsettia (*Euphorbia pulcherrima* Willd.ex Klotzsch cv. Christmas Eve) were used as plant material. The applications were created as BI (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79), BII (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C), BIII (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C), BIV (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D), CF [the full amount of commonly used chemical fertilizer (150 g 100L<sup>-1</sup>), BI+CF, BII+CF, BIII+CF, BIV+CF (by combining with 50% the reduced amount of chemical fertilizer by (75 g 100L<sup>-1</sup>)) and control (uninoculated). The highest Zn, Cu, Pb and Cd (mg kg<sup>-1</sup>) amounts were obtained from BIV application in growth medium. According to CF and control applications, BI+CF application was determined as reducing in the Zn amount of leaf samples of plants 14.47% and 15.70%, respectively. BI application was determined as reducing in the Cd amount of leaf samples of plants 26.58% and 54.69%, respectively when compared to CF and control applications. The highest amount of Pb was determined in plant root samples.

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#### Introduction

Heavy metal pollution is increasing in day by day due to industrialization in all over world. Different agricultural activities like usage of agrochemicals and different industrial activities like waste disposal and deposition of urban sewage sludge accumulate huge amounts of heavy metals to the growth medium and can disrupt human food and environment safety (Saleh et al., 2004). Metals such as zinc, iron, copper,

nickel and manganese play important roles as beneficial or essential micronutrients of microorganisms (Olson et al., 2001; Sakamoto and Bryant, 2001). However, a high concentration of metal ions in growth medium shows serious effects on microbial communities by decreasing diversity and total microbial biomass and changing the community structure (Khan et al., 2009). Therefore, microbial communities are useful indicators of the effect of contamination on growth medium health (Mishra et al., 2008). Bacteria in heavy metal-

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contaminated growth medium is firstly exposed to the heavy metal stress. Also, to have a functional role in remediation, the bacteria must overcome the heavy metal stress. It is known that microorganisms tolerate heavy metals by immobilizing metals on cell surfaces or transforming metals into less toxic forms, for example by acidification, precipitation and oxidation-reduction (Ma et al., 2011).

Rhizosphere is the root surrounding region and has many types of active groups of bacteria (Villacieros et al., 2003) termed as "PGPR" (Plant Growth Promoting Rhizobacteria) (Khatoun et al., 2014). PGPR inhabits in around the root and useful for plant with the enhancement of growth by way of two mechanisms: First is the direct mechanisms such as nitrogen fixation, increasing in availability of nutrients to the plant, growth-regulating agents production, production of plant vitamins and hormones such as gibberellin and cytokinin. The second is the indirect mechanisms includes make iron available, antibiotics synthesis, competing with root inhabiting species (Glick et al., 1999; Verma et al., 2015), causing systemic resistance, and promoting plant resistance to stress conditions caused by non-living factors (Glick et al., 1998). Pal et al., (2004) bacteria developed various resistance mechanisms to adopt the metal contaminated environment.

The solubility of heavy metals in soil is limited due to complexing with organic matter, sorption on clays and oxides, and precipitation as carbonates, hydroxides, and phosphates. This problem can be controlled (Naidu and Harter, 1998) and increase in solubility may be achieved by adding plant growth-promoting rhizobacteria (PGPR) to the soil. Previous studies have also been found no changes or decreases in plant growth and yield with increases in the heavy metal uptake of various

plants (Turan and Angin, 2004; Turan and Esringu, 2007; Angin et al., 2008; Pezzarossa et al., 2009; Gullap et al., 2014).

The objective of this work is to study the effect of different bacteria formulations, chemical fertilizer and their combinations on heavy metals content of poinsettia (*Euphorbia pulcherrima* Willd.ex Klotzsch cv. Christmas Eve) plant and the growth medium.

## Materials and Methods

The research was conducted in climate controlled research greenhouse between July 2015 and July 2017. In the study, rooted cuttings of poinsettia (*Euphorbia pulcherrima* Willd.ex Klotzsch cv. Christmas Eve) were used as plant material. The cultivation medium was prepared by mixing peat in ratio of 2: 1 and pumice as volume (Anonymous, 2010; Anonymous, 2015). Plants were planted in 3.5 liter plastic pots. The applications were created as formulation 1 (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79), formulation 2 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C), formulation 3 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C), formulation 4 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D) (Table 1), the full amount of commonly used chemical fertilizer (100% CF) and by combining the reduced amount of chemical fertilizer by 50% with each bacterial formulation. Bacterial formulations were inoculated in the rooted cuttings of the poinsettia by dipping method and they were planted in pots filled with appropriate growing medium. The study was designed as 3 replicates in randomized experimental design with 1 (variety) x10 (application) x2 (years) in randomized parcel trial design.

**Table 1.** Bacterial isolates used in the study and some biochemical properties (Kotan et al., 2009; Kotan et al., 2010)

Isolate No	MIS Diagnosis Result	SIM	Location	Host	Nitrogen	Phosphate	Siderophore
RK-79	<i>Pantoea agglomerans</i>	0.762	Erzurum	Apple	+	+	-
TV-12E	<i>Paenibacillus polymyxa</i>	0.551	Van	Poaceae	S+	+	-
TV-17C	<i>Bacillus subtilis</i>	0.677	Van	Raspberry	S	W+	-
TV-6D	<i>Bacillus megaterium</i>	0.750	Van	Poaceae	+	+	-
TV-42A	<i>Pseudomonas putida</i>	0.113	Van	Poaceae	W+	W+	+
TV-91C	<i>Bacillus megaterium</i>	0.474	Van	Poaceae	+	W+	-
TV-113C	<i>Kluyvera cryocrescens</i>	0.688	Van	Garlic	+	+	-
RK-92	<i>Pantoea agglomerans</i>	0.889	Erzurum	Pear	+	S	-

(SIM: Similarity index, S: Strong +, W: Weak +; +: Positive, -: Negative)

After planting of rooted cuttings in pots, two different types of fertilizer in a form that can be completely dissolved in water were applied to the pot groups to be applied chemical fertilizer at the determined different doses. These are comprised from "White 15-0-19 + 9CaO + 2MgO + TE, NPK ratio 4: 0: 5" (white composite fertilizer, granule, containing nitrogen, potassium, calcium, magnesium, boron, zinc, iron, copper, magnesium, molybdenum and manganese) and "Blue 18-11-18 + 2.5MgO, NPK ratio 3: 2: 3" (blue composite fertilizer, granule, containing nitrogen, phosphorus, potassium, magnesium, boron, zinc, iron, copper, molybdenum and manganese). These two different chemical fertilizers were given in specified amounts with the irrigation water consecutively (Kofranek et al., 1963; Faust et al., 2001;

Anonymous, 2015). The recommended dose (150 g 100 L<sup>-1</sup>) of these fertilizers for pots, flower beds and all covered seedlings was used in this study.

After 110-120 days from bacterial inoculation, in measurements of heavy metals were made on 10 samples from each application. The amount of heavy metals (Zn, Cu, Pb and Cd (mg kg<sup>-1</sup>) and B (mg kg<sup>-1</sup>) metalloid) in the plant leaves, plant roots and growth medium samples was determined. Plant samples (leaf and root) were oven-dried at 68 °C for 48 h and were then ground. Cu, Zn, Pb and Cd were determined by atomic absorption spectrometry using the methods of AOAC (1990). Boron was determined, after dry-ashing of plant

samples, spectrophotometrically at 550 nm by the curcumin method (Odom, 1992).

At the end of the experiment, growth medium samples were taken from the rhizosphere area, comprised of 3 samples from each application, to represent each application from the cultivation medium. Zn and Cu quantities absorbable by the plant were determined by reading ICP-OES in the percolators extracted according to DTPA method (Lindsay and Norwell, 1978). Total Pb, Cd and B were determined according to AOAC (1990).

Data were treated by the analysis of variance by using the SPSS version 20.0 statistical software package (SPSS Inc., Chicago, IL, USA). For the significance level, 5% has been set to be the maximum acceptable limit to be considered as a significant result.

**Table 2.** The amount of Zn mg kg<sup>-1</sup> in the green and bract leaf, plant root and growth medium samples

Applications	Soluble Zn (mg kg <sup>-1</sup> )			
	Green Leaf	Bract	Root	Growth medium
Control	48.52 cd***	50.46 bc***	59.16 a***	1.79 g***
CF	48.18 cd	51.78 bc	53.68 bcd	2.57 de
BI	49.94 c	48.52 bc	49.34 e	3.20 b
BI+CF	41.21 e	48.95 bc	51.81 de	2.49 de
BII	47.66 cd	50.31 bc	56.32 abc	2.62 de
BII+CF	43.42 de	47.92 c	53.93 bcd	2.26 f
BIII	57.21 ab	52.14 b	58.34 a	2.66 d
BIII+CF	52.59 bc	43.36 d	52.53 cde	2.42 ef
BIV	60.03 a	56.56 a	57.01 ab	3.46 a
BIV+CF	51.96 c	56.03 a	56.85 ab	2.98 c
Mean	50.07	50.60	54.90	2.64

\* P ≤ 0.05; \*\* P ≤ 0.01; \*\*\* P ≤ 0.001; ns: not significant (P ≥ 0.05). The numbers in one column having the same letter are not significantly different

According to the general application mean, the highest mean value for the amount of zinc from the bract leaf samples was determined in BIV (56.56 mg kg<sup>-1</sup>) and BIV + CF (56.03 mg kg<sup>-1</sup>). The amount of zinc in the bract leaves varied between 43.36 mg kg<sup>-1</sup> 56.56 mg kg<sup>-1</sup> according to the applications (Table 2). Zn contents of plants are normally between 5-100 mg kg<sup>-1</sup>, and toxicities are usually seen at values above 400 mg kg<sup>-1</sup> (Mengel and Kirkby, 2001; Güzel et al., 2002; Marschner, 2008). According to these findings, the amount of zinc in the leaves obtained from this study was found to be sufficient or in the appropriate range. Zinc uptake efficiency of plants can vary based on plant varieties, even different genotypes of the same variety (Karaman et al., 2012). In general, the amount of zinc determined in BIV bacterial formulation application may be formed by the effects of some organic compounds. Indeed, some organic compounds, such as the amino acid secreted by the roots of certain plants, decrease level of the pH in the plant's root rhizosphere; so that plant nutrients such as the zinc, manganese, iron and phosphorus can be converted into more soluble and obtainable forms (Marschner, 2008). Di Simine et al. (1998) and Fasim et al. (2002) have documented with the findings that the insoluble Zn compounds are dissolved by bacteria. In this context, the results obtained from the present study can be explained by this finding.

In the experiment, the highest average value in terms of the amount of zinc available for poinsettia roots was determined in control and BIII applications. The amount of zinc

## Results and Discussion

Zinc has effects on nitrogen metabolism, seed maturation and starch formation in plants. It is an important component of various metabolic enzymes and is in the group of immobilized elements in plants; plants need a constant supply of zinc for optimum growth. In addition, it is involved in the metabolism of hormones (auxin) that promote plant growth (Balashouri, 1995; Gardiner and Miller, 2008; Kosesakal and Unal, 2009; McCauley et al., 2009). The amount of zinc in the green leaves varied between 41.21 mg kg<sup>-1</sup> and 60.03 mg kg<sup>-1</sup> according to the applications. In terms of application averages, the highest average value for the amount of zinc was determined in BIV application. The lowest level was obtained from BI+CF application (Table 2).

taken from root samples varied between 49.34 mg kg<sup>-1</sup> and 59.16 mg kg<sup>-1</sup> according to the applications (Table 2). Aliyeva (2014) stated that the Zn concentration in the roots of *Chlamydotis undulata* is always higher than that of the leaves. The Zn amounts obtained in this study are in the normal range according to the range specified by Mengel and Kirkby (2001), Güzel et al. (2002) and Marschner (2008). Also, Di Simine et al. (1998) and Fasim et al. (2002) have shown that the Zn compounds, slow dissolving zinc in soil are dissolved by bacteria. They proved that the dissolution of Zn by microorganisms useful and economical. With these findings, the findings of this study can be supported. The amount of zinc that can be taken from the cultivation media samples varied between 2.26 mg kg<sup>-1</sup>-3.46 mg kg<sup>-1</sup> according to the general average of the applications. The highest amount of zinc was taken from BIV application while the lowest available zinc content was obtained from BII+CF application (Table 2).

Copper (Cu) in plants is important for plant health, plant growth and development. It is involved in the formation and composition of the cell wall, thus affecting lignification (Marschner, 1995). Copper is effective in protein and carbohydrate metabolism. It has a role in symbiotic nitrogen fixation (McCauley et al., 2009; Kacar, 2015). In this study, the highest amount of soluble copper in green leaf samples was found in BII application (15.35 mg kg<sup>-1</sup>) while the lowest soluble copper content was determined in the control (13.14 mg kg<sup>-1</sup>) application. It was found that the differences between the

amount of copper in the bract leaf samples were not statistically significant (Table 3).

The effect of PGPR depends on bacterial strains and population, plant bacterial strain combination, plant genotype, evaluated growth parameters and environmental conditions (Çakmakçı et al., 2006; 2007; Sahin et al., 2004). Cu uptake depends on the amount of soluble Cu in the soil (Turan and Köse, 2004). It was determined that the applications on the amount of copper taken from root samples

were not significant (Table 3). The highest available copper value 2.43 mg kg<sup>-1</sup> BIV application while the lowest available copper was obtained from control application with 1.66 mg kg<sup>-1</sup> in the growing medium (Table 3). It was determined that *Burkholderia pyrrocinia* 13/4, *Paenibacillus macquariensis* 59/8, *Pantoea agglomerans* 5/8, *Stenotrophomonas maltophilia* 21/1 ve *Lysobacter enzymogenes enzymogenes* 9/8 isolates had positive effects on plant vegetative development and nutrient contents (Ertürk et al., 2010).

**Table 3.** Findings of Cu (mg kg<sup>-1</sup>) in the green and bract leaf, plant root and growth medium samples of poinsettia

Applications	Soluble Cu (mg kg <sup>-1</sup> )			
	Green Leaf	Bract	Root	Growth medium
Control	13.14 d*	12.91 <sup>ns</sup>	12.48 <sup>ns</sup>	1.66 e***
CF	13.73 bcd	12.74	12.61	2.02 d
BI	14.79 abc	13.20	12.38	2.14 bcd
BI+CF	14.76 abc	12.09	13.29	2.16 bc <sup>d</sup>
BII	15.35 a	11.95	12.74	2.09 cd
BII+CF	15.14 ab	11.99	12.63	2.20 bc
BIII	13.48 cd	12.39	13.33	2.11 cd
BIII+CF	13.77 bcd	12.88	13.80	2.08 cd
BIV	14.13 abcd	12.13	13.23	2.43 a
BIV+CF	15.03 ab	12.58	13.98	2.25 b
Mean	14.33	12.49	13.05	2.11

In green leaf samples, the highest amount of Pb was taken from CF application while the lowest amount of Pb was determined in BIV application. BI, BII, BIII, BIII+CF and BIV+CF applications were in the same statistical group with BIV application. The maximum amount of soluble Pb in the bracts is in control (0.39 mg kg<sup>-1</sup>) and CF (0.41 mg kg<sup>-1</sup>) applications while the lowest soluble Pb amount was determined in BIV

(0.24 mg kg<sup>-1</sup>) (Table 4). Pb affects plant growth (Wang et al., 2007) in a harmful way by inhibiting many physiological processes (Sinha et al., 2006; Ruley et al., 2004). The amount of Pb obtained from this study is very low. It was observed that this amount was not at the level of toxic effect or at a level that would prevent plant growth and development.

**Table 4.** The effects of the applications on soluble Pb values of the green and bract leaf, plant root and growth medium samples of poinsettia

Applications	Soluble Pb (mg kg <sup>-1</sup> )			
	Green Leaf	Bract	Root	Growth medium
Control	0.42 bc***	0.39 a***	0.36 ab***	0.18 f***
CF	0.50 a	0.41 a	0.40 a	0.26 cd
BI	0.34 de	0.33 b	0.33 bc	0.20 ef
BI+CF	0.44 ab	0.28 bcd	0.34 bc	0.19 ef
BII	0.31 de	0.28 bcd	0.24 e	0.19 ef
BII+CF	0.37 cd	0.29 bc	0.27 de	0.26 cd
BIII	0.30 de	0.25 cd	0.27 de	0.22 de
BIII+CF	0.30 de	0.28 bcd	0.29 cde	0.27 bc
BIV	0.27 e	0.24 d	0.28 cde	0.30 b
BIV+CF	0.31 de	0.33 b	0.32 bcd	0.51 a
Mean	0.35	0.31	0.31	0.26

The effect of the applications in terms of the amount of soluble Pb in the root samples ( $P \leq 0.001$ ) was significant. The highest amount of soluble Pb was taken from CF (0.40 mg kg<sup>-1</sup>) application while the lowest amount of soluble Pb (0.24 mg kg<sup>-1</sup>) was determined in BII application. BII+CF and BIII applications were in the same statistical group with BII application (Table 4). Pb accumulates in stem cells in many plants. Since the plant takes Pb from the soil, the Pb content in the root is related to the amount of Pb in the soil (Raskin and Ensley, 1999). Determination of the maximum Pb content in 100% chemical fertilizer and 50% reduced chemical fertilizer applications is also consistent with this determination. Value

of the highest soluble Pb in the growing media samples was determined in BIV+CF application (0.51 mg kg<sup>-1</sup>) while the lowest soluble Pb amount was obtained in control (0.18 mg kg<sup>-1</sup>) application. BI, BII and BI+CF applications were in the same statistical group with control (Table 4).

Boron, which is a micro plant nutrient, plays an important role in plant cell wall formation, reproduction of plant tissues, transport of sugars through cell membranes, biosynthesis of carbohydrates, nucleic acid, amino acid and protein synthesis. Boron also activates some dehydrogenase enzymes (Stangoulis et al., 2001; McCauley et al., 2009). The highest amount of

boron content in poinsettia green leaf samples was found in BIV+CF application while the lowest boron content was obtained from the control application. Control application and BII application were statistically found in the same group. The amount of boron in the bract leaf was determined as the highest average in BIV+CF application. The lowest boron level was determined in the control application and control application is in the same group with CF and BI applications (Table 5). In general, the amount of boron nutrients required for the development of many plants is between 6 and 60 ppm (Jones and Jacobsen, 2001; Epstein and Bloom, 2005). The highest boron content for root samples was obtained from

BIV+CF application. The lowest boron content for root samples was obtained from control and BI applications (Table 5). Microelements such as Ca, Mo and B play an important role in the pigmentation and growth of bracts of poinsettia (Arreola et al., 2008). The highest available boron value was obtained from BIII+CF application (0.86 mg kg<sup>-1</sup>). The lowest available boron amounts were obtained from CF (0.46 mg kg<sup>-1</sup>), control (0.47 mg kg<sup>-1</sup>), BI+CF (0.50 mg kg<sup>-1</sup>) and BI (0.53 mg kg<sup>-1</sup>) (Table 5). Zulueta-Rodriguez et al. (2014) reported that *Pseudomonas putida* rhizobacterium was effective in increasing in the coloration and anthocyanin pigmentation of poinsettia varieties.

**Table 5.** The effects of the applications on soluble B values of the green and bract leaf, plant root and growth medium samples of poinsettia

Applications	Soluble B (mg kg <sup>-1</sup> )			
	Green Leaf	Bract	Root	Growth medium
Control	21.28 e <sup>***</sup>	21.84 e <sup>**</sup>	22.72 d <sup>***</sup>	0.47 d <sup>***</sup>
CF	24.08 cd	23.57 de	24.06 cd	0.46 d
BI	24.34 cd	22.87 de	23.15 d	0.53 d
BI+CF	23.79 cd	27.40 bc	26.45 bc	0.50 d
BII	22.50 de	27.20 c	26.28 bc	0.61 c
BII+CF	26.72 ab	29.27 b	27.52 b	0.64 c
BIII	24.26 cd	24.52 d	24.56 cd	0.72 b
BIII+CF	26.03 bc	27.56 bc	24.81 cd	0.86 a
BIV	26.97 ab	29.05 bc	27.43 b	0.73 b
BIV+CF	28.51 a	32.05 a	31.22 a	0.71 b
Mean	24.85	26.53	25.82	0.62

**Table 6.** Findings of Cd (mg kg<sup>-1</sup>) in the green and bract leaf, plant root and growth medium samples of poinsettia

Applications	Soluble Cd (mg kg <sup>-1</sup> )			
	Green Leaf	Bract	Root	Growth medium
Control	1.58 e <sup>***</sup>	1.55 de <sup>***</sup>	1.31 de <sup>***</sup>	0.24 f <sup>***</sup>
CF	2.56 a	2.36 a	2.27 a	0.34 e
BI	1.16 g	1.14 g	1.12 f	0.37 cd
BI+CF	1.95 bc	1.38 f	1.60 bc	0.42 bcd
BII	1.35 f	1.47 ef	1.12 f	0.40 cde
BII+CF	2.07 b	1.81 b	1.76 b	0.47 ab
BIII	1.35 f	1.21 g	1.24 ef	0.39 cd
BIII+CF	1.84 cd	1.68 c	1.73 b	0.47 ab
BIV	1.38 f	1.20 g	1.46 cd	0.45 ab <sup>c</sup>
BIV+CF	1.70 de	1.63 cd	1.66 b	0.50 a
Mean	1.69	1.54	1.53	0.40

According to the applications, cadmium values varied between 1.16 mg kg<sup>-1</sup> and 2.56 mg kg<sup>-1</sup> in the green leaf samples of poinsettia while the available cadmium values in the bract leaves ranged from 1.14 mg kg<sup>-1</sup> to 2.36 mg kg<sup>-1</sup>. In green leaf samples, the highest available cadmium level was found in CF application and the lowest available cadmium amount was found in BI application. The least amount of cadmium in the bracts was determined in BI, BIII and BIV applications (Table 6). Cadmium is a heavy metal cation that causes phytotoxicity in plants (Shah and Dubey, 1998a, 1998b). As a result of this study, the highest Cd content was determined in plant leaves grown with 100% chemical fertilizer application. It can be said that it is expected to obtain less Cd amount in bacterial formulation applications, based on previous studies. Burd et al. (1998) reported that inoculation of seeds of canola and indian mustard with the strain *Kluyvera ascorbata* SUD165, one of the plant growth promoting

rhizobacteria, protected plants against to Ni, Pb and Zn toxicity by producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme and siderophore. It has been reported that various PGPR strains have the ability to immobilize Cd in nutrient medium (Belimov et al., 1998) and soil (Pishchik et al., 2002) as well as providing resistance to Cd (Belimov et al., 2005). As a result of this study, no indication of Cd's toxic effect was observed in any application. Plant capacities, such as depositing heavy metals and tolerating excess, are special characteristic of the plant species (Baker, 1987; Antosiewicz, 1992). According to the findings of these researchers, it can be concluded that the capacity of poinsettia to accumulate Cd heavy metal is low. The highest amount of cadmium in root samples was determined in CF (2.27 mg kg<sup>-1</sup>) application. The lowest available cadmium content was obtained from BII and BI (1.12 mg kg<sup>-1</sup>) (Table 6).



As a result of this study, the highest Cd content was determined in plant roots grown with 100% chemical fertilizer application. The toxic level of Cd may be due to natural soil properties, applications such as agricultural, manufacturing, mining, or the use of metals contained in pesticides and fertilizers in agricultural soils (Radotić et al., 2000). Since less Cd content obtained from BI, BIII and BIV bacterial formulations in the leaf samples and from the BI and BII bacterial formulations in the root samples, it was caused to be considered as ecological applications of these bacterial formulations in the present study. The highest available cadmium value in growing media samples was 0.53 mg kg<sup>-1</sup> BIV+CF application while the lowest available cadmium amounts were obtained from control application with 0.24 mg kg<sup>-1</sup> (Table 6). As a result of this study, no indication of Cd's toxic effect was observed in any application.

High amount of chemical fertilizers and various pesticides are used in the cultivation of poinsettia. Such applications may result in the increase in heavy metals particularly Cd, Pb, and As (Nouri et al. 2008; Atafar et al., 2010). Long-term use of excessive chemical fertilizers and organic manures in the bare vegetable field and the greenhouse vegetable field contributed to the accumulation of heavy metals in the soils (Huang and Jin, 2008). High fertilizer applications and acid atmospheric deposition, combined with insufficient liming, may also cause a decrease in pH and thus increase in heavy metal availability, aggravating the problem of deteriorating food quality, metal leaching, and impacting on soil organisms (De Vries et al., 2002).

In conclusion, plants inoculated with plant growth-promoting rhizobacteria have helped to taken from growth medium having heavy metals in growth medium. Some heavy metal resistant to bacteria are predicted to be able to useful for enhancement of plant growth with recolonization of plant's rhizosphere region in metal polluted soil. For this purpose, more bacterial strains should be tested and the resistance characteristics of the bacteria to heavy metals should be identified and tested on poinsettia and other ornamental plants in further studies.

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## RESEARCH ARTICLE

### Agricultural Loan and Agricultural Production Value in Turkey

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#### ABSTRACT

One of the major tools in agricultural finance is agricultural loans. Therefore, it is important to investigate the relationship between agricultural loans and agricultural production. In this study we aim to determine whether there is a causality relationship between the agricultural loan and agricultural production value. For this purpose we use the time series data for the years of 2005-2018. In the study, we use Phillips-Perron unit root test to determine the stationarity levels of the variables examined. After we examine the stationary levels of time series, we perform Granger causality test to detect the causality relationship between agricultural loans and agricultural production. As a result of the Granger causality test, we determine that there is a unilateral causality relationship from the agricultural loan variable to the agricultural production value variable, that is, it can be said that agricultural loans affect the value of agricultural production. For this reason, we can state that facilitating the use of loans in the agricultural sector, and increasing the lending institutions will contribute to the increase of agricultural production value in meeting the input needs of the producers effectively.

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#### Introduction

The agricultural sector is a vital role in supplying raw materials to agriculture-based industries, the nutrition of the population, and increasing the export income for the country. For agricultural activities, modern agriculture practice is essential. Modern agriculture is important for the economic growth and development of the country. If the producers provide financial opportunities, it is possible for them to purchase agricultural inputs, to make new investments and so they use advanced production technologies (Sjah et al., 2003). However, as the application of modern agricultural technology to increase agricultural production requires intensive capital use, the financing needs of the farmers and thus the demand for loans increases (Schultz, 1964; Mellor, 1966; Johnston and Cownie, 1969; Zuberi, 1989). It can be mentioned that especially small farmers can increase the production value with agricultural loans. So, the loan is an important tool for

modern agricultural production systems.

Financial institutions play an important role in providing financial support to the real sector in developing countries. Financial support is an important issue for the sustainability of agricultural activities. The agricultural loans are considered in providing financial support to farmers for their activities. Farmers receive loans according to their different needs and aims. However, in general, these aims can be distinguished into two groups as production and investment loans. The production loans are used by producers to increase their production and the investment loans are used by producers to increase and improve their production (Karacan, 1991). The use of production loans can be exemplified by purchasing the necessary assets, seeds, and breeds, increasing the assets of animals. Investment loans are used for the equipment of the enterprise, buildings and facilities, land acquisition and efficiency, and improvement. Loan availability allows both

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higher consumption and the use of more purchased inputs, which can be said to increase the welfare of farmers. Therefore, the marginal contribution of the loans brings the input levels closer to optimal levels. Thereby it rises the output because the amount of land is constant and the productivity increases (Feder et al., 1990). So, it is stated that agricultural loans serve as a bridge between the income and expenditures of the farmer and it is an essential ingredient in the growth strategy of the agricultural sector.

In the studies which are investigating the relationship between agricultural production and agricultural loans, it is understood that there is a relationship between these two variables. Azimi (2013) says that the loan positively affects the production and employment process. According to Terin et al. (2014) study results, there is unilateral causality from agricultural production to agricultural credit use. Chandio et al. (2017) assert that formal loan plays an important role in the development of agriculture and ultimately in the development of the economy. They also show a positive correlation between loan supply and the increase in agricultural production in the country. Ansari (2001) states that farmers need financial resources to increase agricultural productivity and to obtain loans under easy terms and conditions. Therefore, it can be stated that the relationship between agricultural production and agricultural loan utilization, which is thought to be effective in increasing or sustaining agricultural production, should be investigated.

The main developments in recent years are the expansion in loan volume for agriculture and the sectoral orientation of private sector banking. However, the most important thing is the relationship between the loan expanding and growing of the agricultural sector (Güneş et al., 2017). So, in this study, it is aimed to examine the relationship between the agricultural loan and agricultural production value in the time period 2005-2018.

Agricultural loan develops along with internal and external factors such as structural status of agricultural enterprises, production and market conditions, farmer purchasing power, and parity (Güneş et al., 2017).

The agricultural enterprises are in the form of small family enterprises and that a certain period of time is required in order to obtain the product in the agricultural sector. That is, the lack of time coherence between income and expenditure requires the need for a loan in agriculture important (Turkey Agricultural Finance Summit, 2017). Producers' income from crop and animal production is related to their ability to provide production factors (Özden et al, 2012). On the other hand, agricultural loans are important financial instruments in providing production factors.

When the structure of agriculture in Turkey are examined, the total agricultural area of 23.180 (thousand ha) (TURKSTAT, 2018). Some field crop production quantity such as wheat, barley, corn, sugar beet, potato respectively; 20 000 000 tons, 7 000 000 tons, 5 700 000 tons, 18 900 000 tons, 4 550 000 tons. In 2018 total fresh fruits and vegetable production are respectively; 20 494 028 tons and 30 032 727 tons (TURKSTAT, 2018). Organic agricultural products' total quantity is 2 371 612

tons (transition period included). On the other hand, there are 8 419 204 cattle, 7 030 297 cattle crossbreeds, 1 593 005 domestic cattle, and 178 397 buffaloes. In the presence of small ruminants, there are 32 513 293 domestic sheep, 2 681 679 merinos, 10 698 553 head hair goats, 223 874 head angora goats. In 2018, the number of tractors is 1 332 139. According to the Turkish Statistical Institute (TURKSTAT) 2018 data, the share of agriculture in Gross Domestic Product (GDP) is 5.8%, including forest and fisheries. The agricultural sector with 216.6 billion GDP value has an important place for Turkey's economy. In the agricultural sector, which is economically and socially important, resources must be used effectively (Güneş et a., 2017). Therefore, in the agricultural sector, financial instruments are important in the efficient use of resources.

At Agricultural Finance Summit (2017) it was compared the share of agriculture in GDP and the share of loan volume in the developing countries. As a result, this comparison, it was seen that the share of agriculture in loan volume was lower than the share of agriculture in GDP. Also, in the prepared report, it was expressed that the agriculture of Turkey has a similar situation with developing countries and noted that the banking system is still unable to access adequate funding. The factors which affect the agricultural financing need in the Agricultural Finance Summit report listed as:

1. Agricultural enterprises are in the form of small family businesses. Due to a significant rate of the business that are more family-run agricultural businesses in Turkey, it is inadequate in terms of business size and capital accumulation. These enterprises with insufficient agricultural income and equity need other sources of funding. On the other hand, the small and divided structure of agricultural enterprises creates problems in the structure of the enterprise and hinders the development of enterprises and the creation of new financing opportunities in agricultural markets.

2. In the agricultural sector, where the products are mostly sold once a year, but the whole year is spent in the agriculture sector, the amount of usable capital is often insufficient since the turnover of capital is slow and it is difficult to create capital by saving. This inadequacy largely hinders the realization of the necessary activities to increase production, the rationalization of the enterprises, and thus the increase of the income of the farmer. The fact that agricultural production depends on natural resources which creates high risk and uncertainty.

3. As the innovations experienced in the industry and finance sector day by day require the renewal of the methods used in the preparation of the market and product supply as well as the mechanization in the agricultural sector, rapid mechanization and the adaptation of the farmers to the changes in the consumer preferences, they create new expense items in the agricultural sector and create the need for financing.

4. The fluctuations in the market prices that arise due to the supply and demand elasticities of agricultural products have a high impact on the income of agricultural enterprises. The farmers, who can not obtain the expected income, need foreign capital to continue their activities.

Yılmaz (2008) states that the share of the Republic of Turkey Ziraat Bank in total agricultural loans in 2003 was 88.15% and that of private banks was 11.85%. In addition, in 2004, the share of private banks in used agricultural loans increased to 18.81%, and in 2005, 26.77%. In Turkey Agricultural Finance Summit (2017) report, it was stated that the ratio of private banks had in 2017 to 31.8% in the year-end. In 2018, the total amount of agricultural loans extended to 77.8 billion Turkish Liras (TL) and Republic of Turkey Ziraat Bank (Agricultural Bank) had a share of approximately 80% with

62.2 billion TL. Private banks were also included in total agricultural loans with approximately 20%. As can be seen from the ratios of total agricultural loans for some years the Republic of Turkey Ziraat Bank has a significant share in providing agricultural financing. It is observed that the rate of private banks has increased in providing agricultural loans by years. Republic of Turkey Ziraat Bank gave almost all agricultural loans in Turkey until the mid-2000s (Güneş and Artukoğlu, 2010).

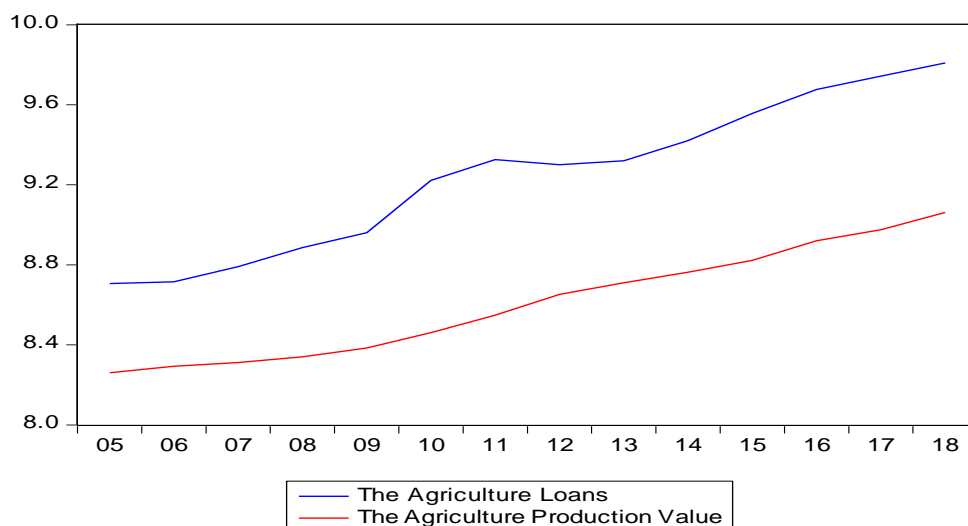


Figure 1. The trend of non-logarithmic time series in 2005-2018 period

After 2002, agricultural loans have started to increase, especially with the provision of loans to the agricultural sector by private banks and improvements in loan terms (Terin et al., 2014; Duramaz and Taş, 2018). There has been a significant increase in agricultural loan balances due to the interest shown by private banks in the sector and the subsidized loan application that has been implemented since 2004. Agricultural loan balance, which was 10 billion TL in 2007, reached 22.8 billion TL in 2010 and 61.3 billion TL in 2015 (Ünlüer and Güneş, 2016). Figure 1 shows the trend of the agricultural production value and agricultural loans between 2005 and 2018. When Figure 1 is examined, we can see that the value of agricultural production increases between the 2005- 2018 years and the amount of agricultural loans increases in years. But the agricultural loans are higher between 2009 and 2012 than from the other years.

The study is planned in four sections. In the introduction section of the research, the importance of agricultural loans in the agricultural sector and its effect on production, and information about agricultural loans in Turkey are explained. In the second section, the materials and methods of the study are mentioned. In the third section, the findings of the study are interpreted and discussed. Finally, the study is completed with conclusion.

## Materials and Methods

The aim of this study is to determine the relationship between agricultural production value and agricultural loans

for the 2005-2018 time period at an annual frequency. We use agricultural production value and agricultural loan data.

In this study, when we form the time series of agricultural loans provided by the banking sector, we do not exclude the 2008 global crisis data from the scope of analysis. Because in 2008, there was an expansion of loan volume (Hedlund and Kahn, 2009; Boeri and Guiso, 2007; Zandi, 2008). In order to see the effect of this loan volume expansion on the agricultural production value, we include the 2008 global crisis year in the scope of analysis. As a matter of fact, in the studies examining the relations between the agricultural loans and agricultural production value (Duramaz and Taş, 2018; Terin et al., 2014), we see that the 2008 global crisis year was included in the data.

The agricultural loan variable covers banking sector agricultural loans of Development and Investment Banks, Deposit Banks, and Participation Banks. Agricultural production value is the value of agricultural products produced in a year in TL. We obtain agricultural production value and banking sector agricultural loan data from the Republic of Turkey Central Bank Electronic Data Distribution System (EDDS). Time series are included in the analyses in logarithmic form.

The functions of the deposit banks, participation banks and, development and investment banks are different between each other. However, in this study, we include the total of the loans given by these banks in TL in the data. Furthermore, such

as the Republic of Turkey Central Bank and the Banks Association of Turkey institutions declare the agricultural loans given by the banks. In this study, only the agricultural loans given by the banks operating in the Turkish banking sector in TL are taken into consideration.

If the average of a time series does not change over time, it is considered that these series are stationary. Mean and variance of non-stationary time series change over time. If the time series is not stationary, they are made stationary taken the difference from the first and second or higher levels. The stationarity of time series is investigated by unit root tests in the literature (Gujarati 1995). In this study, we use the Phillips-Perron unit root test, which takes structural breaks into consideration, to determine the stationarity levels of time series. In the Phillips-Perron unit root test, the null hypothesis is that the variable contains unit root at the level value. As the alternative hypothesis is that the variable does not contain unit root at the level value.

After determining the stationarity levels of the time series included in the study, we use the Granger causality test to examine whether there is a causality relationship between the variables and to research the direction of causality relationship if there is any causal relationship between the agricultural production value and the agricultural loans.

When the direction of the relationship between economic variables can not be determined by economic theory, the existence and direction of the interaction between the variables can be detected by Granger (1969) causality test. In this test, the variables are not separated as a dependent and independent variable. The interaction between variables can be analyzed simultaneously in the Granger causality test. For this reason, we use the Granger causality analysis in this study (Doğan et al., 2016).

The Granger causality test starts with the determination of the optimum lag length that minimizes the Akaike Information Criterion (AIC) or Schwartz Information Criterion (SIC) obtained by regressing the dependent variable with its own lagged values (Yapraklı and Güngör 2007). In the Granger causality test, the presence of the causality relationship (→ indicates the direction of the causality relationship) requires

**Table 2.** Phillips-perron unit root test results

Variables	Level Values		First Difference Values		Second Difference Values	
	Constant	Constant/Trend	Constant	Constant/Trend	Constant	Constant/Trend
Agricultural Loans	0.22(6)	-2.17(3)	-3.77(11) <sup>(b)</sup>	-3.72(3) <sup>(c)</sup>	-	-
Agricultural Production Value	1.67(1)	-3.42(2)	-1.82(1)	-2.25(0)	-4.1(2) <sup>(b)</sup>	-4.42(3) <sup>(c)</sup>

\*<sup>(b)</sup> and <sup>(c)</sup> indicate statistical significance at 5% and 10% levels.

\*Values in parentheses represent the optimum lag lengths for the Phillips-Perron unit root test.

The results of the Phillips-Perron unit root test in Table 3 show that the null hypothesis that the agricultural loan variable contains unit root at level value is rejected and the variable is stationary in the first difference. Furthermore, the findings reported in Table 3 indicate that for the agricultural production value variable, the null hypothesis, which the variable contains unit root at the level value, is rejected and the variable becomes stationary in the second difference as

the following hypothesis to be rejected.

$$\text{For } Z \rightarrow P: H_0 : \sum_{i=1}^r \lambda_i = 0 \tag{1}$$

$$\text{For } P \rightarrow Z: H_0 : \sum_{i=1}^s \phi_i = 0 \tag{1}$$

In Equality (2) and Equality (3), there is a mathematical representation of the Granger causality test applied in this study.

$$\text{Agricultural Loans} = \theta_0 + \sum_{i=1}^n \theta_1 \text{Agricultural Loans}_{t-1} + \sum_{i=1}^n \delta_1 \text{Agricultural Production Value}_{t-1} + \varepsilon_t \tag{2}$$

$$\text{Agricultural Production Value} = \theta_0 + \sum_{i=1}^n \theta_1 \text{Agricultural Production Value}_{t-1} + \sum_{i=1}^n \delta_1 \text{Agricultural Loans}_{t-1} + \varepsilon_t \tag{3}$$

**Results and Discussion**

In this part of the study, firstly we present descriptive statistics of related variables to examine the relationship between agricultural production value and banking sector agricultural loans. Then, we examine the stationarity levels of related variables by the Phillips-Perron unit root test, and finally, we use the Granger causality test to determine whether there is a causal relationship between the variables. Descriptive statistics of agricultural loans and agricultural production value time series are reported in Table 1.

**Table 1.** Descriptive statistics

Variables	Mean	Standart Deviation	Minimum	Maximum
Agricultural Loans	9.25	0.38	8.7	9.8
Agricultural Production Value	8.61	0.27	8.26	9.06

According to the descriptive statistics in Table 1, we can say that the agricultural loan variable fluctuates more than the agricultural production value variable. Similarly, the average of the agricultural loan variable is greater than the average of the agricultural production value variable. After we present the descriptive statistics, we perform the unit root test to determine the stationarity levels of the variables and report in Table 2.

[2]. After the determination of stationarity by the Phillips-Perron unit root test, we perform the Granger causality test and present it in Table 3.

The findings in Table 3 indicate that there is a unilateral causality relationship from the agricultural loan variable to the agricultural production value variable. In this case, it can be said that agricultural loans affect the agricultural production value. Such that, our results are in line with theoretical



expectations; but also, our results are opposite with the claim of Terin et al. (2014). Because Terin et al. (2014) argue that agricultural production affects agricultural loans.

In the related literature, the loan increases agricultural production and the effect of the loan on agricultural

production is positive and significant (Saleem and Jan ,2011; Chandio et al., 2016). Guirkingner and Boucher (2007) find that if all credit restrictions were removed in Peru, the agricultural production value in Peru would increase by 26%. These findings are consistent with the findings that we obtain in our study.

**Table 3.** Granger causality test results

Dependent Variable-Independent Variable	F Statistic Value	Probability Value
Agricultural Loans-Agricultural Production Value	3.87	0.08
Agricultural Production Value-Agricultural Loans	0.03	0.85

\*The optimum lag length is 1, based on AIC and SIC

## Conclusion

The agricultural loan has an important role in the development of the agricultural sector, especially in developing countries. Because, it is one of the financial instruments needed in agricultural enterprises to benefit from new production technologies and marketing opportunities. In this study, it is aimed to present the relationship between agricultural loans and agricultural production value. As a result of the study, it is concluded that there is a relationship between agricultural loans and agricultural production value. The Granger causality analysis shows that the changes in agricultural loans affect the changes in the agricultural production value. That is, the direction of the relationship between the two variables is from agricultural loans to agricultural production value. For this reason, it is thought that loans will contribute to the increase of agricultural production value in facilitating the use of loans in the agricultural sector, increasing the institutions providing loans, supplying the input needs of the producers, marketing the products, and expanding the area of activity or investment. As a result, the findings show that there is an effect from the loan used to agricultural production value is in line with theoretical expectations. It can be said that loan expansion will increase the welfare of farmers as it allows both higher consumption and the use of more purchased inputs. The contribution of the study to the literature can be expressed as follows. This study is one of the few research to examine the relationship between the agricultural production value and agricultural loan in Turkey. This aspect of the study is expected to fill an important gap in the literature. In addition, the study has the ability to show evidence for future studies on determining factors affecting agricultural production value. Another contribution of the study to the literature is to provide evidence for detecting the relationship between the agricultural loan and agricultural production value for policymakers in decision making.

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## RESEARCH ARTICLE

### Effects of Bacteria and Iba on the Rooting of Citrange Citrus Rootstocks Cuttings

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#### ABSTRACT

In this study, the effects of *Agrobacterium rubi* A-18 and *Bacillus* OSU-142 bacterial strains alone and in combination with 1000, 2000, 4000 ppm IBA on rooting of Carrizo citrange and Troyer citrange citrus rootstock in softwood, semi-hardwood and hardwood cuttings were investigated. In the case of IBA solution, *Agrobacterium rubi* A-18 and *Bacillus* OSU-142 were prepared in solution at a concentration of  $1 \times 10^9$  bacteria / ml and applied to cuttings of citrus. Application were performed in the mist propagation system. Cuttings kept in the fogging environment for 3 months removed at the end of this period, and their rooting rates (%), callus formation rates (%) and survival rates (%) determined. Based on the results of the study, the survival rates and callus formation rates of the cuttings of citrus were generally high at the end of the rooting period. The highest rooting rates in Carrizo citrange cuttings were detected in 4000 ppm IBA and OSU-142 + A-18 (13.33%) treatments for softwood cuttings, 4000 ppm IBA + OSU-142 (20.00%) in semi-hardwood and hardwood cuttings. In the Troyer citrange, OSU-142 + A-18 (6.67%) treatments for softwood cuttings, in 1000 ppm IBA for semi-hardwood, and in 4000 ppm IBA (13.33%) and 1000 ppm IBA treatment for hardwood cuttings, 2000 ppm IBA and OSU-142 (13.33%) the highest rooting has been treatments. According to the cuttings pick-up period, the rooting rates of Carrizo citrange are not different, at the Troyer citrange in the semi-hardwood and hardwood cuttings was higher. As a result, it could be state that plant growth promoting bacteria and IBA applications have not effect on rooting in the softwood, semi-hardwood and hardwood cuttings of the Carrizo citrange and Troyer citrange citrus rootstocks.

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#### Introduction

Turkey is one of the rare countries where a combination of many types of fruit grown in world and, also one of the major producers of many fruit species (Gerçekçiöğlü et al. 2008).

Citrus group includes citrus fruits of high economic value such as citrus, orange, mandarin, bergamot, grapefruit and lemon. They are produced economically, and they are extremely important for human health. Citrus fruits, which are described as vitamins stores in winter months, are consumed

extensively as fruit juice. Citrus is cultivated between 40° north and 40° south latitude in the world, and its production is constantly increasing (Mendilcioğlu, 1999).

Citrus, whose homeland is China, Southeast Asia and India, can grow in tropical and subtropical climatic areas, and can be grown commercially in regions where the temperature does not fall below -4°C.

In Turkey, reaching about 5 million tons of citrus is done in coastal areas of the Mediterranean and Aegean regions of production. After a maximum of apples and grapes are grown

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in Turkey and also made the most exports of citrus fruits. Commercial sense in the production of Turkey is examined, as well as most types of citrus produced in the world, respectively, orange, mandarin, lemon and grapefruit.

Citrus farming, whose homeland is Southeast Asia, began in the US in the modern sense in the 19th century and spread rapidly. It is produced economically in the Northern Hemisphere, North and Central America and the Mediterranean countries, and in the Southern Hemisphere in South America, South Africa and Oceania. The world's largest citrus producing countries; Brazil, USA and China. Turkey is among the first 10 countries in the world citrus production (FAO, 2019).

Turkey is located in the northern border areas of the world citrus production. Turkey has extremely convenient to citrus growing areas in terms of ecological conditions. Turkey citrus production 4.769.772 tons in 2017(TUIK, 2019).

The prevalence of foreign fertilization has brought vegetative propagation methods to the forefront in most of the fruit species and varieties. Thus, expansions resulting from seed propagation can be prevented and all the properties of the variety can be preserved. Vegetative propagation; cutting, dipping, root and bottom shoots, or one or more of the tuber propagation methods are used, but today, especially in fruit growing method is used more widely by propagation (Rom, 1987; Hartmann et al. 1990).

Like other fruit species, the production of citrus species does not directly involve seed propagation. As the production of cutting is not very successful in this type of production is based on the reproduction of grafting. Grafting is based on the cultivation of different plants on the same trunk, including rootstock and scion. There are two elements in grafting and reproduction, rootstock and scion. While the scion forms the crown part of the tree, the rootstock forms the subsoil part of the tree and assumes the soil to hold onto the soil and undertakes tasks such as the uptake and transport of water and nutrients. In fruit growing, rootstocks are as important as the varieties grafted on them. As a matter of fact, although a fruit rootstock is not expected to have the characteristics of a standard variety, the rootstocks should have good performance in the special relations formed under the ground and the scion. Roots are the living part that undertakes first degree responsibility in the life of trees (Gülcan, 1991).

Rootstocks used in fruit production are classified as generative and vegetative rootstocks according to their production methods. Because of the generative way, namely seed rootstock production has been opened, there has been rapid increase in the use of clone rootstocks in fruit sapling production in recent years. The use of clone rootstocks is increasingly common in the production of citrus fruit species in order to increase yield and quality and to tolerate many abiotic and biotic stresses. Among the subtropical climate fruit trees, the most common use of clonal rootstocks in the world is citrus.

The rootstocks used in fruit growing are divided into two as seedling and clone rootstocks. Most of the fruit species are

seedling rootstocks. The common drawback of almost all of these is that they show a large amount of variation. This situation adversely affects the homogeneity of the tree development. It is also known that heterogeneous depressions have different behaviors in terms of rootstock and mismatch and environmental adaptation (Gülcan, 1991).

Different rootstocks are used in the production of citrus. The most important of these are citrus and hybrids, trifoliolate orange and hybrids, mandarin and mandarin likes, lemons and their relatives, limes and relatives, oranges, citremon (trifoliolate orange X lemon), sitrumelo (trifoliolate orange X grapefruit), sweet lime, grapefruit, yuzu, volkameriana, macrophylla, citranges (orange X trifoliolate oranges). In recent years, citranges have also started to be used (Mendilcioğlu, 1999).

Citrus is used as rootstock in citrus plants are experiencing difficulties in root rooting. To solve the problem of rooting amino acid (Pedrotti et al. 1994), indole acetic acid (De Klerk et al. 1997; Ahmad et al. 2005), some vitamins (Antonopoulou et al. 2005) applications are made. In addition to these, it has been reported in many studies that it has been presented as a solution to the problem of rooting with rhizobacteria that increase plant growth, which has recently become widespread (Larraburu et al. 2007; Teixeira et al. 2007).

In this study, the effects of *Bacillus* OSU-142 and *Bacillus* A-18 bacterial strains and indole butyric acid applications on the rooting of Carrizo and Troyer citrus rootstocks were investigated.

## Materials and Methods

### Materials

This study was carried out in the heated glass greenhouse in Adana between 2017- 2018. Carrizo and Troyer citrange (*Citrus sinensis* X *Poncirus trifoliata*) rootstocks were used in the study. The cuttings used in the study were obtained from Alata Horticultural Research Institute (Mersin). Carrizo citrange resistant to drought and nematod. It can withstand pH up to 7.6. It has a positive effect on the quality of grafted mandarins. Growth force is greater than Troyer. For tristeza resistance is better than orange (Mendilcioglu, 1999). Troyer citrange very similar to trifoliolate orange. It is used as rootstock in Aegean Region. Creates 80-100% nucellar plant. It can grow at pH 8-8.5 and is resistant to dry soil, lime and cold. Development is good, especially in satsuma mandarin is very positive effect on fruit quality (Mendilcioglu, 1999). In this study *Agrobacterium rubi* A-18 and *Bacillus* OSU-142 bacterial strains which were determined to produce auxin by in vitro studies were used. Bacteria were obtained from Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering.

### Methods

The cuttings were prepared in July (2017) as softwood cutting, October (2017) as semi-hardwood cutting and January (2018) as hardwood cutting. The cuttings used in the research were obtained from trees from Alata Horticultural Research

Institute. The cuttings were prepared as 4-knot, 2-knot leafless, top 2-knot leaf planted. *Agrobacterium rubi* A-18 and *Bacillus* OSU-142 bacteria strains were applied to these cuttings with 1000, 2000 and 4000 ppm IBA alone and in combination. Applications to cuttings are given below.

1. Control
2. 1000 ppm IBA
3. 2000 ppm IBA
4. 4000 ppm IBA
5. Application of OSU-142
6. Application of A-18
7. Combination of OSU-142 + A-18
8. 1000 ppm combination of IBA + OSU-142
9. Combination of 2000 ppm IBA + OSU-142
10. Combination of 4000 ppm IBA + OSU-142
11. Combination of 1000 ppm IBA + A-18
12. Combination of 2000 ppm IBA + A-18
13. Combination of 4000 ppm IBA + A-18

IBA was applied to the prepared cuttings by fast dipping in solution and bacterial strains were prepared in suspension at a concentration of  $1 \times 10^9$  bacteria / ml (Pırlak and Baykal, 2011). The applied cuttings were placed in a mist propagation unit with temperature of 25°C, 90-95% relative humidity and perlite. Cuttings kept in mist propagation environment for approximately 3 months were removed at the end of this period, rooting rates (%), callus formation rates (%) and survival rates (%) were determined (Bhusal et al. 2001). While determining the survival rates of the cutted cuttings, it was examined whether the tissue under the steel shells were alive or not, and they were cross-sectioned from the eyes on the cutting.

### Statistical Analysis

Trial; two factors (applications, cuttings retrieval period) according to the completely randomized design, three replicates and 5 cutting in each repetition have been established. The data obtained were subjected to arc sinus (angle) transformation and evaluated by SPSS statistical program and Duncan Multiple Comparison Test was applied.

## Results

### The Effects of Applications on the Cutting Rooting of Carrizo Citrange

The effects of bacteria and IBA applications on the rooting of softwood, semi-hardwood and hardwood cuttings in Carrizo citrange were significant (Table 1). The viability rate of softwood cuttings was 73.33% in the control application and the viability rate was lower than the control in applications other than 1000 ppm IBA. The lowest viability rates were determined in OSU-142 + A-18 and 1000 ppm IBA + A18 (40.00%) and highest in 1000 ppm IBA application (86.67%). The callus rate, which was 66.67% in the control application, increased in 1000 ppm IBA application (86.67%). The lowest callus formation rates were found in 4000 ppm IBA + OSU-142 and

1000 ppm IBA + A-18 (26.67%). The differences between the effects of applications on rooting of green cuttings were insignificant. Rooting rates were generally low, the highest rooting applications occur with a ratio of 13.33% of 4000 ppm IBA and OSU-142 + A-18 (Table 1).

In practice, most of the Carrizo citrange semi-hardwood cuttings remained alive. The viability rate of 100.00% in the control was lower than the control in 2000 ppm IBA (93.33%) and A-18 (86.67%) applications and all the cuttings maintained their viability in other applications. In callus formation, while some of the applications were significant in the same group as control, some of them decreased significantly compared to control. The lowest callus rates were determined in 1000 ppm IBA and 4000 ppm IBA + A-18 (66.67%). Despite the high rates of viability and callus formation in Carrizo citrange semi-hardwood cuttings, the rooting rates remained below the expected level. Rooting rate of 6.67% in the control was found to be the same as the control in 6 applications, only OSU-142 + A-18 (13.33%), 1000 ppm IBA + OSU-142 (13.33%) and 2000 ppm IBA + OSU-142 (20.00%) applications increased compared to control. 3 applications (1000 ppm IBA + A-18, 2000 ppm IBA + A-18, 4000 ppm IBA + A-18) did not occur rooting (Table 1).

The viability and callus formation rates of hardwood cuttings were generally high. While the viability rate was 100.00% in 9 applications with control application, viability was found to be lower than control in 4 applications (2000 ppm IBA, OSU-142, A-18, 1000 ppm IBA + A-18). The rate of callus formation was found to be 100.00% in 5 applications (4000 ppm IBA, OSU-142 + A-18, 1000 ppm IBA + OSU-142, 2000 ppm IBA + OSU-142) with the control and decreased in the other applications compared to the control. Rooting rate of Carrizo citrange cuttings was found to be low in woody cuttings as in softwood and semi-hardwood cuttings. While rooting does not occur in the control application, rooting has occurred at different rates in 8 applications, the highest rooting was detected in 2000 ppm IBA + OSU-142 application (20.00%) (Table 1).

When the effects of bacteria and IBA applications according to cutting uptake periods are examined in Carrizo citrange rootstock, it is seen that the differences between the effects of applications on viability and callus formation are statistically significant and the effects on rooting are insignificant (Table 1). The average viability of softwood cuttings was found to be statistically lower than that of semi-hardwood and hardwood cuttings. While the viability rate of softwood cuttings was 60.59%, it was 98.46% for semi-hardwood cuttings and 96.41% for hardwood cuttings. Similarly, in callus ratios, softwood cuttings lagged behind semi-hardwood and hardwood cuttings. The ratio of callus was found to be 49.47% in softwood cuttings, 88.21% in semi-hardwood cuttings and 87.69% in hardwood cuttings. Rooting rates of the cuttings obtained in three different periods were found to be very low and no statistical difference was found between the periods.

**Table 1.** The effects of applications on the cutting rooting of Carrizo citrange

	CARRIZO CITRANGE								
	SOFTWOOD CUTTINGS			SEMI-HARDWOOD CUTTINGS			HARDWOOD CUTTINGS		
	Viability rate (%)*	Callus formation rate (%)	Rooting rate (%)	Viability rate (%)*	Callus formation rate (%)	Rooting rate (%)	Viability rate (%)*	Callus formation rate (%)	Rooting rate (%)
Control	73.33 b**	66.67 b	0.00	100.00 a	100.00 a	6.67 b	100.00 a	100.00 a	0.00 b
1000 ppm IBA	86.67 a	86.67 a	0.00	100.00 a	66.67 c	6.67 b	100.00 a	80.00 b	0.00 b
2000 ppm IBA	60.00 bcd	53.33 bc	6.67	93.33 b	93.33 a	6.67 b	93.33 ab	93.33 ab	6.67 ab
4000 ppm IBA	66.67 bc	53.33 bc	13.33	100.00 a	100.00 a	6.67 b	100.00 a	100.00 a	6.67 ab
OSU-142	66.67 bc	53.33 bc	6.67	100.00 a	80.00 b	6.67 b	86.67 b	80.00 b	6.67 ab
A-18	66.67 bc	66.67 b	6.67	86.67 b	80.00 b	6.67 b	86.67 b	80.00 b	6.67 ab
OSU+A-18	40.00 e	33.33 cd	13.33	100.00 a	100.00 a	13.33 ab	100.00 a	100.00 a	13.33 ab
1000 ppm IBA+OSU-142	53.33 cde	53.33 bc	6.67	100.00 a	100.00 a	13.33 ab	100.00 a	100.00 a	13.33 ab
2000 ppm IBA+OSU-142	66.67 bc	33.33 cd	6.67	100.00 a	100.00 a	20.00 a	100.00 a	100.00 a	20.00 a
4000 ppm IBA+OSU-142	60.00 bcd	26.67 d	0.00	100.00 a	100.00 a	6.67 b	100.00 a	80.00 b	6.67 ab
1000 ppm IBA+A-18	40.00 e	26.67 d	6.67	100.00 a	80.00 b	0.00 b	86.67 b	80.00 b	0.00 b
2000 ppm IBA+A-18	60.00 bcd	60.00 b	6.67	100.00 a	80.00 b	0.00 b	100.00 a	80.00 b	0.00 b
4000 ppm IBA+A-18	46.67 de	33.33 cd	0.00	100.00 a	66.67 c	0.00 b	100.00 a	66.67 b	0.00 b
LSD	16.00	18.47	N.S.	14.27	17.94	20.28	17.48	22.26	17.21
				Viability rate (%)	Callus formation rate (%)	Rooting rate (%)			
				SOFTWOOD CUTTINGS	60.59 b	49.47 b	5.64		
				SEMI-HARDWOOD CUTTINGS	98.46 a	88.21 a	7.18		
				HARDWOOD CUTTINGS	96.41 a	87.69 a	6.15		
				LSD	12.72	13.65	N.S.		

\*Statistical analysis have been carried out using arc sin values.

\*\*Values shown in different letters in the same column are different at 0.05 (Duncan test)

### **The Effects of Applications on the Cutting Rooting of Troyer Citrange**

Plant growth-promoting bacteria and IBA applications on effects of rooting of Troyer citrange rootstock softwood, semi-hardwood and hardwood cuttings are given in Table 2. The effects of the applications on the viability, callus formation and rooting of the steels were found to be statistically significant.

Most of the Troyer citrange softwood cuttings remained viable at the end of the rooting period. In the control, the viability rate increased from 66.67%, but the effects of OSU-142 + A-18 and 4000 ppm IBA + OSU-142 applications where only the highest viability rates were determined (93.33%) were statistically different from the control group (Table 2). The callus forming cuttings ratios were lower than the viability, except that OSU-142 + A-18 had no effect on callus formation compared to control. The highest callus formation with 93.33% OSU-142 + A-18 application, the lowest occurred with 40.00% A-18 application (Table 2). Although most of the Troyer citrange softwood cuttings maintained their viability and formed callus, rooting did not occur except OSU-142 + A-18 application, and the rooting rate was as low as 6.67%.

The viability rates of the semi-hardwood cuttings were found to be high, and the application of 1000 ppm IBA + A-18, where only 80.00% viability was determined, was statistically different from the other group. Similarly, callus formation was

found to be high in semi-hardwood cuttings. Callus rate of 80.00% in control was found to be close to control in IBA and bacterial applications, and 1000 ppm IBA application with the highest rate of callus formation with only 100.00% rate was statistically different from other applications. Similar to softwood cuttings, semi-hardwood cuttings have high viability and callus formation rates, but rooting rates are well below satisfactory levels. Only 4 applications were rooting with the control. They are 6.67% control and 2000 ppm IBA and 13.33% with 1000 ppm and 4000 ppm IBA applications (Table 2).

Viability ratios were also high in hardwood cuttings. Applications generally had a positive effect on viability. The viability rate of 80.00% in control was found to be the same as control in OSU-142 and 1000 ppm IBA + A-18 applications, but higher in control in other applications. Callus formation is also high in hardwood cuttings. In general, no effect on callus formation was observed, and only 1000 ppm IBA (100%) and 4000 ppm IBA (93.33%) increased callus rates compared to control. As with Troyer citrange green and semi-hardwood cuttings, the positive effects of applications on rooting have not been determined. The rooting rates were low in 5 applications with the control, while the rooting rates in other applications were 0.00%. Rooting occurred in applications with 6.67% control and 4000 ppm IBA and 13.33% with 1000 ppm IBA, 2000 ppm IBA and OSU-142 (Table 2).

**Table 2.** The effects of applications on the cutting rooting of Troyer citrange

	TROYER CITRANGE								
	SOFTWOOD CUTTINGS			SEMI-HARDWOOD CUTTINGS			HARDWOOD CUTTINGS		
	Viability rate (%)*	Callus formation rate (%)	Rooting rate (%)	Viability rate (%)*	Callus formation rate (%)	Rooting rate (%)	Viability rate (%)*	Callus formation rate (%)	Rooting rate (%)
Control	66.67 b**	66.67 bc	0.00 b	100.00 a	80.00 bc	6.67 ab	80.00 c	80.00 c	6.67 ab
1000 ppm IBA	73.33 b	66.67 bc	0.00 b	100.00 a	100.00 a	13.33 a	100.00 a	100.00 a	13.33 a
2000 ppm IBA	73.33 b	73.33 bc	0.00 b	100.00 a	80.00 bc	6.67 ab	100.00 a	80.00 c	13.33 a
4000 ppm IBA	73.33 b	73.33 bc	0.00 b	100.00 a	93.33 ab	13.33 a	100.00 a	93.33 ab	6.67 ab
OSU-142	80.00 b	53.33 cd	0.00 b	100.00 a	66.67 c	0.00 c	80.00 c	66.67 c	13.33 a
A-18	66.67 b	40.00 c	0.00 b	93.33 a	73.33 bc	0.00 c	93.33 b	73.33 c	0.00 b
OSU+A-18	93.33 a	93.33 a	6.67 a	100.00 a	80.00 bc	0.00 c	100.00 a	80.00 c	0.00 b
1000 ppm IBA +OSU-142	80.00 b	66.67 bc	0.00 b	100.00 a	80.00 bc	0.00 c	100.00 a	80.00 c	0.00 b
2000 ppm IBA +OSU-142	73.33 b	66.67 bc	0.00 b	100.00 a	80.00 bc	0.00 c	100.00 a	80.00 c	0.00 b
4000 ppm IBA +OSU-142	93.33 a	73.33 bc	0.00 b	100.00 a	80.00 bc	0.00 c	100.00 a	73.33 c	0.00 b
1000 ppm IBA +A-18	73.33 b	66.67 bc	0.00 b	80.00 b	66.67 c	0.00 c	80.00 c	66.67 c	0.00 b
2000 ppm IBA +A-18	66.67 b	53.33 cd	0.00 b	100.00 a	93.33 ab	0.00 c	100.00 a	86.67 bc	0.00 b
4000 ppm IBA +A-18	86.67 ab	80.00 b	0.00 b	100.00 a	80.00 bc	0.00 c	100.00 a	80.00 c	0.00 b
LSD	22.16	18.25	10.09	18.00	29.03	20.18	10.09	21.68	22.57
				Viability rate (%)	Callus formation rate (%)	Rooting rate (%)			
				SOFTWOOD CUTTINGS	76.92 b	67.18 b	0.51 b		
				SEMI-HARDWOOD CUTTINGS	97.95 a	81.03 a	3.08 a		
				HARDWOOD CUTTINGS	93.33 a	80.00 a	4.10 a		
				LSD	11.93	5.07	2.51		

\*Statistical analysis have been carried out using arc sin values.

\*\* Values shown in different letters in the same column are different at 0.05 (Duncan test)

The effects of treatments on viability, callus formation and rooting were found to be statistically significant compared to the cutting uptake periods in the Troyer citrange rootstock. The viability, callus formation and rooting rates were higher in the semi-hardwood and hardwood cuttings than the softwood cuttings. The average viability was 76.92% for softwood cuttings, 97.95% for semi-hardwood cuttings and 93.33% for hardwood cuttings; callus formation rates were 67.18% for softwood cuttings, 81.03% for semi-hardwood cuttings, 80.00% for hardwood cuttings; rooting rates were 0.51%, 3.08% and 4.10% respectively.

## Discussion

In this study, the effects of plant growth promoting rizobacteria and IBA applications on rooting, callus formation and survival rates of Carrizo citrange and Troyer citrange rootstocks in softwood, semi-hardwood and hardwood cuttings were investigated. The highest rooting rates were 4000 ppm IBA and OSU-142 + A-18 (13.33%) in Carrizo citrange softwood cuttings, 4000 ppm IBA + OSU-142 (20.00%) in semi-hardwood and hardwood cuttings; Troyer citrange softwood OSU-142 + A-18 (6.67%), 1000 ppm IBA and 4000 ppm IBA (13.33%) and 1000 ppm IBA, 2000 ppm IBA and OSU-142 applications in semi-hardwood cuttings (13.33%) was determined (Table 1, 2). Similar results were obtained in a study conducted on M9 apple rootstock cuttings, while bacterial and IBA applications increased the viability and callus formation rates of the

cuttings, it was determined that they had no effect on rooting (Pirlak and Baykal, 2009). When the cuttings are placed in a suitable environment for rooting, callus layer forms at the bottom of the cuttings. Conductive tissue cambium and callus tissue formed in the adjacent phloem region may in some cases consist of various cells in the cortex and core. The protective layer formed by the callus tissue delays the decay of the cutting from the bottom. In some cases, it is shown that the callus layer helps the cutting to absorb water (Hartmann et al. 1990). However, there are different views on the relationship between callus tissue and root formation. According to Girouard (1967), xylem elements that differ in the dense callus tissue generally seen in the cuttings of hard-rooted plants determine the starting point of root formation. Hartmann et al. (1990) states that callus formation and root formation are independent events. Again, Tayfun (1995) in a study made by kiwifruit, while a low rooting in the wood cuttings, a high callus formation occurred in the same conditions. The researcher interpreted this situation as inhibiting rooting and root development due to excessive callus formation.

Due to its many advantages, cutting propagation is widely used in fruit growing as in most plant species. These advantages include a small body part and a large number of homogeneous plants in a small area, being cheap, quick and easy. In addition, this propagation method is less likely to cause soilborne diseases into plants. In spite of all the positive properties of cutting propagation, the biggest obstacle that restricts its use is that the cutting cannot be rooted due to the

low regeneration ability of some species (Rugini and Fedeli, 1990; Webster and Looney, 1996).

The low rooting rates in the citrus rootstock cuttings examined are related to the species characteristics. The rooting ability of cuttings in fruit species shows great differences even between different species and varieties within these species. Accordingly, species are classified as very easy-rooted, hard-rooted and very hard-rooted. Citrus species in this grouping is often included in the hard-rooted (Hartmann et al., 1990). As a matter of fact, positive results have been obtained in studies investigating the effects of bacterial applications on genetically rooting species (Nagarajan et al., 1989; Bassil et al., 1991; Jacob and Hamdam, 1992; Hatta et al., 1996; Sarmast et al., 2012; Arıkan et al., 2015; Kinik and Celikel, 2017).

In general, in citrus fruits propagated by grafting, very good results except for lemon have not been obtained in steel propagation so far (Cooper, 1935). In the study examining the effects of IBA and Paclobutrazol on steel rooting in Valencia orange cultivar, the highest steel rooting (19.6%) was obtained from 500 ppm IBA + Paclobutrazol (Habermann et al., 2006). In the study which investigated the effects of IBA applications on cutting rooting in different citrus rootstocks, trifoliolate orange, Carrizo citrange, Cleopatra mandarin, Citrumelo 1452 rootstocks control and different rooting applications could not be detected (Uzun and Seday, 2011). In the study examining the effects of IBA and cycloposphamide applications on rooting of Citrus jambhiri cuttings, the highest rooting rate (8.2%) was obtained by using IBA and cycloposphamide together (Singh et al., 1987).

Although the rooting rate of the cuttings is very low, it is seen that the rooting of the semi-hardwood and hardwood cuttings is higher than the softwood cuttings. It is stated that rooting is better if cutting is taken in a certain season or month of each plant species grown with cutting (Güleryüz, 1987). In a study conducted by Pırlak (1997) cranberry, it was determined that the rooting rates of cuttings taken at different periods were different.

## Conclusion

In conclusion, in this study, the effects of IBA and bacterial applications on cutting rooting in Carrizo citrange and Troyer citrange rootstocks were not sufficient. This study mainly focuses on the effects of plant growth enhancing bacteria which are used as an alternative in cutting rooting studies in recent years. In different studies, it can be investigated whether there will be an increase in rooting rates by experimenting with different breeds of these bacteria and their combinations with different growth regulators.

In addition, the viability and callus rates in the cuttings are quite high, suggesting that the rooting rates may increase if the 3-month period generally used in cutting rooting applications is increased slightly.

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## RESEARCH ARTICLE

### Mix Farming Based on Sago Palm in Meranti Island District, Riau Province, Indonesia

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#### ABSTRACT

Sago can be used as raw material for sugar and bioenergy on a national scale because Indonesia has huge sago area especially in the eastern part of Indonesia. Sago can grow well in peat soil. The aim of this research is to develop peat soil optimally at Tanjung Peranap Village, Meranti Island District, Riau Province. The research involved land owner in the region burned down in 2016, extension service and local government. Research form activities indicates that the local people income increase because they harvested cayenne chili, big red chili, sweet corn, baby corn, corn kernel, green kale and watermelon. Their income was Rp 7200000, Rp 2625000, Rp 2774400, Rp 4368000, Rp 2995230, Rp 5400000 and Rp 4900000 respectively. Various income from mixed cropping can change the farmer mind. They realize that mixed cropping can be reliable as an income source and change their activity from destroyed the forest and mangrove to cultivated mixed cropping. It can minimize the environmental damage.

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#### Introduction

Sago is a palm plant that has high starch production potential as well wide area. Production of sago starch reaches 200-400 kg palm<sup>-1</sup> (Bintoro et al., 2010). Indonesia is the country that has the widest sago area in the world, which is 5.5 million ha (Bintoro et al., 2016). Of these areas, most are in Papua Province and West Papua of 5.2 million ha (Djoefrie et al., 2014), the rest is spread over several islands large and entering the periphery and border areas (Bintoro et al., 2017).

Sago starch generated has many derivative products that can be developed in the future.

Utilization of sago starch in Indonesia has been used for household purposes and industry. Sago starch can be used as noodles, sago rice, liquid sugar, bioethanol, biofoam, environmentally friendly plastics and pharmaceuticals (Ramadhan et al., 2015; Karouw et al., 2015; Komarayati et al., 2011; Kamsiati et al., 2017; Pandey et al., 2015). Waste results sago pith dissolution can be used as animal feed, mushroom and crop media organic fertilizer (Bintoro et al., 2010). Sago starch content is healthier than other starch,

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because sago starch has a low glycemic index so good for of prediabetes (Hariyanto et al., 2017).

Sago is one of the plants that thrive on peatlands. Ecosystems wet peat in accordance with sago growing environment. Sago growing on the land peat is largely not done by community maintenance. The habit difficult to change because sago is a perenial plant that takes time a long harvest, which is about 10 years old. No maintenance of sago on peatlands have an impact on low and unsustainable sago growth sago land becomes unproductive plus the economic problems of society namely the debt bondage system. Communities in the peatlands are familiar with the debt bondage system, due to increasing economic needs, while employment not available. Communities in the peatlands, especially the coastal areas have been converted mangrove loggers. It has a negative impact on the coastal environment there is abrasion. The big problems that occur in peatlands are related to the ecosystem peat fire. Fires have already hit the existing peat and non-peatlands in Indonesia by 2015 with an area of 2 million ha (BNPB, 2015). Problems it should be anticipated so as not to reoccur one of them with effort utilization of sago area with cultivation technique appropriate with peat ecosystem.

Sago newly planted area with a spaced distance between sago plants can cultivate horticultural crops, fishing and livestock activities. Efficiency land use in sago plantation can increase economic income community from the sale of non-sago products. Integrated farming system with sago main commodity suitable for development on peatland. Institutional strengthening through farmer groups can support the sustainability of the model. Meranti Island District are one of the sago-producing areas in the peatlands the largest in Riau Province, but the people's economy has not yet been felt maximum. Therefore, the integrated agricultural model of sago, duck, cattle, fish and horticultural crops needs to be done and applied in community sago plantation. The purpose of such activities is to develop integrated agriculture in the community, especially sago-producing areas for the economy of the community increased.

## Materials and Methods

The intercropping activities are conducted in Tanjung Peranap Village, Meranti Island District, Riau Province, Indonesia. Activities are held in July-December 2017. The area used is burnt area of 1 ha with coordinate point 00° 55'23.3" N, 102° 27'11.5" E. Local farmers are included in the activities and given authority in the maintenance of all commodities being piloted.

The main cultivated crop is sago. Sago seedlings planted with a distance of 10 m x 10 m with total seedlings planted as many as 100 plants. The row inside the sago plant intercropping with sweet corn, corn kernel, chili, watermelon, green kale, catfish, cattle and ducks.

Selection criteria good sago seedling i.e. seedling weight 5-7 kg, shape 'L', not attacked by pests and disease. Sago seedling is cutting on the part of the petiole and the leaf midrib.

Cutting works to reduce transpiration (water loss) during nursery and stimulate the growth of new shoots. Cutting is also done on the root contained in the rhizome, because the root is no longer functioning in the absorption of nutrients. Pruning the roots will stimulate the growth of new roots that function for water absorption and nutrients during the growth of seedlings in the nursery. The horticultural planted i.e. corn and sweet corn. Spacing applied to sweet corn is 70 cm x 70 cm with the number of seeds as many as three seeds per hole. Spacing applied to corn that is 70 cm x 45 cm with the number of seeds as much as three seeds per hole. Planting distance of cayenne pepper is 40 cm x 25 cm, big red chili 40 cm x 25 cm, green kale 10 cm x 5 cm and watermelon 50 cm x 10 cm. The area of cayenne pepper is 2700 m<sup>2</sup>, big red chili 900 m<sup>2</sup>, green kale 30 m<sup>2</sup> and watermelon 400 m<sup>2</sup>.

The land is first cleaned from the bush manual (cleared), after which it is planted without land preparation. Planting corn hole made using a cane, after which the seeds are inserted and applied for insecticides preventing pests in corn seeds. Maintenance of corn includes embroidery, weed and fertilizer control. Embroidering is done at 2 MST (weeks after planting). Weed control is done manually, by weeding and weeds out on the ground. Dominant weeds that grow on maize fields are weed ferns (ex: *Nephrolepis biserrata*). Fertilization consists of giving lime 5.49 farming ton ha<sup>-1</sup> and NPK 300 kg ha<sup>-1</sup> fertilizer with two applications. Provision of fertilizer with the system groove, ie fertilizer is applied in a groove made 7 cm from the row of planting, after that the grooves are closed again to prevent fertilizer evaporation.

## Results and Discussion

### *Intercropping Sago, Fish, Livestock and Crops/Horticulture*

The planted horticultural crops such as chilies (cayenne pepper and big red chili), green kale and watermelon. Chili production is well and suitable to developed on the peatland (Figure 1). Chili can be harvested at the three months after planting so that the people can get the income every three months from the sale of chili. The harvest time of green kale relatively faster than the others, that is three weeks after planting (Figure 2). If making sustainable cropping system arrangements, the people can sell their crops every day. Watermelon can start to harvest at the three months after planting, so they can sell every three months by arranging the sustainable cropping system. The advantages of watermelon post-harvest are easier because watermelon can last for more than a month (Figure 3).

At the eight weeks after planting (MST), vegetative observations were made on 10 random sample of sweet corn (Table 1). The average of plant height and leaf number respectively 76.90 cm and 6.10 sheet. If compared sweet corn growth on the peatland with on the mineral soil (optimum condition), the peatland is lower than mineral soil. Peat soil is a nutrient-poor soil, besides that many other limiting factors, affect plant growth and development, such as soil pH, water table, low soil porosity, and low saturation of the soil base.

The low soil pH in peatland needs maximum calcium oxide to increase soil pH level into neutral.



Figure 1. Chilies ready to harvest



Figure 2. Green kale



Figure 3. Watermelon

Table 1. Sweet corn growth at 8 MST

Sample	Height	Leaf number (sheet)
1	64	5
2	79	7
3	73	6
4	99	8
5	71	6
6	85	7
7	60	4
8	92	8
9	77	5
10	69	5
Mean	76.90	6.10
Standard deviation	12.25	1.37
Coefficient variability	15.93	22.46

### Economic Revenue Result of Intercropping

#### a. Sweet corn

Land area = 800 m<sup>2</sup>

Population = 1632 plants

1 kg = 4 cobs

Total cob production (kg) = (1632 x 2): 4 = 652.8 kg

Cob production is = 85%

Cob yield = 554.8 kg

Sweet corn price per kg = Rp 5000

Total revenue = Rp 2774400 (USD 201.73)

#### b. Corn

The maize cultivation system consists of 3 seeds per planting hole, with the aim to produce corn feed, baby corn and animal feed. Live fodder can be used for wool at the age of 8 MST, so the rupiah value is not calculated.

##### 1. Baby corn

Harvest age of 70 days after planting

Land area = 3700 m<sup>2</sup>

Population = 11746

1 kg = 16 cobs

Total cob production (kg) = 11746:16 = 734.12 kg

Cob production is = 85%

Cob yield = 624 kg

Baby corn price per kg = Rp 7000

Total revenue = Rp 4368000 (USD 317.60)

##### 2. Corn kernel

Harvested at 85 days after planting

Land area = 3700 m<sup>2</sup>

Population = 11746

Per cob = 80 g corn kernel

Cob production is = 85%

Total corn kernel (kg) = 9984.1 kg

Corn kernel price per kg = Rp 3000

Total revenue = 2995230 (USD 217.79)

#### c. Chili

Results from chili:

The total land area of 3600 m<sup>2</sup> of chili, comprising 2700 m<sup>2</sup> of cayenne chili and 900 m<sup>2</sup> of big red chili.

##### 1. Cayenne chili

Land area = 2700 m<sup>2</sup>  
Plant spacing = 40 cm x 25 cm  
Plant population = 27000  
1<sup>st</sup> (50 kg), 2<sup>nd</sup> (70 kg), 3<sup>rd</sup> (80 kg) and 4<sup>th</sup> (40 kg) harvest  
Total harvest = 240 kg  
Cayenne chili price per kg = Rp 30000  
Total revenue = Rp 7200000 (USD 523.52)

#### 2. Big red chili

Land area = 900 m<sup>2</sup>  
Plant spacing = 40 cm x 25 cm  
Plant population = 27.000  
1<sup>st</sup> (10 kg), 2<sup>nd</sup> (30 kg), 3<sup>rd</sup> (20 kg) and 4<sup>th</sup> (15 kg) harvest  
Total harvest = 75 kg  
Big red chilli price per kg = Rp 35000  
Total revenue = Rp 2625000 (USD 190.87)

#### d. Green Kale

Green kale harvested 25 days after planting and harvesting can be done 2 time period.

Land area = 30 m<sup>2</sup>  
Plant spacing = 5 cm x 10 cm  
Plant population = 600  
Production success is = 90%  
Total 1 bunch of green kale = 4 stems  
Production of green kale = 135 bunch  
Green kale price per bunch = Rp 2000  
Total income = Rp 2700000  
Total revenue 2 period = Rp 5400000 (USD 392.64)

#### e. Watermelon

Land area = 400 m<sup>2</sup>  
Plant spacing = 50 cm x 10 cm  
Population = 8000 plants  
Total harvest = 70 kg  
Watermelon price per kg = Rp 7000  
Total revenue = Rp 4900000 (USD 356.29)

### **Livestock and Sago Waste**

Livestock husbandry with integration model between cattle and agricultural crops are popular program nowadays. Cattle integration with sago plant is a new thing. Sago plant produces starch as food raw materials and pulp as waste. Sago waste that not been used will throw away and contaminate the adjacent environment, especially water environment and will kill water biota.

Sago waste still consists of starch and can be used as energy resources for cattle. Cattle can consume sago waste and reduce environmental pollution. Sago waste lack is low protein levels, low crude fiber, and low-fat level. Protein levels are a key factor for cattle's growth and progress beside energy levels. Feed ingredients for cattle can be made from local resources raw material. Source of local feed is:

1. Sago waste can be used as an energy source because of high-level starch. The result of proximate analyze from sago waste shows that level of carbohydrate is 90%, consist of 2% of crude fiber and 88% starch level. The protein level of sago waste is 2% and fat level 4%.

2. Trash fish have very high protein level, about 60% and can be used as protein, mineral, calcium, and phosphor source.
3. Rice bran as energy, fiber, and vitamin B source.
4. Vegetables, especially kale and leaf taro as a source of fiber, vitamin C, vitamin A, and vitamin B.
5. Ashes from sago bulk burning as a source of calcium, magnesium, and micro mineral.

Weakness from duck and cattle cultivation are water quality. Water at Tanjung Peranap Village is peatlands water. These water have acidity level 4.0 - 5.0 acid water make feed can't be digested, kill microbes in a cow stomach, and make micro mineral tied and unused. The micro-mineral can increase reproduction process. Lacks micro mineral disturb reproduction process.

A society that hasn't raised duck intensively doesn't have experience. The other weaknesses are low educational background. To handle those problems, supervising is held to increase their knowledge. Several strategies that can be done for village society to increase their income are:

1. Available land can be used for cattle and sago integration farming. Sago waste can use as cattle feed sources combining with trash fish. These activities will make land more productive.
2. Rainwater must be collected and used as a source of cattle drinking water. The other solution is reducing acidity level through water treatment process.
3. Trash fish and rice bran that abundant at some season must be preserved and stored for famine season.
4. Supervising for village society is needed because cattle husbandry in the cage is a new thing for them.

Duck feed that given is a mixture from sago waste (35%), trash fish (50%), rice bran (10%), vegetable (5%), and ashes (0.1%). Every duck fed as much 250 g day<sup>-1</sup> for 2 times. Mixtures feed consists of 30 % protein. These levels are high and enough to fulfill nutrition. Trash fish are protein, calcium, and phosphor sources. Female duck needs high calcium to formed egg skin. Concentrate feed from the factory can be used as a substitute if there is no trash fish available. Sago waste will be used as energy source, and there is no problem with its availability.

The cattle will be given fermented sago waste (Figure 4). This is substitute feed for the cattle. Drinking water for a cow is peatlands water. Peatlands water has acidity level at 4.0 to 5.0. This is not a problem to the cattle. The cattel have rumen that has a function as feed fermentation place before chewed back (ruminant). Rumen fluid is acid, so acid water as drinking water is allowed as long as the water not contaminated by feces and urine.



Figure 4. Feed from sago waste

Sago waste utilization as cattle feed will decrease environmental pollution. Sago waste fermentation process do with simple technology that can be applied in village society. Sago waste fermented process not added probiotic and other carbohydrate sources as like molasses and drops of sugarcane. Sago waste just added with urea as a nitrogen source and ashes as mineral sources. Addition of each urea and ashes are about 1% of sago waste mass. Feed consist of sago waste given as much as 50 % of its natural feed.

Sago waste can be used as an energy source because still have high starch levels. The sago waste proximate analysis result shows that carbohydrate levels are 90%, consists of 2% crude fiber and 88% of starch. Protein level analysis result shows that protein and fat level are very low, 2% and 4%. This level of protein is too low to be used as feed. Sago waste that has been fermented can increase its protein level and increase productivity, especially weight gain.

#### **Development of Catfish (*Clarias sp.*) in Peatlands**

Rural development on peatland in this activity is applied one of them through the cultivation of fisheries. The abundance of water in peatlands is a potential for aquaculture activities. The acidic water of peat (reaching pH 4.0) becomes one of the obstacles to fish farming, not all fish survive and grow in the environment. Therefore, the adjustment of catfish in peat water and the provision of feed made from local as a substitution of plant feed become the main thing to consider the solution.

Initially, the pond for fish cultivation using a ground pond measuring 7 x 10 m long and with a depth of 1.5 m. The pond is made in the middle of peatland and around it is made a ditch as a waterway to enter. But problems arise because the farmers are still not able to understand the instructions well from technical experts related to the provision of water quality formula, feed and sorting caused by the size of the pond.

Solutions to overcome these problems is to create a tarpaulin pond size 3 m x 4 m or smaller pool size. This is so that farmers are easy to apply with appropriate dosage.

Implementation of activities during July-December 2017 has shown a change better. This is seen in cultivated fish managed to experience weight gain and length. Movement of catfish agile forward and down. Fish treated with mastery succeeds so as to allow fish to adapt to the appropriate pH to grow. Parameters used as a measure of growth and development of catfish culture are water quality, growth (weight and length), sorting, fish health, reservoir and pond construction (water discharge hole).

One of the efforts to increase the oxygen demand in the waters, namely by the addition of water plants based on peat air. The next treatment for water quality in accordance with life is to start the provision of animal waste (ducks and cattle). The given dose is 10kg (10m<sup>2</sup>)<sup>-1</sup>. The dirt used does not affect the toxicity of the fish and is then fermented. Furthermore, the salt administration is intended to kill harmful pathogenic bacteria for fish.

Water quality that is not in accordance with the standard of cultivation (acid) becomes the main limiting factor in fish farming in peatlands. It needs a solution to eliminate or minimize the influence of the main limiting factors in peatlands. Efforts are made by making a reservoir or treatment pond or treatment to adjust the water conditions into water ready for cultivation.

The concept of this raw water is the catchment of rain in the hope that the water is not acidic and uses water from peatland which is pH sour. The pond is made up of two mutually similar sizes of length, width and height: 2 m, 1 m, and 1 m. The pool material used is a tarpaulin A 20 which is thicker and has a durability of more than 4 years.

The treatment provided in the reservoir pool includes the application of agricultural lime and the addition is gradually increased every 5 days until the water quality changes for the better according to fish farming standards. In addition, salt administration of 3 kg once a week until the pool water is ready for use. The formula is given in a homogenized way first into the next bucket inserted into the standard water pond gradually. Giving is done alternately from one pool to another pool.

In the fish farming activities, the main factor that has the most influence on the growth, health and quality of fish is the environment which in this case is water as a medium of cultivation. Therefore, water becomes the most important thing to be prepared. Including in this case water that in fact is sourced from peatlands. Peatlands are a source of acidity in water used in tarpaulin catfish farming ponds. Originally pH water cultivation reached 4 so that when imposed for the maintenance of fish it affects the death, the minimum damage to organs. While in the pH range 5 impact on damage to fish organs, minimum growth disturbance occurs somatic and gonads. At pH 6 the impact of growth disturbance of fish, the minimum lust eating downhill.

The challenge in this Tanjung Peranap Village is the absence of electricity so that the need for oxygen for fish becomes a limiting factor other than water for cultivation sources. Dissolved oxygen has a vital role to water aquaculture activities namely improving the quality of water and appetite, as well as chemical reactions in the waters. Automatic peat water management cannot rely on the supply of aerators, so a formula that includes physics, chemistry and biology is required in order for water to be suitable for cultivation. The water quality treatment formula that was administered was considered successful, as evidenced by the pH value at the beginning of fish stock 4.5 and after treatment on day 8 of water pH to 7.0 or normal.

Catfish is inserted in the condition of water protected from the sun, with use a barrier above the pool. The barrier used is a sago leaf placed over a pond. The fish fries are 3-4 cm in size with a density of 6.000 catfish fry ( $12 \text{ m}^2$ )<sup>-1</sup>. Catfish are fish that tend to be active at night or called nocturnally. Feeding is not appropriate time active fish, it will have an impact on the lack of appetite. If outside the active period of catfish, then most likely fish to eat but not optimally absorbed by the fish's hull or eaten food will be spewed when the fish at the bottom of the pond. In these activities to fit Standard Operating Procedure (SOP) and growth can be better, then feeding time adjusted to catfish metabolic clock, from the best time of absorption to the lowest that is at: at 05.00, 21.00, 01.00 and 18.00 WIB (Western Indonesian Time). Fourth of those timing founded by research from various variables, which is an interval of stomach emptying, feed absorb enzyme optimization and speed to take food on the water surface.

Catfish sampling activity held every week. These activities are done in order to know progress and growth generally, finds out if there are troubles or incompatibility indication between water; feed, and fish qualities, and finds out exactness fish treatment; does fish feed already fulfill the requirement of fish seed growth.

Sampling performed the amount of fish at one-kilogram mass. Decreases in fish number mean fish have good growth, the same amount of increase in fish number mean some problem in the cultivation process.

Fish health determines life sustainability, if fish does not health then will susceptible to disease. A fish disease that occurs in peatlands caused by highly acidic water. These water will make fish skin scratch. Acidic water influence fish health quality. At the beginning of the activity, partially catfish skin somewhat peeling and the other parts are died with swollen gill.

Catfish sorted to decrease the probability of inequality growth. Catfish tend to cannibal and have different fast growth between the seed up to 30% of the population. This condition will make bigger catfish eat the smaller catfish and spend the feed faster than it should. Development of catfish seeds depends on catfish raising management (including fish sorted and water replacement).

Tarpaulin pool at peat lands doesn't have water resources as like inland. Peatlands do not have electric current so for

water circulation depend on human strength. Water replacement performed with pipe usage around the pool and using the basket for discharge water. Discharge water from tarpaulin pool streamed to soil/land pool. Water replacement streamed from reservoir pool near from tarpaulin pool.

Addition of aquatic plants needed to assist reduction process of ammonia level at tarpaulin pool and land pool. Addition of aquatic plants amounted to 50% of water surface area to accelerate the ammoniac decomposition process. Aquatic plants can be functioned as natural feed for catfish at night. Type of aquatic plants that added to the pool is *Eichhornia crassipes*. These plants have proper morphology to absorb water.

### **Sago Farmer Institutional**

Institutional in agriculture environment formed with the purpose to optimize farmer work in the structured organizational framework. The organizational framework needs to be equipped with work distribution that can be measured and evaluated. Organizational reinforcement at the research project is important to be implemented so introduction program can run simultaneously, effective, and optimal.

In order to reinforcement farmer organizational framework at Tanjung Peranap Village, reserch team assisted by four field agricultural instructor from Meranti islands. These activity beginning with formed farmer workgroup. These workgroups consist of thirty people, but as time goes by a decrease to sixteen people with gender ratio 1:1.

Approach to understanding rationalization that research activity will give economic benefit and good impact on the natural environment is needed. Society at Tanjung Peranap Village is the first party that affected if peatland is broken. The society expected to be independent through these research activities, as like independent to fulfill needs and from persuasion to the damaging environment.

Reinforcement of farmer organizational framework become important to maintain farmer trust and commitment, so the farmer will consistent with farming, became sustainability farmer and totally stop for damaging forest or mangrove.

### **Conclusion**

The intercropping can be found people mindset for conducting integrated farming system and a long time can be increased economic people. It has been changed people mindset in a framework that mangrove destroys become an integrated farming system, until can be decreased environmental damage. The people economic income from selling cayenne chili, big red chili, sweet corn, baby corn, corn kernel, green kale and watermelon is Rp 7200000 (USD 523.52), Rp 2625000 (USD 190.87), Rp 2774400 (USD 201.73), Rp 4368000 (USD 317.60), Rp 2995230 (USD 217.79), Rp 5400000 (USD 217.79) and Rp 4900000 (USD 356.29) respectively. The founding organization farmer system based on sago palm in

Tanjung Peranap Village and presented by Effort Property Village Agency (BUMDES).

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## RESEARCH ARTICLE

### Some Morphological Characteristics of Gene Pool from the Hybridization of Local Tomato Genotypes and Some Commercial Types

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#### ABSTRACT

Determination of different gene sources is important for plant breeding studies; therefore, local genotypes are of interest. In this study, collected genotypes were hybridized with commercial genotypes in order to transfer some traits such as disease resistance and long shelf life to local genotypes. After that, obtained genotypes were self pollinated twice and gene pool was created according to some morphological traits. Nine different groups were created from combinations according to result of clustering analysis. Result of principal component analysis (PCA) revealed that total rate of 65.208% variation was observed. As a result of the research, half way materials were acquired that are thought to be used in obtaining qualified variety or varieties.

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#### Introduction

Tomato, one of the economically most significant products, has importance not only for economy but also for human diet. It is the one of the most produced vegetables in both Turkey and the world. World tomato production is 177 042 359 tons and China is the biggest producer in the world. It is followed by India, USA and Turkey, respectively. China produces 50 540 000 tons which constitutes 28.54% of world total tomato production. In the year of 2000, Turkey had 8 890 000 tons tomato production whereas, in 2018 with an increase of approximately 40%, this number reached up to 12.15 million tons (Anonymous, 2020) and amounted to 6.86% of world production (Anonymous, 2018). There are many reasons for the

increase of tomato production in Turkey. Some of the reasons are breeding studies conducted to develop required quality and standard varieties (Sönmez and Ellialtıoğlu, 2014) and improved culture practices. Many breeding studies are carried out in line with demands of the consumer such as disease resistance and long shelf life. In breeding studies, it is important to obtain and identify local genotypes.

Rodríguez et al. (2019) evaluated the differences in genotypic homogeneity and heterogeneity for three genetically different tomato groups. They studied twenty-four hybrids, seventeen landraces varieties, and six advanced lines (F8). They found significant differences ( $p \leq 0.05$ ) between genetic groups for all variables evaluated. Except for the days

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when the fruits ripen in the fifth branch. Six hybrids, three local varieties and two advanced lines showed remarkable agronomic responses in yield per plant. In another research; Leal et al. (2019) reported that a good germplasm can be achieved by using commercial tomato (*Solanum lycopersicum* L.) hybrids for obtaining new tomato inbred lines. Aim of their study was to investigate the stability of commercial F<sub>1</sub> hybrids in climatic conditions in mountainous regions, to predict genetic parameters, and to evaluate the breeding potential of F<sub>1</sub> hybrids according to the agronomic performance of the F<sub>3</sub> generation. According to their results, while there were significant differences in most F<sub>1</sub> and F<sub>3</sub> generations, only one variable showed significant difference in the F<sub>2</sub> population.

Qaryouti et al. (2007) performed the characterization of 44 landrace tomato populations collected from Jordan according to IPGRI criteria. They determined that there was a high variation among tomatoes in terms of vegetative, yield and fruit characteristics.

In Argentina, Hu et al. (2012) examined 67 different tomato samples in terms of morphology and genetic diversity. They stated that according to cluster analysis 3 groups were formed both morphologically and molecularly and the populations collected before 1960 showed more variation than those collected later. In another research, Osei et al. (2014) reported that a total of 216 tomato accessions obtained from Ghana, Korea, Taiwan and Burkino Faso were examined for 18 morphological features. At the end of the study, it was stated that there were 10 factor groups and 58.09% cumulative variation occurred in PCA analysis. In the clustering analysis, it was reported that the samples were divided into two groups with a similarity ratio of 0.86. Henareh et al. (2015) examined morphological characteristics of 97 different tomato populations collected from Iğdir province of Turkey and from different parts of Iran. In principal components analysis, they determined that the variation of the first three main component among samples was 71.6%. The first principal component constituted 50% of the total variation and the yield value showed a high correlation with this component, therefore, they stated that breeders can use the characteristics of this component as selection criteria. On the other hand, Bhattarai et al. (2016) studied 71 tomato samples.

According to their clustering analysis, it was stated that 6 different groups were formed and 5 main components explained more than 92% of the variation in the principal component analysis. In another study, comparison of quality characteristics between three tomato hybrids and their six maternal and paternal individuals were conducted. According to results, it was stated that by hybridization studies, a variation can be created in quality properties such as lycopene content, sugar composition and color. Although their taste, smell and aroma properties are good, local varieties are not preferred for commercial production due to their low yields, short shelf life, low disease resistance and deformed fruit shape. It is thought that local tomato varieties can gain a place in the market if their undesirable characteristics get eliminated. For this reason, it was aimed to collect and identify local tomato genotypes as well as to create a gene pool by hybridization of local tomato genotypes, and to utilize this gene pool in breeding programs.

## Materials and Methods

### Material

Plant material of this study consist of 136 Genotypes that reached to S<sub>2</sub> stage and are obtained by hybridization of 11 local and 6 commercial tomato varieties collected from different regions of Turkey.

### Method

Seedlings belonging to genotypes were planted to greenhouse in February 2013. Some morphological characterization measurements and observations were performed according to UPOV criteria. Measurements and observations are given in Table 1. Means of all observations and measurements obtained in this study are presented and interpreted as tables. To determine the relationship between the genotypes and investigated properties, all data obtained were analyzed using the Ward method in the JMP computer program for clustering analysis. Principal Component Analysis (PCA) and factor analysis were also performed with the same program.

**Table 1.** Some morphological characteristics of tomato genotypes

Morphological Features	Group	Scoring	S <sub>2</sub>		
			Genotype	Percentage (%)	
Anthocyanin formation in seedlings	Absent	1	6	4.41	
	Present	9	130	96.29	
Plant growth type	Determinate	1	90	66.7	
	Indeterminate	9	46	33.82	
Plant growth power	Few	3	0	0	
	Medium	5	120	88.97	
	Many	7	16	11.02	
Stem internode length	Short	3	5 cm <sub>≤</sub>	32	23.52
	Medium	5	6-10	102	75.55
	Large	7	11 <sub>≥</sub>	2	1.48
Stem internode thickness	Thin	3	5 mm <sub>≤</sub>	15	11.82
	Medium	5	6-10 mm	41	30.37
	Thick	7	11 mm <sub>≥</sub>	80	59.25

Table 1. (continued)

Morphological Features	Group	Scoring	S <sub>2</sub>		
			Genotype	Percentage (%)	
Stem pubescence	Absent	1	0	0	
	Few	3	0	0	
	Medium	5	120	88.23	
	Intensive	7	16	11.85	
	Very intensive	9	0	0	
Leaf attitude of petiole of leaflet in relation to main axis	Semi- erect	3	45	33.08	
	Horizontal	5	91	66.91	
	Semi- drooping	7	0	0	
Leaf length	Short	3	10 cm ≤	42	30.88
	Medium	5	10-15 cm	90	66.6
	Long	7	15 cm ≥	4	2.96
Leaf width	Narrow	3	5 cm ≤	52	38.23
	Medium	5	5-10 cm	83	61.48
	Broad	7	10 cm ≥	1	0.74
Green color intensity of leaf;	Light	3		13	9.55
	Medium	5		93	68.38
	Dark	7		30	22.05
Flower color	Yellow	1		136	100
	Orange	9		0	0
Peduncle length	Short	3	1cm ≤	0	0
	Medium	5	1-2 cm	128	94.11
	Long	7	2cm ≥	8	5.88
Inflorescence type	Simple	1		104	76.47
	Mixed	2		32	23.53
	multiple	3		0	0
Flower pubescence	None or very little	1		3	2.20
	Present	9		133	97.80
Fruit weight		1	35gr <	2	1.47
		2	35-70gr	26	19.11
		3	70-105gr	43	31.61
		4	105-140gr	34	25
		5	140-175gr	22	16.17
		6	175gr >	9	6.61
Fruit height		1	15 mm ≤	0	0
		2	15-30 mm	2	1.47
		3	30-45 mm	70	51.47
		4	45-60 mm	52	38.23
		5	60-75 mm	10	7.35
		6	75-90 mm	2	1.47
		7	90 mm ≥	0	0
Fruit width		1	15 mm ≤	0	
		2	15-30 mm	0	
		3	30-45 mm	48	35.29
		4	45-60 mm	45	33.08
		5	60-75 mm	20	14.70
		6	75-90 mm	21	15.44
		7	90 mm ≥	2	1.47
Fruit shape in longitudinal section	Flattened	1		38	27.94
	Slightly flattened	2		78	55.14
	Circular	3		13	9.55
	Rectangular	4			
	Cylindrical	5		0	0
	Elliptic	6		0	0
	Heart-shaped	7		0	0
	Ovate	8		7	5.14
	Pear-shaped	9		0	0
Fruit color (at maturity)	Light red	3		37	27.20
	Red	5		96	70.58
	Pink	7		3	2.20

Results and Discussion

136 genotypes were measured and observed at S<sub>2</sub> stage and the results were indicated below.

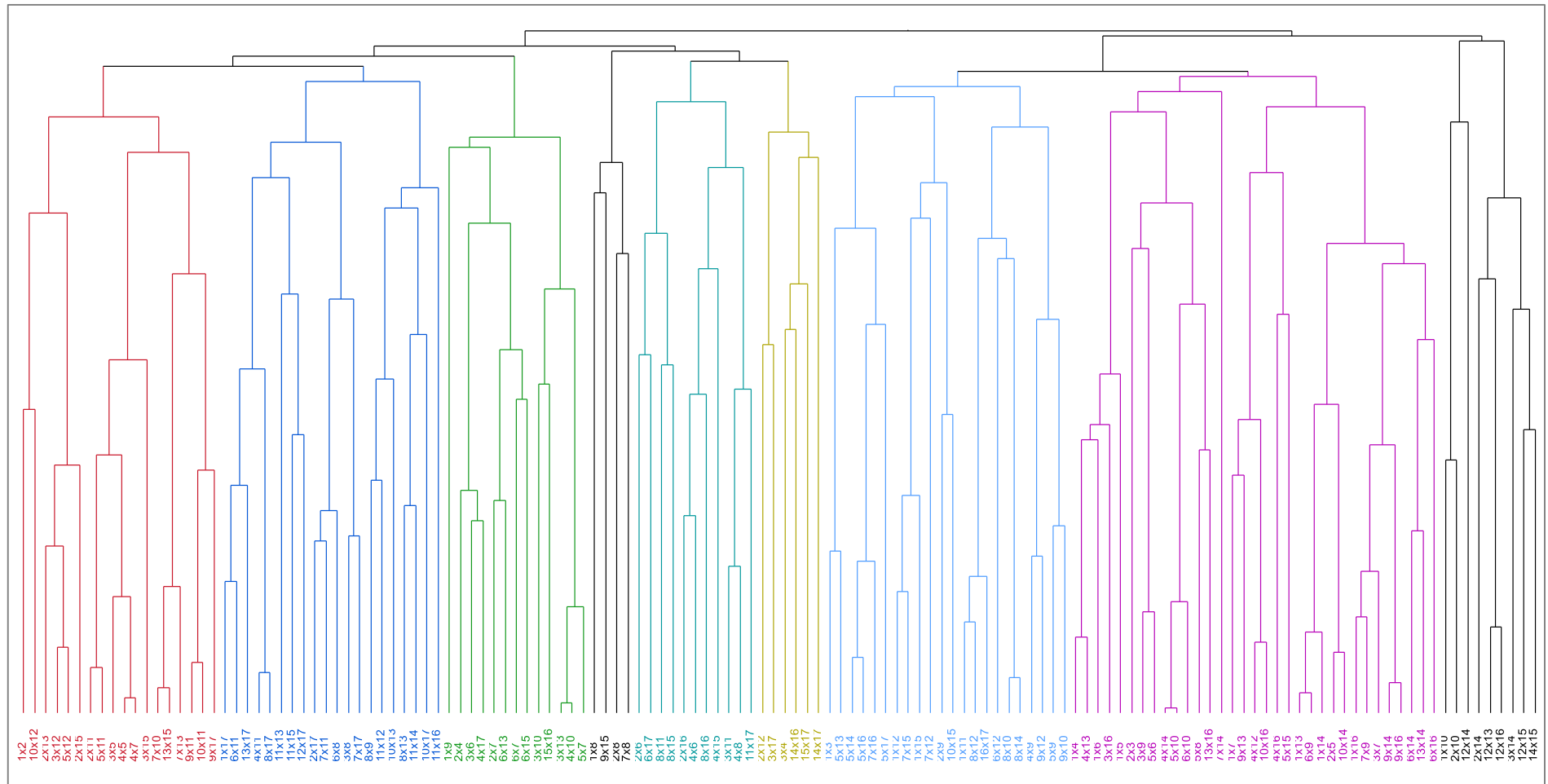


Figure 1. Dendrogram of hybrids

Anthocyanin formation in seedlings: There was no anthocyanin formation in 6 (4.41%) of 136 genotypes, while it was present in 130 genotypes (96.29%). The absence of anthocyanin formation can be used as an indicator of resistance to some disease factors such as male infertility and fusarium (Masuda et al. 2000). Plant growth power: 120 genotypes (88.97%) out of 136 were observed to have “moderate” plant growth power whereas, it was “high” in other 16 genotypes (11.02%). Criteria such as plant growth power and stem thickness are considered to be important elements in endurance of the plant against environmental factors (Peralta and Spooner, 2005). We obtained supporting measuring data. Plant growth type were observed as “indeterminate” in 46 genotypes (33.82 %) and “determinate” in 90 genotypes (66.17 %) out of 136 genotypes. In another study, Oğuz (2010) observed 32 “determinate” and 56 “indeterminate” genotypes in 88 genotypes. Stem Internode length were measured to be short for 32 genotypes (23.52%), “medium” for 102 genotypes (75.55%) and “large” for 2 genotypes (1.48%) out of 136 genotypes. Stem Internode thickness of the 136 genotypes were classified as “thin” for 15 genotypes (11.82%), “medium” for 41 genotypes (30.37%), and “thick” for 80 genotypes (59.25%). In plants; internode thickness and plant growth power are important criteria in evaluation of effective resistance of the genotype to environmental factors (Peralta and Spooner 2005).

Stem pubescence were determined as “medium” in 120 genotypes (88.23%) and “intensive” in 16 genotypes (11.85%) out of 136 genotypes. Çukadar and Dursun (2012) observed as “few” in 24 genotypes, “medium” in 23 genotypes and “intensive” in 1 genotype. Leaf attitude of petiole of leaflet in relation to main axis was identified as “semi-erect” in 45 genotypes (33.08 %) and “horizontal” in 91 genotypes (66.91%). Leaf length were measured as “short” (30.88 %) for 42 genotypes, “medium” for 90 genotypes (66.6%) and “long” for

4 genotypes (2.96 %). On the other hand, in their study, Çukadar and Dursun (2012) measured 3 genotypes as “short”, 24 genotypes as “medium” and 21 genotypes as “long”. Their results support our findings. Green color intensity of leaf was classified as “light” in 13 genotypes (9.55 %), “medium” in 93 genotypes (68.38 %) and “dark” in 30 genotypes (22.05%). Peduncle length were measured as “medium” in 128 genotypes (94.11%) and “long” in 8 genotypes (5.88 %). Inflorescence type was classified as “simple” for 104 genotypes (76.47%) and “mixed” for 32 genotypes (23.53%). Similarly, Oğuz (2010) described 52 genotypes as “simple” and 35 genotypes as “mixed”. Flower pubescence was classified as “none or very little” in 3 genotypes (2.20 %) and “present” in 133 genotypes (97.80 %).

Fruit weight was observed as  $35g \leq$  in 2 genotypes (1.47 %), 35-70g in 26 genotypes (19.11%), 70-105 g in 43 genotypes, (31.61%) 105-140 g in 34 genotypes (25 %), 140-175g in 22 genotypes (16.17%) and  $175g \geq$  in 9 genotypes (6.61%). Fruit height was measured as 15-30 mm for 2 genotypes (1.47 %), 30-45 mm for 70 genotypes (51.47 %), 45-60 mm for 52 genotypes (38.23%), 60-75 mm for 10 genotypes (7.35 %) and 75-90 mm for 2 genotypes (1.47 %). Fruit width was measured as 30-45 mm in 48 genotypes (35.29%), 45-60 mm in 45 genotypes (33.08 %), 60-75 mm in 20 genotypes (14.70 %) and 75-90 mm in 2 genotypes (1.47%).

Fruit shape in longitudinal section was classified as “flattened” in 38 genotypes (27.94%), “slightly flattened” in 78 genotypes (55.14 %), “circular” in 13 genotypes (9.55%) and “ovate” in 7 genotypes (5.14%). Fruit color (at maturity) was identified as “light red” for 37 genotypes (27.20%), “red” for 96 genotypes (70.58 %) and “pink” for 3 genotypes (2.20%). The amount of water-soluble dry matter of 136 genotypes ranged from 2.6 to 4.8. Gölükçü et al. (2010) found that the amount of water-soluble dry matter ratio was between 3.65-7.20%.

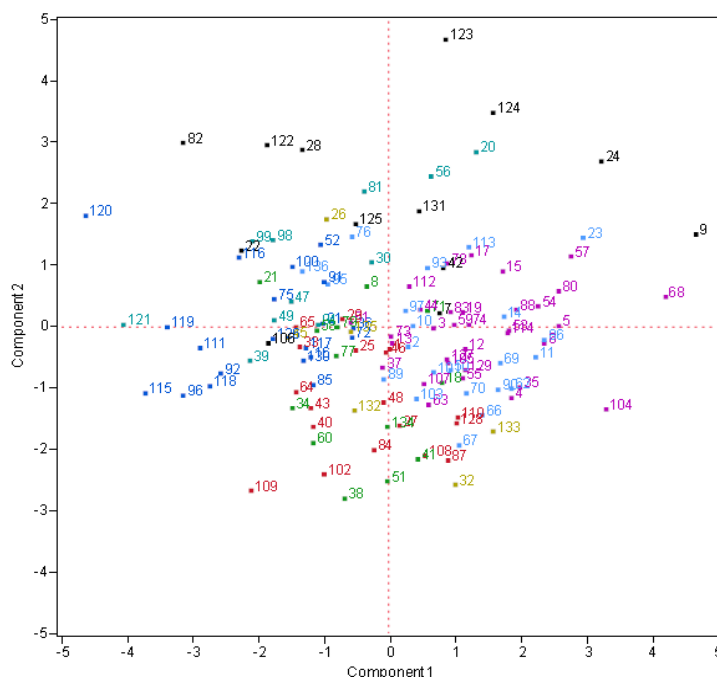


Figure 2. Discriminant analysis between tomato genotypes on the basis of morphological characters

**Table 2.** Some morphological characteristics of tomato genotypes

Factor	1	2	3	4	5	6	7	8	9
Essence value	2.7807	1.7526	1.5504	1.2943	1.1929	1.1772	1.1485	1.1120	1.0330
Cumulative variation	13.907	22.666	30.419	36.890	42.855	48.741	54.483	60.043	65.208
Anthocyanin formation in seedlings	0.1126	0.5375	-0.3016	-0.3503	-0.2033	0.1419	-0.0263	0.2580	-0.0989
Plant growth power	-0.0007	0.3029	0.2325	0.5953	-0.1463	0.1749	0.1552	-0.1396	-0.0681
Plant growth type	-0.3526	0.6736	0.1053	0.0188	0.0555	0.1771	-0.1039	-0.1645	0.0483
Stem internode length	0.0388	-0.0728	-0.1433	0.6204	0.2386	-0.0336	0.0092	0.1783	-0.0835
Stem internode thickness	0.3326	-0.1459	0.0344	-0.2487	0.3603	0.1302	0.2428	-0.0116	0.5116
Stem pubescence	-0.1279	0.1716	-0.1902	-0.3558	0.1400	-0.5102	0.4391	-0.0434	-0.2502
Leaf length	-0.1141	0.1543	0.7047	-0.0363	0.0788	-0.1570	-0.0951	-0.1202	-0.0691
Leaf width	0.0202	0.0162	0.7903	0.0109	-0.0224	0.0268	0.0460	0.1281	0.0781
Leaf attitude of petiole of leaflet in relation to main axis	-0.0356	0.0287	0.1365	-0.4220	0.0076	0.5026	0.2093	0.2049	-0.2948
Green color intensity of leaf	-0.0466	0.1627	-0.0401	0.1232	0.7399	-0.0741	-0.2085	0.2062	-0.0172
Inflorescence type	0.0509	0.7372	0.1837	0.1155	-0.0136	-0.0759	0.0602	-0.0789	0.1248
Flower pubescence	0.1455	-0.2526	0.1336	0.0257	0.6175	0.1054	0.0664	-0.2464	-0.0117
Peduncle length	-0.0275	0.1079	-0.1231	0.0612	0.0619	0.5773	0.0687	0.0294	-0.0295
Fruit weight	0.8691	0.0256	-0.0507	-0.1109	0.0937	0.0893	-0.1058	-0.1054	-0.0395
Fruit width	0.8387	-0.0646	-0.0228	-0.0150	0.1661	-0.0352	-0.0055	0.1777	-0.1066
Fruit height	0.7766	-0.0666	-0.0343	0.1694	-0.1536	-0.0211	-0.0364	0.0175	0.0508
Fruit shape in longitudinal section	-0.1673	0.1706	0.0146	-0.0102	-0.0824	-0.0893	-0.0004	0.0621	0.8265
Fruit color (at maturity)	-0.0873	-0.0345	-0.0105	0.0820	-0.1203	0.0741	0.8574	-0.0071	0.0745
Water soluble dry matter	-0.1644	0.1270	0.2980	0.2020	0.1540	-0.4320	0.2414	0.3530	-0.0226

In Figure 1, it can be seen that 9 different clusters are formed according to classification made in accordance with 21 characteristics examined of 136 genotypes as S<sub>2</sub> stage. When we examined the figure, it was observed that 1x2-1x3 (14.20) combination was in the farthest distance from these genotypes. In addition, 1x3 combination was found to be at a far distance (12.45) from 1x10 combination. The closest distance was obtained from the combination of 4x14-5x10 (1.26). Combinations of other genotypes were found to be between these two extreme values. As all of the combinations of flower color yielded the same result, it was excluded from factor analysis. Data obtained from remaining 20 properties revealed that genotypes are grouped in 9 factors. As a result of Principal Component Analysis (PCA) of investigated characteristics from combinations, there was a variation at the rate of 65.208%.

## Conclusion

One of the most important stages in intensive and long-term breeding studies is to determine suitable parents to the purpose. In this study, local varieties were hybridized with commercial varieties that are highly appreciated by consumers in terms of taste, smell and aroma. In conclusion, a gene pool that can be potentially evaluated in terms of breeding was obtained.

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## RESEARCH ARTICLE

### Domestic Consumption Pattern of Cereal Commodities in Nigeria

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#### ABSTRACT

The present research empirically study the demand pattern of cereal commodities for domestic use in Nigeria using time series data which spanned from 1966 to 2018. The data covered the domestic quantities and price series of the five most important domestically used cereal commodities. The sources of the data were FAO and USDA databases and the collected data were analyzed using descriptive statistics and linear approximate almost ideal demand system (LA/AIDS). The empirical evidence showed that all the considered goods were normal goods and mostly a necessity except for wheat which is a luxury. In addition, domestic consumption of these commodities was sensitive to changes in their respective prices. With wheat been a luxury commodity, it can be inferred that an increase in per capita income would tend to bring about a paradigm shift from the consumption of staple foods to non-staple food (wheat). This indicates that households tend to diversify their diet composition. Also, it was observed that most of the good pairs are complementary (uncompensated cross-price elasticity) while on the other hand (compensated cross-price elasticity), most of the good pairs are substitutes. The empirical evidence showed the income effect which owes to price changes for all the selected commodities to be moderate. Therefore, onus lies on the policymakers to embark on mass production of wheat in order to free the country from wheat food insecurity bondage caused by external influence, an impediment to the barometer that strikes a balance between the forces of demand and supply.

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#### Introduction

Theil (1965) and Mudassar *et al.* (2012) reported that in applied economics the topic of consumer demand analysis is one of the oldest. In Nigeria as well as across the globe, for the past few decades, consumers' demand for food commodities and its dynamic has cut the attention of many researchers. The recent unprecedented rise in the country's food prices has renewed the interest of researchers in re-conducting an empirical detail analysis of consumers' food demand. Despite the substantial progress made by the country in enhancing its per capita consumption for major food

items viz. cereals, pulses, animal protein, fish and vegetables, unfortunately, evidence from the agglomerated data revealed that low incomes and diet deficiencies have succeeded in forming a vicious cycle around the large proportion of the nation's population (FAO, 2018). Considering food security, this development is a major setback to the not long ago positive picture of the total calories and protein intakes of the country. It is a known fact that the large share of the total household income is spent on food items, and inequalities continue to persist in food consumption across the existing income categories in the country.

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One of the biggest challenges facing Nigeria is how to feed its buoying population of approximately 180 million. Succinctly, policymakers are faced with the dilemma of adopting a liberal trade policy vis-à-vis intervening in markets to ensure food security, especially for the poor. With the accelerated growth rate, the major questions before the Nigerian policymakers are: What will happen to the country's demand for food in particular and agricultural products in general? Will the country be able to feed itself or continue to subsist on importation? These are pertinent matters that will continue to re-echo since the nation is faced with the problem of demand deficit due to the continuous growth in its population.

Therefore, it is very important to conduct a study on the current specific demand elasticity estimates, as income and price elasticities of demand would not only increase our horizon of understanding the country's economic behavior but can also enhance our vision on the matrix of policy. Haq *et al.* (2011) reported that empirical research on food consumption patterns can provide evidence on consumers' responsiveness to price and expenditure changes that are useful in designing a country's food policies. Elasticity estimates of price and income for different classes of foods can help in setting administered prices and in designing subsidy and tax policies as well as in determining the impacts of these policies on poverty. To formulate a long-term policy on food security and poverty reduction in a developing country, there is a need to understand how different classes of households respond to changes in the prices of different food items (Haq *et al.*, 2011; Mudassar *et al.*, 2012). It is against this background that this research was conceptualized to determine the domestic consumption pattern of cereal goods in Nigeria. Thus, the outcome of this research would fill the information gap concerning the domestic consumption pattern of cereal commodities in Nigeria.

### Materials and Methods

The present research used time series data which covered domestic consumptions and prices for wheat, rice, maize, sorghum and millet. The data were sourced from the data bank of FAO and USDA. The collected data were analyzed using descriptive statistics and linear approximate almost ideal demand system (LA/AIDS). The price data were deflated using the consumer price index (CPI) of the country so as to eliminate the problem of inflationary trend.

### Empirical Model

Following Anwarul-Huq *et al.* (2004); Awal *et al.* (2008) Babar *et al.* (2011), using the budget share form, the LA/AIDS model is given below:

$$\omega_i = \alpha_i + \sum_j \gamma_{ij} \ln P_j + \beta_i \ln \left[ \frac{X}{P^*} \right] + \varepsilon_i \quad (1)$$

$$\ln P^* = \sum_j w_j \ln P_j \quad (2)$$

The restrictions on the parameters of the AIDS equation (1) are:

$$\sum_i \alpha_i = 1, \sum_i \beta_i = 0, \sum_j \gamma_{ij} = 0, \text{ (Adding - up condition, Engel Aggregation)} \quad (3)$$

$$\sum_j \gamma_{ij} = 0 \text{ (homogeneity condition)} \quad (4)$$

$$\gamma_{ij} = \gamma_{ji} \text{ (Symmetry condition)} \quad (5)$$

Where,  $\omega_i$  = budget share of the  $i^{\text{th}}$  commodity (i.e.  $\omega_i = P_i Q_i / X$ );  $P_j$  is the price of the  $j^{\text{th}}$  commodity;  $X$  = total household expenditure on all the food items considered for the study;  $P^*$  = stone price index;  $\varepsilon_i$  = stochastic term, and it is assumed to be zero and has constant variance;  $\alpha_i$  = intercept;  $\gamma_{ij}$  = price coefficient; and,  $\beta_i$  = expenditure coefficient. Blanciforti and Green (1983); Awal *et al.* (2008) stated that the model that uses Stone's geometric price index is referred to as the "Linear Approximate Almost Ideal Demand System (LA/AIDS)".

The demand elasticities are calculated as the functions of the estimated parameters and they have standard implications.

The expenditure elasticity ( $\epsilon_i$ ) which measures the sensitivity of demand in response to changes in consumption expenditure is specified as follow:

$$\epsilon_i = 1 + \left( \frac{\beta_i}{\omega_i} \right) \quad (6)$$

$$\epsilon_i = \frac{MBS}{ABS} \quad (7)$$

Price elasticity is estimated in two ways viz. uncompensated (Marshallian) elasticity that contains both price and income effects, and the compensated (Hicksian) elasticity which contain only price effect.

The uncompensated own-price elasticity ( $\epsilon_{ii}$ ) and the cross-price elasticity ( $\epsilon_{ij}$ ) measures how a change in the price one product affects the demand of itself and that of the other products respectively, with the total expenditure and other prices being held constant i.e. *ceteris paribus*. The Marshallian own and cross-price elasticities are shown below (Babar *et al.*, 2011):

$$\epsilon_{ii} = \left( \frac{\gamma_{ii}}{\omega_i} \right) - (\beta_i + 1) \quad (8)$$

$$\epsilon_{ij} = \left( \frac{\gamma_{ij}}{\omega_i} \right) - (\beta_i \omega_i / \omega_j) \quad (9)$$

The Hicksian own and cross-price elasticities ( $\epsilon_{ii}^*$  and  $\epsilon_{ij}^*$ ) which measures the price effects on the demand assuming the real expenditure ( $X/P^*$ ) is constant is given as follows (Babar *et al.* 2011):

$$\epsilon_{ii}^* = \left( \frac{\gamma_{ii}}{\omega_i} \right) + (\omega_i - 1) \quad (10)$$

$$\epsilon_{ij}^* = \left( \frac{\gamma_{ij}}{\omega_i} \right) + \omega_j \quad (11)$$

Besides, the compensated price elasticity can be estimated by using  $\epsilon_i$ ,  $\epsilon_{ii}$  and  $\epsilon_{ij}$ , and the permutation is as follow:

$$\epsilon_{ij}^* = \epsilon_{ij} + \epsilon_i^* \omega_i \quad (12)$$

Babar *et al.* (2011) reported that the sign of the estimated  $\epsilon_{ij}^*$  indicates the substitutability or complementarity between the destinations under consideration. A commodity pair is denoted as a complement or substitute if their compensated cross-price elasticity is negative or positive respectively.

Based on the value of expenditure elasticity, a food item is classified as a necessity/necessary commodity ( $0 < \epsilon_i < 1$ ), a luxury commodity ( $\epsilon_i > 1$ ) or a Giffen / inferior commodity ( $\epsilon_i < 0$ ).

In absolute term, the demand for a particular commodity is price elastic (inelastic) if the elasticity value of its own-price is larger than unity (less than unity).

The Hicksian elasticity indicates the change in demand for a commodity due to a price variation, when the real expenditure change caused by the aforementioned price variation is compensated by an expenditure variation so that satisfaction/utility is kept constant.

## Results and Discussion

### Summary Statistics of the Variables

A cursory review of the summary statistics showed the country average annual budget shares on wheat, rice, millet, maize and sorghum to be 0.117, 0.171, 0.191, 0.257 and 0.264 respectively, with a conditional expenditure of ₦438.23million (Table 1). On average, wheat, rice, millet, maize and sorghum accounted for 11.7%, 17.1%, 19.1%, 25.7% and 26.4% respectively, of the average annual expenditure incurred by the country on cereal commodities. Sorghum and maize had an overwhelming dominance in the domestic consumption expenditure of the studied area. Thus, these average annual budget shares for the country are true 95% of the time within the intervals of  $0.117 \pm 0.038$ ,  $0.171 \pm 0.047$ ,  $0.191 \pm 0.052$ ,  $0.257 \pm 0.066$  and  $0.264 \pm 0.0499$ ; and an annual conditional expenditure within ₦438.23±637. The implication is that the

country would expend ₦0.117, ₦0.171, ₦0.191, ₦0.257 and ₦0.264 on the domestic consumption of wheat, rice, millet, maize and sorghum respectively, for a naira budget on these cereal crops.

It can be observed that sorghum had the highest proportion while wheat has the least proportion. On the average, the quantity consumed was observed to be highest for sorghum (5.3 thousand MT), then trailed by millet (4.1 thousand MT) and then wheat with the least (1.495 thousand MT). Therefore, it can be inferred that sorghum is consumed more in the country possibly because of its low price in relation to that of the other available cereals in the market. In addition, the domestic use of sorghum for both human and animal consumptions in the country is high when compared to the other cereal commodities. Sorghum is processed into flour, paste, snacks, confectionaries etc and consumed by humans while the raw material is consumed directly by the large stock of livestock which abounds in the country especially the northern axis of the country. The reason for the least share proportion of wheat is because of its high cost as the bulk of the domestic supply is imported.

The standard deviation results of the budget shares showed high inconsistencies in the consumption of all the cereal commodities under the study. In addition, the variation in the annual average expenditure been high implied that the country exhibits inconsistent behavior on the expenditure of commonly consumed cereal commodities during the period of study. Thus, this may be due to the imbalance in the supply and demand of cereal commodities which are relatively unstable in the studied area.

Table 1. Summary statistics of the variables

Items	Mean	SD	Minimum	Maximum
<b>Quantity (thousand Million metric ton)</b>				
Rice	2130.48	16.23.22	250	5800
Millet	4128.94	1842.49	910	9064
Sorghum	5296.96	2160.52	2298	9950
Maize	3939.52	2553.20	933	8900
Wheat	1494.94	1208.80	131	4160
<b>Budget share</b>				
$\omega_{rice}$	0.1704504	0.0465497	0.062282	0.404545
$\omega_{millet}$	0.1913878	0.05169	0.034063	0.420037
$\omega_{sorghum}$	0.2642629	0.0499325	0.155066	0.407234
$\omega_{maize}$	0.2565193	0.0661397	0.125155	0.382357
$\omega_{wheat}$	0.1173795	0.0378641	0.030053	0.33247
<b>Prices (₦)</b>				
$P_{rice}$	19028.65	23741.41	110	75138
$P_{millet}$	15091.81	20503.02	40	73913
$P_{sorghum}$	15112.19	20584.57	35	79452
$P_{maize}$	16878.71	22925.06	46	82452
$P_{wheat}$	19413.17	24121.27	75	80500
<b>Annual expenditure (₦)</b>				
Rice	74,696,665	109,051,261	27500	3.34E+08
Millet	83,872,077	142,656,107	69880	6.7E+08
Sorghum	115,808,182	174,466,505	113260	7.35E+08
Maize	112,414,725	167,912,959	43746	6.18E+08
Wheat	51,439,337	77,877,946	9825	2.85E+08
EXP	438,230,987	7.249E+14	294867	2.58E+09

Source: Authors' own computation, 2020

Note:  $\omega$  and P means budget share and Price respectively.

Furthermore, the marginal budget shares of the country's cereal consumption are 0.175 for wheat; 0.150 for rice; 0.21 for maize, 0.11 for sorghum and 0.157 for millet (Table 2). This implies that if the annual income is increased by 100 percent the budget shares for wheat, rice, maize, sorghum and millet would increase by 17.46%, 15.03%, 20.597%, 11.36% and 15.72%, respectively.

**Table 2.** Marginal budget share (marginal propensity to consume) for the selected food items

Commodity	ABS	MBS	MBS%
$\omega_{rice}$	0.17045	0.150319	15.03188
$\omega_{millet}$	0.191388	0.157238	15.72379
$\omega_{sorghum}$	0.264263	0.113593	11.3593
$\omega_{maize}$	0.256519	0.20597	20.59696
$\omega_{wheat}$	0.11738	0.174634	17.46344

Source: Authors' own computation, 2020

Note: ABS and MBS means Average Budget Share and Marginal Budget Share respectively.

### Demand Pattern of Cereal Consumption

#### OLS estimates of LA/AIDS model

A perusal of the Table showed the OLS estimates of the LA/AIDS model for the commodities to be reliable for predictions as evident from their respective diagnostic statistics viz. the LM test statistics for homoscedasticity and autocorrelation which were different from zero at 10% degree of freedom. In addition, the Durbin-Watson statistics were within a plausible or acceptable region. All the subsequent diagnostic tests viz. test for a structural break or change in the parameters (Cusum test), adequacy of the model specification

(RESET test), Arch effect test and Chow test are within the acceptable margin. Of the thirty parameter estimates, fifteen were observed to be different from zero at 10% error gap (Table 3). Based on the consumer theory, the estimated results were consistent as there is no evidence of violation of the homogeneity and symmetry properties as indicated by the significance of the Chi-square test statistics.

The coefficient of multiple determination values ranged from 0.7588 to 0.4377 with maize having the upper limit while millet has the lower limit. It was observed that seventeen out of the thirty parameter estimates were within the acceptable margin that is different from zero at 10% degree of freedom. The empirical evidence showed that sorghum and millet witnessed a positive trend growth as evidenced by the significance of their respective intercept parameters. Thus, it implies the presence of exogenous growth in sorghum and millet, independent of price and income movement. It was observed that the budget share of wheat decreased with increases in its own-price and price of rice while it increased with an increase in the price of millet. The budget share of rice decreased with an increase in the price of wheat, and increased with increases in its own-price and price of sorghum. For maize, its budget share decreased with increases in the prices of millet and wheat while it increased with an increase in the price of rice. The budget share of sorghum is inversely related to its own-price and that of millet, and directly related to that of maize. Besides, the budget share of millet decreased with an increase in the price of rice and increased when there is an increase in the price of wheat.

**Table 3.** Parameter estimates of the LA/AIDS

Price items	Rice	Millet	Sorghum	Maize	Wheat
<i>Intercept</i>	0.0473(0.2029) 0.23 <sup>NS</sup>	0.6113(0.3335) 1.83*	1.7080(0.2677) 6.38***	0.2918(0.2795) 1.04 <sup>NS</sup>	-0.2643(0.1982) 1.33 <sup>NS</sup>
$P_{rice}$	0.0376(0.0179) 2.10**	-0.0604(0.0294) -2.05**	-0.0321(0.0236) 1.36 <sup>NS</sup>	0.1458(0.0246) 5.92***	-0.0445(0.0175) 2.55**
$P_{millet}$	-0.0086(0.0278) 0.31 <sup>NS</sup>	0.00498(0.0456) 0.11 <sup>NS</sup>	-0.0653(0.0366) 1.78*	-0.09397(0.0383) 2.46**	0.0687(0.0271) 2.53**
$P_{sorghum}$	0.0465(0.0262) 1.78*	-0.0557(0.0430) 1.29 <sup>NS</sup>	-0.1880(0.0345) 5.45***	0.0319(0.0361) 0.88 <sup>NS</sup>	0.0414(0.0256) 1.62 <sup>NS</sup>
$P_{maize}$	-0.0326(0.0293) 1.11 <sup>NS</sup>	-0.0113(0.0481) 0.24 <sup>NS</sup>	0.1448(0.0386) 3.75***	-0.0269(0.0403) 0.67 <sup>NS</sup>	0.0039(0.0286) 0.13 <sup>NS</sup>
$P_{wheat}$	-0.0358(0.0123) 2.92***	0.0801(0.0201) 3.98***	0.0050(0.0162) 0.31 <sup>NS</sup>	-0.0572(0.0169) 3.38***	-0.0411(0.01197) 3.43***
<i>Expenditure</i>	-0.0105(0.0226) 0.47 <sup>NS</sup>	-0.0366(0.0371) 0.99 <sup>NS</sup>	-0.1476(0.0298) 4.96***	-0.0339(0.0311) 1.09 <sup>NS</sup>	0.0316(0.0221) 1.43 <sup>NS</sup>
$R^2$	0.7433	0.4377	0.6117	0.7588	0.6299
<i>F-statistic</i>	19.785***	5.318***	10.76***	21.49***	11.62***
<i>Autocorrelation (LM)</i>	12.012(0.112) <sup>NS</sup>	14.62(0.146) <sup>NS</sup>	30.56(0.105) <sup>NS</sup>	0.247(0.264) <sup>NS</sup>	16.72(0.212) <sup>NS</sup>
<i>D-W statistic</i>	1.97	2.02	2.12	2.05	2.35
<i>Heteroscedasticity (LM)</i>	12.32(0.420) <sup>NS</sup>	16.76(0.159) <sup>NS</sup>	32.19(0.225) <sup>NS</sup>	13.90(0.21) <sup>NS</sup>	13.49(0.334) <sup>NS</sup>
<i>Arch LM test</i>	2.34(0.125) <sup>NS</sup>	25.81(0.104) <sup>NS</sup>	4.094(0.663) <sup>NS</sup>	2.42(0.61) <sup>NS</sup>	17.40(0.235) <sup>NS</sup>
<i>Normality test (<math>\chi^2</math>)</i>	7.083(0.028)**	5.517(0.063)*	7.347(0.025)**	5.547(0.062)*	3.141(0.207) <sup>NS</sup>
<i>RESET test</i>	0.286(0.595) <sup>NS</sup>	0.345(0.234) <sup>NS</sup>	0.246(0.981) <sup>NS</sup>	1.412(0.255) <sup>NS</sup>	1.575(0.217) <sup>NS</sup>
<i>CUSUM test</i>	1.651(0.106) <sup>NS</sup>	-1.059(0.296) <sup>NS</sup>	-0.873(0.387) <sup>NS</sup>	-0.037(0.97) <sup>NS</sup>	0.226(0.290) <sup>NS</sup>
<i>Chow test</i>	0.52(0.812) <sup>NS</sup>	0.321(0.938) <sup>NS</sup>	1.365(0.251) <sup>NS</sup>	0.758(0.625) <sup>NS</sup>	1.039(0.783) <sup>NS</sup>

Source: Authors' own computation, 2020

Note: Values in ( ) are standard deviation while \*\*\* \*\* \* <sup>NS</sup> means significant at 1%, 5%, 10% and non-significant, respectively

### Expenditure elasticity

A cursory review of the expenditure elasticity conformstothea *priori* expectation i.e has the expected sign (Table 4). The expenditure elasticitiesof all the cereal commodities are positive while that of the own-price elasticitiesare negative across the selected commodities. Because of the economic situation in Nigeria, the expenditure elasticities of these selected commodities are relatively high. This implies that all of the selected commodities are very important foods given that they fulfilled the fundamental needs of the populace as many of the households, especially the poor are constrained with tight budgetary allocation. The empirical evidence showed the commodities viz. rice, maize, sorghum and millet to be necessities i.e. necessary goods as evidenced from their respective expenditure elasticities which were less than unity, while wheat is a luxury good as revealed by its expenditure elasticity which is greater than unity. For the necessary commodities, these results conform to the findings of Makama *et al.* (2017) while for the wheat it contradicts their findings as they classified wheat to be a necessary commodity. However, these researchers did not include millet in their study. In addition, all the selected commodities are normal goods as evidenced by their respective income elasticities which were positively signed. Thus, it is expected that wheat will experience an increase in demand when the gross per capita income increases in tandem with the overall growth of the country's economy or GDP. Because of the low purchasing power (poverty) in the country, people would respond more towards the consumption of wheat as their income increases. Therefore, it can be inferred that households would tend to diversify their diets as their per capita income increases, thus anincrease in the consumption of non-staple food rather than staple foods. In relative terms, a decrease in the per capita real income will lead to a reductionin the per capita expenditure allocation on wheat in the studied area.The income elasticities of the necessary goods were inelastic while that of the luxury commodity was elastic, indicating that a percent change in the per capita income would lead to a less and greater than proportional changes in the demand for the necessary goods (former) and the luxury good (latter), respectively. A percent increase in the per capita income would lead to 1.488%, 0.803%, 0.882%, 0.430% and 0.822% increases in wheat, rice, maize, sorghum and millet respectively.

### Own-price elasticity

The results of the Marshallian (uncompensated) own-price elasticity is consistent with the economic theory which stipulated an inverse relationship i.e. negativity of the elasticity coefficient, indicating that the demand curve is negatively sloped. Besides, the Hicksian (compensated) own-price elasticity conforms to the *a priori* expectation as all the selected commodity elasticity coefficients were negatively signed. The negative signs displayed by both the uncompensated and compensated own-price elasticity showed the inverse relationship between the price of a normal commodity and its demand (Table 4). Between the uncompensated and compensated own-price elasticities, a

substantial difference was observed, thus indicating a substantial income effect. This is contrary to the findings of Makama *et al.* (2017) who found substitution effect i.e. price effect to be stronger than income effect.

It was observed that the own-price elasticities of wheat, rice and sorghum were different from 10% degree of freedom, implying that the consumers were quite responsive to changes in price while adjusting their consumption of the corresponding commodity. The demand for rice and millet react in-elastic to their own-prices while for the remaining selected cereals its response was elastic as indicated by their respective coefficients which were less than unity for the former and greater than unity for the latter. Therefore, it can be inferred that rice and millet are less sensitive to price change while sorghum, maize and wheat are highly sensitive to changes in the price level.

The uncompensated own-price elasticity is composed of two components viz. price or substitute effect and income effect. The uncompensated own-price elasticity estimate for wheat demand showed that if the price of wheat plummets by 10% the demand for wheat per capita would surge by 16.67%. Of this increase in per capita demand, 15.70% is purely due to price/substitution effect as indicated by the compensated own-price elasticity. Furthermore, the income effect due to the decrease in the price accounted for the remaining 0.97% (i.e. 16.67-15.70) increase in the wheat demand due to the increase in the real income as the absolute amount of the money income remains unchanged. If an increase in per capita income by 10% is accompanied by a 10% decrease in the price of wheat, thus the demand for wheat would increase by 31.55% (i.e. 16.67+14.88).

For rice, maize, sorghum and millet, if their respective prices (own-price elasticities) plummeted by 10%, their respective per capita demands would increase by 5.67%, 11.23%, 15.79% and 9.39% respectively. Of these total increases in the per capita demands, 4.89%, 9.85%, 14.67% and 7.71% in respect of rice, maize, sorghum and millet owe purely to price/substitution effect as evidenced by their respective compensated own-price elasticities. The income effect due to the decrease in the prices accounted for the remaining 0.79%, 1.38%, 1.11% and 1.69% increase in the demand for rice, maize, sorghum and millet respectively, which owe to the increase in real income as the absolute amount of the money income remains unchanged. Therefore, if an increase in the per capita income by 10% is accompanied by a 10% decrease in the prices for rice, maize, sorghum and millet, their demands respectively would increase by 14.49%, 19.26%, 20.08% and 17.61%.

However, an increase in the per capita income represents a shift in the demand curve for wheat that normally leads to an increase in the price of the reference commodity. Thus, information on the supply elasticity of wheat is very necessary for the estimation of the resultant equilibrium level for wheat consumption in the studied area. The income effect due to price changes for all the selected commodities was moderate. This owes to the fact that the shares of these selected commodities in the household expenditure were almost

moderate, thus, changes in their prices had moderate effects on the real income.

In absolute terms, the compensated own-price elasticities were less than the uncompensated ones, an indication that a rise or fall in the price of the respective commodities would have a considerable effect on the real expenditure. Also, it

implies that the price responsivenesses of the different food items were income-dependent, such that when income is held constant i.e. *ceteris paribus*, the responsiveness of households to the prices of food tends to be low. Generally, all the food crops with in-elastic own-price elasticity both for uncompensated and compensated means they are integral items of the household's diet.

**Table 4.** Expenditure (income) elasticity, Marshallian and Hicksian own-price elasticities

Commodity	Expenditure	Marshallian own-price	Hicksian own-price
Rice	0.881891	-0.56704	-0.48853
Millet	0.821567	-0.93913	-0.77049
Sorghum	0.429849	-1.57858	-1.46728
Maize	0.80294	-1.12263	-0.9845
Wheat	1.487776	-1.66662	-1.57037

Source: Authors' own computation, 2020

### Cross-price elasticity

Cursory reviews of the matrices of the uncompensated and compensated cross-price elasticities show most of the cross-elasticity values in absolute terms to be lower than the values of the expenditure and own-price elasticities. The Marshallian cross-price elasticity shows the "gross" cross-effect that encompasses both the price/substitution and income effects (Table 5), while the Hicksian cross-price elasticity represents the pure price effect i.e. only the substitution effect or the net effect of change in price on demand (Table 6). Of the ten uncompensated cross-price elasticities, four were positively signed while six were negatively signed; indicating the former to be gross substitutes and the latter to be complementary goods. The reverse is the case for the compensated cross-price elasticity as six out of the ten cross-price elasticities were positive while the remaining four were negative, signifying that the former are gross substitutes while the latter are

complementary commodities. The empirical evidence showed some of the signs of the cross-price elasticities for uncompensated and compensated to be contrary. For example, the uncompensated cross-price elasticity showed millet and rice to be complementary goods while the compensated cross-price elasticity revealed that they are substitutes. In other words, the total effect of a change in the price of millet on the demand for rice indicated that millet and rice are 'gross' complements while the compensated cross-price elasticity been positive, implying that millet and rice were 'net' substitutes.

Anwarul-Huq *et al.* (2004); Awal *et al.* (2008) posited that the uncompensated cross-price elasticity is ambiguous and suggested that when information about substitution possibility is required, compensated cross-price elasticity should be used i.e. is the most appropriate. Though, strong expenditure effects play a role.

**Table 5.** Marshallian cross-price elasticity for the selected food items

Items	$D_{rice}$	$D_{millet}$	$D_{sorghum}$	$D_{maize}$	$D_{wheat}$
$D_{rice}$	<b>-0.56704</b>	-0.27827	-0.07322	0.864997	-0.73125
$D_{millet}$	-0.07193	<b>-0.93913</b>	-0.13525	-0.50582	0.961708
$D_{sorghum}$	0.552522	-0.22517	<b>-1.57858</b>	0.236432	0.512643
$D_{maize}$	-0.34548	-0.00889	0.657343	<b>-1.12263</b>	-0.02435
$D_{wheat}$	-0.39487	0.461058	0.056313	-0.31945	<b>-1.66662</b>

Source: Authors' own computation, 2020

Note: Own-price elasticities are written in bold letters

**Table 6.** Hicksian cross-price elasticity for the selected food items

Items	$D_{rice}$	$D_{millet}$	$D_{sorghum}$	$D_{maize}$	$D_{wheat}$
$D_{rice}$	<b>-0.48853</b>	-0.20513	-0.03495	0.936479	-0.59881
$D_{millet}$	0.109091	<b>-0.77049</b>	-0.04701	-0.34101	1.267093
$D_{sorghum}$	0.780858	-0.01246	<b>-1.46728</b>	0.444326	0.897852
$D_{maize}$	-0.19377	0.116937	0.731288	<b>-0.9845</b>	0.231587
$D_{wheat}$	-0.33781	0.454981	0.084123	-0.26751	<b>-1.57037</b>

Source: Authors' own computation, 2020

Note: Own-price elasticities are written in bold letters

## Conclusion

Based on these findings it can be inferred that rice and millet are integral items of household diets as both the uncompensated and compensated own-prices are inelastic. All the selected commodities were normal goods with almost all been necessary goods except for wheat that is a luxury commodity. In tandem with the overall economic growth of the country, wheat is expected to witness an increase in demand when the gross per capita income increases. Therefore, it is very obvious that the households diversify their diets as their per capita income increases, thus leading to more consumption of non-staple food and less of staple foods. Sorghum tends to have the highest budgetary share; an indication that the good has many domestic purposes viz. food paste, confectionaries, food flour and animal feeds etc. The empirical evidence showed the income effect which owes to price changes for all the selected commodities to be moderate. Also, for the uncompensated cross-price elasticity more than half of the cross-price elasticities in the matrix were complimentary goods while the reverse was the case with the compensated cross-price elasticity. Therefore, the onus lies on the policymakers to swiftly intervene by increasing the production of wheat so as to contain the excess of external influence on the food security of wheat which is the bane of the imbalance in the market forces.

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## RESEARCH ARTICLE

### Economic Analysis and Marketing Margins of Chickpea Prices in Turkey

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#### ABSTRACT

The study examined the producer-consumer prices and marketing structure of the chickpeas in Turkey, with a focus on the production-consumption, foreign trade, price fluctuations and marketing between the years 2003-2017. Despite the reduction in acreage and production rates in Turkey, an increase was observed in the production. In this study, marketing margins of chickpea are calculated according to current and real prices (2017=100). Producer and consumer chain indexes are also calculated according to current prices and compared with annual inflation rates. In fifteen years, the average yield was determined as 113 kg da-1, and the average consumption per person was 6.5 kg year. In fifteen years, the producer earned from 1 kg chickpea production increased by 55% and the amount paid by the consumer increased by 86%. In the fifteen-year period, prices have been in real favor for the producers and against the consumer. The model related to chickpea production function was estimated and agricultural real worker price was found to be effective on chickpea production.

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#### Introduction

The leguminous crops, which have been cultivated around the world for many years, have a great importance in terms of satisfying the need for plant-based protein in human nutrition. Particularly high content of crude protein containing legumes is particularly rich in basic amino acids such as Lysine, Leucine, Isoleucine, A, B vitamins and mineral substances and is especially important in meeting the protein requirements of developing countries (Şehirali, 1988).

Chickpea is a leguminous plant used in human and animal nutrition and green fertilization, both in our country and in the world, especially in the Near East, Far East, Mediterranean, South America and Central American countries (Eser, 1976; Reddy and Singh, 1984; Reddy and Kabbabeh, 1985).

Edible beans- an important food source for low-income people in many developing countries, has also an important place in many family's daily consumption in Turkey (Uzunöz, 2009).

In terms of cultivation area in Turkey, legumes take the second most important place after cereals. Of the nine varieties produced, the most grown are chickpeas, beans and lentils. As of 2017, legumes are cultivated in approximately 0.79 million hectares and constitute 2% of the total cultivated area. In 2017, 1.2 million tons of legumes were produced. While total leguminous cultivation area constitutes 50% of the chickpea cultivation area, 40.3% of the total legumes production is chickpea production (TSI, 2019).

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Approximately 84% of the worldwide pulses produced are intended to meet the domestic demand of countries. The remaining 16% is included in world trade (Anonymous, 2017). The chickpea, which is included in the legumes product group, is grown in 2016 in 12.7 million hectares in the world. The country with the highest cultivation area in the world is India with 8.4 million hectares. India is followed by Pakistan with 1.0 million hectares and Australia with 677 thousand hectares. Turkey ranks 7th with 352 thousand hectares (FAO, 2019).

In 2016, the world's production of chickpeas was 12.1 million tons and the average yield was 956 kg ha<sup>-1</sup>. In World chickpea production, India ranks first with 7.8 million tons of production in 2016, Australia second with 875 thousand tons, Myanmar third with 559 thousand tons, Turkey fourth with 455 thousand tons and Ethiopian fifth with 444 thousand tons. Pakistan ranks second in the cultivation area and ranked seventh with 286 thousand tons of production due to the low yield (304 kg ha<sup>-1</sup>) (FAO, 2019).

The highest yields in 2016 were China (5177 kg ha<sup>-1</sup>), Israel (4148 kg ha<sup>-1</sup>) and Moldova (3945 kg ha<sup>-1</sup>), respectively. Turkey (1293 kg ha<sup>-1</sup>) has a yield above the world average (FAO, 2019).

The countries that import chickpea in 2016 are India, Bangladesh, Pakistan, UAE and Algeria, respectively. Turkey ranks 10<sup>th</sup> in the world chickpea imports with 30 thousand tons (FAO, 2019). The countries exporting chickpeas in 2016 are Australia, Russia, Canada, Argentina and India, respectively. Turkey ranks 11th in export with 23 thousand tons (FAO, 2019).

When it comes to the chickpeas producer prices in 2016, Iraq (1380 \$ ton<sup>-1</sup>), Iran (1330 \$ ton<sup>-1</sup>) and Turkey (958 \$ ton<sup>-1</sup>) have the highest chickpea producer prices respectively, while Bangladesh (503 \$ ton<sup>-1</sup>), Australia (583 \$ ton<sup>-1</sup>) and Mexico (618 \$ ton<sup>-1</sup>) (FAO, 2019) have the lowest producer prices, respectively. In this study, the economic status of chickpea was examined taking into consideration producer-consumer prices and the marketing situation. In addition, factors affecting chickpea production were tried to be measured by estimating the model of chickpea production function.

## Materials and Methods

### Material

In this study, the production amount of chickpeas, acreage and yield quantities during 2003-2017 period were obtained from Turkish Statistical Institute (TSI) while the producer prices of chickpeas (those which farmers have), retail prices (consumer), and the import-export volumes were taken from TSI, the Food and Agriculture Organization (FAO), the Union of Chambers and Commodity Exchanges of Turkey (UC CET), Turkey Exporters Assembly (TEA), the Institute of Agricultural Economics and Policy Development (IAEPD). The price of fertilizer, fuel price, agricultural worker price was taken from the web site of TSI and IAEPD, while the amount of precipitation was taken from the General Directorate of Meteorology (GDM) website. In addition, various publications and resources were also used.

## Method

By considering producer and consumer price indexes (2017 = 100), current prices of chickpea producers and consumers have been converted into real prices. The year-to-year fluctuations in prices were first shown as absolute values, then expressed as a percentage of the first two years of comparison. Averages values were calculated without considering the percentages obtained (Dağdemir and Birinci, 1999; Altundağ and Güneş, 1992). The difference between the prices paid by the farmer (producer prices) and the prices paid by the consumer is calculated as "Marketing Margin" (Aşkan and Dağdemir, 2015; Topcu, 2003).

While calculating the chain price index, the current prices of producers and consumers were calculated comparatively year-by-year-basis by taking into consideration the term between 2003 and 2017. There is no basic year in the chain price index. The index for any year is based on the price of the previous year. The main objective in the chain price index is to examine the annual changes in the price at the time to determine how much the prices would increase or decrease in the next year compared to the previous year (Dağdemir, 1998).

While the model regarding chickpea production function was estimated, the series were tested based on linear, double logarithmic and semi-logarithmic models one by one. In the analysis, double logarithmic model (log-log) fitted best among selected model. Prices were taken into account in real terms (2017 = 100). Durbin-Watson test was used to determine whether there was an autocorrelation problem in the time series analysis of the established models and no autocorrelation problem was found.

The estimated model for chickpea production function is as in formula 1.

$$\text{LogCPA}_{(t)}: \alpha + \beta_1 \text{LogFP} + \beta_2 \text{LogLP} + \beta_3 \text{LogDP} + \beta_4 D + \epsilon \quad (1)$$

*CPA: Chickpea Production Amount (ton)*

*FP: Fertilizer Reel Price (TL ton<sup>-1</sup>)*

*LP: Agricultural Labor Real Price (TL per month)*

*DP: Diesel Reel Price (TL lt<sup>-1</sup>)*

*D: Dummy Variable (The average rainfall for the years 2003-2017 is 627.3 mm. According to years "0" below the average, "1" above the average was accepted.)*

## Results and Discussion

In Turkey, 600 thousand tons of chickpea production were obtained in 0.63 million hectares in 2003, acreage was reduced to about 0.4 million hectares with a decline of 59% in 2017, and production declined to 470 thousand tons with 28% decrease. In contrast to the decline in production areas and production, the average increase in yield has increased from 950 kg ha<sup>-1</sup> to 1190 kg ha<sup>-1</sup>. Between 2003 and 2017, the average planting area was 468.5 thousand hectares, the average production was 522.3 thousand tons and the average yield was 1130 kg ha<sup>-1</sup>. The year with the highest yield was 2015 with 1280 kg ha<sup>-1</sup>, while the lowest yield was in 2003 with 950 kg ha<sup>-1</sup> (Table.1).



**Table 1.** Chickpea plantings, production and yield condition in Turkey (2003-2017)

Years	Planting Area (ha)	Quantity (ton)	Yield (kg ha <sup>-1</sup> )
2003	630 000	600 000	952
2004	606 000	620 000	1023
2005	557 800	600 000	1075.6
2006	524 367	551 746	1052.2
2007	503 675	505 366	1003.4
2008	505 165	518 026	1025.5
2009	455 934	562 564	1233.9
2010	455 690	530 634	1164.5
2011	446 413	487 477	1092
2012	416 242	518 000	1244
2013	423 557	506 000	1194.6
2014	388 518	450 000	1158.2
2015	359 304	460 000	1280.2
2016	359 529	455 000	1265.5
2017	395 310	470 000	1188.9
<b>Average</b>	<b>466 500</b>	<b>522 320.9</b>	<b>1130.23</b>

Source: TSI, 2019

In 2017, the total consumption of chickpea was 536 955 tons. In 2003, per capita consumption was 6.11 kg per year, and 6.64 kg per year in 2017. The highest average consumption rate of chickpea was 7.17 kg in 2004 and the lowest in 2016 with 5.79 kg. The average consumption in Turkey between the years 2003 and 2017 seems close. Average consumption is kept close to each other because of the decrease in production (28%), increase in productivity (25%) and imports (22000%) between 2003-2017 (Table 2).

While the chickpea qualification rate was 98.3% in 2016, it decreased by 87.5% in 2017. Between the years of 2003-2017, the export average is 67 540 tons, while the import is 21 887 tons (Table 2).

**Table 2.** Chickpea production, consumption and marketing in Turkey (2003-2017)

Years	Production (ton)	Consumption (ton)	Per Capita Consumption (kg/year)	Imports (ton)	Exports (ton)
2003	600 000	410 399	6.11	41	189 642
2004	620 000	487 473	7.17	546	133 073
2005	600 000	477 053	6.93	646	123 593
2006	551 746	448 943	6.44	1 881	104 684
2007	505 366	441 350	6.25	5 176	69 192
2008	518 026	438 449	6.13	8 760	88 337
2009	562 564	478 459	6.59	4 404	88 509
2010	530 634	481 427	6.53	7 586	56 793
2011	487 477	467 723	6.26	8 451	28 205
2012	518 000	527 602	6.98	34 939	25 337
2013	506 000	543 875	7.09	56 875	19 000
2014	450 000	473 000	6.09	41 000	18 000
2015	460 000	474 834	6.03	37 306	22 472
2016	455 000	462 471	5.79	30 446	22 975
2017	470 000	536 955	6.64	90 241	23 286

Source: Original calculations

In general, chickpea prices are on an upward trend. Producer prices of chickpeas, which were 1.13 TL kg<sup>-1</sup> in 2003 in current prices, increased by 483% and became 5,46 TL kg<sup>-1</sup> in 2017. In consumer prices, the price of 1.62 TL kg<sup>-1</sup> in 2003 increased by 555% to 9 TL kg<sup>-1</sup> in 2017. The highest increase in chickpea producer prices was 48% in 2017 up from 3.68 TL kg<sup>-1</sup> to 5.46 TL kg<sup>-1</sup> over the previous year. The highest increase in consumer price increased by 32% in 2012 and prices increased from 4.76 TL kg<sup>-1</sup> to 6.28 TL kg<sup>-1</sup> (Table 3).

The marketing margin is the difference between the price paid by the consumer and received by the producer for one kg of chickpeas. In other words, the marketing margin is the value taken by intermediaries. When the current prices are taken into consideration, the rates reached by the intermediaries according to the years vary between 28% and 59% and the rates obtained by the farmers vary between 41% and 72% (Table 3).

**Table 3.** Chickpea marketing margins by current prices in Turkey (2003-2017)

Years	Producer Prices (TL kg <sup>-1</sup> )	Consumer Prices (TL kg <sup>-1</sup> )	Marketing Margin	Passing the Producer (%)	Passing the Tool (%)
2003	1.13	1.62	0.49	70	30
2004	1.25	1.74	0.49	72	28
2005	1.27	2.28	1.01	56	44
2006	1.14	2.63	1.49	43	57
2007	1.24	2.94	1.70	42	58
2008	1.49	3.37	1.88	44	56
2009	1.44	3.35	1.91	43	57
2010	1.60	3.61	2.01	44	56
2011	2.11	4.76	2.65	44	56
2012	2.68	6.28	3.60	43	57
2013	2.46	6.03	3.57	41	59
2014	2.33	5.39	3.06	43	57
2015	2.61	6.00	3.39	44	56
2016	3.68	7.25	3.57	51	49
2017	5.46	9.00	3.54	61	39

Source: Original calculations

When the real prices of chickpea are examined, the producer real price of chickpea was 3.53 TL kg<sup>-1</sup> in 2003 and 5.46 TL kg<sup>-1</sup> in 2017. When the consumer real prices are analyzed, the consumer real price, which was 4.84 TL kg<sup>-1</sup> in 2003, was determined as 9.00 TL kg<sup>-1</sup> in 2017. Consumers' chickpea buying parity decreased by 86%. The producer's earnings from 1 kg chickpea production increased by 55% over the course of fifteen years. The figure that the consumer pays for 1 kg of chickpea has increased by %86. In the five-year period, it was in favor of the producers in real terms and against the consumer (Table 4).

According to the current prices of chickpea, producer and consumer chain indexes were calculated and compared with the inflation rates in table 5. As a result of this comparison, it was determined that the prices of chickpeas remained below the inflation rate in 2004, 2005, 2006, 2009, 2013 and 2014. In 2004, 2009, 2013 and 2014, the purchasing power of the consumers increased and decreased in other years.

**Table 4.** Chickpea marketing margins by real prices in Turkey (2003-2017)

Years	Producer Prices (TL kg <sup>-1</sup> )	Consumer Prices (TL kg <sup>-1</sup> )	Marketing Margin	Passing the Producer (%)	Passing the Tool (%)
2003	3.53	4.84	1.31	73	27
2004	3.59	4.70	1.11	76	24
2005	3.37	5.69	2.31	59	41
2006	2.76	5.98	3.21	46	54
2007	2.76	6.29	3.52	44	56
2008	3.01	6.39	3.39	47	53
2009	2.74	6.28	3.54	44	56
2010	2.80	6.23	3.43	45	55
2011	3.47	7.40	3.93	47	53
2012	4.04	9.20	5.16	44	56
2013	3.45	8.45	5.00	41	59
2014	3.00	6.85	3.85	44	56
2015	3.13	7.25	4.12	43	57
2016	4.09	8.40	4.31	49	51
2017	5.46	9.00	3.54	61	39

Source: Original calculations

**Table 5.** According to chickpeas producer-consumer chain indexes in Turkey the current prices and annual inflation rates

Years	Producer Chain Index	Producer Index Difference	Producer Price Index	Consumer Chain Index	Consumer Index Difference	Consumer Price Index
2003	100	-	13.9	100	-	18.4
2004	110.6	10.6	13.8	107.4	7.4	9.3
2005	101.6	1.6	2.7	131	31	7.7
2006	89.7	-10.3	11.6	115.4	15.4	9.7
2007	108.7	8.7	5.9	111.8	11.8	8.4
2008	120.1	20.1	8.8	114.6	14.6	10.1
2009	96.6	-3.4	5.9	99.4	-0.6	6.5
2010	111.1	11.1	8.9	107.8	7.8	6.4
2011	131.8	31.8	13.3	131.9	31.9	10.5
2012	127	27	2.5	131.9	31.9	6.2
2013	91.7	-8.3	7.0	96	-4	7.4
2014	94.7	-5.3	6.4	89.4	-10.6	8.2
2015	112	12	5.7	111.3	11.3	8.8
2016	140.9	40.9	9.9	120.8	20.8	8.5
2017	148.4	48.4	15.5	124.1	24.1	11.9

Source: Original calculations

According to the signs determined by the chickpea production function, it is observed that there is an inverse relationship between the production of chickpeas and the real price of fertilizer and the real price of agricultural workers, and there is a correct relation between diesel real price and rainfall amount (dummy). It is seen that there is a correct relationship between chickpea production and diesel real price and it does not comply with economic theory. As a result of the use of time series data, the signs of the coefficients may have negative results.

In the model, the value of R<sup>2</sup> was high (0.810) and according to the F test, the predicted model for chickpea production function was statistically significant at 1% (P=0.001) significance level. Again, the real price of agricultural workers from the independent variables was statistically significant at the significance level of 1% and it was found that other independent variables were insignificant. When we increase agricultural labor costs by 1% according to the agricultural labor coefficient in the model, a decrease of 0.373% in

chickpea production is foreseen.

The Ministry of Agriculture and Forestry supports the farmers with diesel and fertilizer. It is observed that these two inputs are not effective in the production model. Again, it is seen that the increase in the amount of precipitation enhances the production of chickpeas, which is statistically insignificant.

**Table 6.** Regression analysis results about chickpea production function

LogCPA	Coefficients	Standard Error	P (t)	P (F)
$\alpha$	15.7454 ***	0.6015	0.000	
LogFP	-0.1019	0.1142	0.393	
LogLP	- 0.3730 ***	0.0772	0.001	0.001
LogDP	0.1155	0.1541	0.471	
D	0.0078	0.3260	0.816	

\*\*\*, \*\*, \* ==> Significance at 1%, 5%, 10% level

## Conclusion

In chickpeas, there was an increase in the amount of consumption due to the population increase. On the contrary, there has been a decrease in production amount over the years. There has been a decrease in exports and an increase in imports by years. Over the past fifteen years, Turkey has decreased to position importer from an exporter in chickpeas. Diesel and fertilizer support are given to farmers by the Ministry of Agriculture and Forestry and it has been determined that it is not effective in increasing production. In order to reach the self-sufficiency level in chickpeas, different policies should be implemented.

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## RESEARCH ARTICLE

### Hydraulic Conductivity Values of Soils in Different Soil Processing Conditions

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#### ABSTRACT

Hydraulic conductivity is an important indicator of water movement and pore structure in soil. Therefore, it is important to determine soil physical and hydraulic properties under different land use conditions. The present study was conducted under three different landuse; dry farming (D), irrigated land (I) and pastureland (P). Three samples were collected from each field (9 samples in total). Infiltration measurements were also tested at each sampling point in each field. The results of this study showed that although hydraulic conductivity was not significantly different under dry and irrigated agricultural lands, significant differences were observed between the pastureland and the tilled areas. Soil water infiltration was positively correlated with soil organic matter, aggregate stability and hydraulic conductivity, whereas infiltration was negatively correlated with bulk density. The lowest infiltration rate was found under pastureland compare to those are the highest under the irrigated lands. Therefore, increasing the organic matter content of the local soils will make significant contributions to sustainable soil management.

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#### Introduction

The water use efficiency is increasingly important due to changes in the frequency and intensification of regional droughts under climate change scenarios. To develop climate change friendly management practices, effective management of irrigation water makes soil physical and hydraulic properties crucial especially those, soil hydraulic conductivity, texture, clay type, aggregate stability, and hence pore size distribution (Öztekin et al., 2007). Karahan and Erşahin (2016) documented the significance of soil texture, pore size distribution and total porosity are important to estimate the hydraulic state of the soil. Gülser et al. (2007) specifically mentioned that saturated hydraulic conductivity found to be most effective on water management strategies besides other soil physical properties.

Soil hydraulic conductivity can be defined as the ability of the water or a solution to pass through the soil pores at the

particular time scale. Hydraulic conductivity is related to physical properties such as soil particle size distribution, shapes of the soil particles, effective porosity, and thus aggregation (Rosas et al., 2014). Moreover, hydraulic conductivity changes depend on the properties of the porous medium, and density and viscosity of the liquid (water or water solution) (Schwartz and Zhang, 2003; Ishaku et al., 2011). Besides on field experiments, laboratory studies also reported the soil hydraulic state as a soil health indicator (Boadu, 2000).

In addition to soil and environmental conditions, different management practices also make soil physical and hydraulic state crucial due to their direct effect on determining water usage and indirect impacts through modifying other soil properties. Osunbitan et al. (2005) reported significant differences in saturated hydraulic conductivity, soil volume weight and water retention as influenced by different soil tillage practices. Öztekin and Erşahin (2006), investigated the

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spatial variation of saturated hydraulic conductivity in soils under different tillage conditions and reported that saturated hydraulic conductivity in the tilled areas showed 2.5 times more variability than no-till areas. They stated that the reason of saturated hydraulic conductivity in the tilled areas is very large due to the compaction of the soil as a result of field traffic.

## Materials and Methods

### Study Sites and Soil Sampling

The study fields were located at Bigadiç district of Balıkesir province. The district of Bigadiç is located on Balıkesir-İzmir intercity road (38 kilometers from the city center) in the South-West Marmara region. The altitude of the field is 180 meters and the surface area is 1007 km<sup>2</sup> (Anonymous 2019a).

The sampling area is in the transition zone from the marine climate to the continental climate. The study field has continental climate characteristics. Summers are warm in the region; winters are dryer and rainfall is moderate. It has Mediterranean climate in terms of precipitation and continental climate in terms of temperature (Anonymous, 2019a). According to the eighty-year average data measured by Balıkesir Meteorological Station, the average temperature is 14.6 °C and the average annual rainfall is 583.2 mm. The highest precipitation falls in December (95.2 mm) (Anonymous, 2019b).

Soil samples were taken from 3 different points under irrigable cultivated agricultural areas, non-irrigated dry areas, and pastures (9 samples in total). Deteriorated soil samples were taken from each point at 3 replications at a depth of 0-20 cm and carried to the laboratory.

## Study Analysis

Infiltration values of the soils were measured by infiltrometer (Hussen, 1999) in 2 replications at 9 points established in the field in dry, irrigated and pasture areas.

Soil texture (Gee and Bauder, 1986), pH measurements (McLean, 1982), CaCO<sub>3</sub> (Nelson, 1982), organic matter (Nelson and Sommers, 1982), aggregate stability (Kemper and Rosenau, 1986), hydraulic conductivity (Demiralay, 1993), bulk density (Tüzüner, 1990), particle density (Blake and Hartge, 1986), porosity (Danielson and Sutherland, 1986) and inflation rate determination (Hussen, 1991) were determined on soil samples.

## Statistical Analysis

Data were analyzed by ANOVA, and Duncan's multiple range test to comparison of means under different land use with a significant value of  $\alpha < 0.1$  (SPSS 1999). A principle component analysis was applied by using  $\alpha < 0.05$  and SAS JMP software.

## Results and Discussion

Data showed that D2 and D3 points in dry farming area and P1 sampling point in pasture area were classified as sandy clay loam texture, while the remaining samples were in sandy loam texture class. Texture plays a key role owing to its impacts on bulk density, hydraulic conductivity, aggregate stability, porosity and cation exchange capacity (Aksakal, 2004; Barik, 2011). In addition, the roughening of the soil texture helps soil to provide a better growing environment, decreases the resistance to tillage, and hence improve soil structure (Özdemir et al., 2018).

Table 1. Analysis findings obtained from soil samples

Samples Number	D1	D2	D3	I1	I2	I3	P1	P2	P3	
Texture	Clay (%)	14.09	27.60	25.70	13.71	11.84	13.45	21.58	11.19	11.25
	Silt (%)	12.45	25.88	21.50	21.61	20.63	15.26	18.20	23.34	19.39
	Sand (%)	73.46	46.52	52.80	64.68	67.53	71.18	60.22	65.67	69.36
Bulk density, g (cm <sup>3</sup> ) <sup>-1</sup>	1.20	1.23	1.21	1.17	1.18	1.14	1.08	1.01	1.16	
Particule density, g (cm <sup>3</sup> ) <sup>-1</sup>	2.53	2.54	2.53	2.50	2.48	2.54	2.54	2.44	2.61	
Porosity (%)	52.65	51.49	52.17	53.22	52.43	55.12	57.49	58.61	55.56	
Organic matter (%)	2.15	2.01	2.09	2.01	1.93	1.79	1.99	4.22	2.88	
Aggregate stability (%)	46.03	32.13	32.17	21.62	16.86	17.22	61.42	88.28	75.85	
Lime (%)	1.09	1.09	1.31	1.28	1.40	1.46	2.80	2.58	3.47	
Hydraulic conduct, cm h <sup>-1</sup>	6.04	4.63	5.09	5.25	4.55	4.75	7.35	8.98	9.56	
pH (1:2.5 soil:water)	5.79	5.80	5.79	5.85	5.39	5.72	6.98	6.87	6.91	

Table 2. Changes between land samples taken and land use status (p<0.05)

Usage	Organic matter (%)	Aggregate stability (%)	Bulk density g (cm <sup>3</sup> ) <sup>-1</sup>	Particle density g (cm <sup>3</sup> ) <sup>-1</sup>	Porosity (%)	Hydraulic conductivity cm h <sup>-1</sup>	pH (1:2.5)	Lime (%)
Dry	2.08b	36.78b	1.21a	2.53	52.12b	5.25b	5.65b	1.16b
Irrigation	1.91b	18.57c	1.16a	2.53	53.52b	4.85b	5.79b	1.38b
Pastureland	3.03a	75.18a	1.08b	2.51	57.23a	8.63a	6.92a	2.95a

Organic matter content of these soils varies between 1.79% and 4.22%. The SOM content was slightly above 2% under the

dry agricultural management (Table 1) which can be classified as moderate. In all irrigated fields, SOM content was lower

than 2% and classified as low. This can be because of the high mineralization which is can be attributed to the intensive cultivation of anchor plants in aqueous conditions (Tümsavaş, 2003). Soils under pasture were found to have moderate and rich SOM contents. According to multi-comparison analysis of SOM content, there were not any significant differences under dry agriculture in comparison to those under irrigated fields, while SOM content under pasture was significantly different ( $p < 0.05$ ). It can be stated that SOM and nitrogen contents decreases with increases in frequency and intensification of tillage applications and enhanced effect of mineralization (Balesdent et al., 2000). SOM content was around and below 2% in all agricultural areas. Similarly, SOM under agriculture were classified as poor (Ülgen and Yurtsever, 1974). Overall SOM contents of this study area can be classified low (Tümsavaş, 2003).

Even though soil bulk density was not significantly different under dry agriculture in comparison to those under irrigated management, pasture significantly increased soil bulk density ( $p < 0.05$ ). Soil bulk density is a variable showed that no-till areas had lower bulk density due to better development of the structure. This might be due to differences in pore size distribution and SOM contents. In dry and irrigated agricultural

fields, intensive tillage management significantly decreased the soil porosity. This finding was also reported by Barik et al. (2014) in a study.

There were not observed significant differences in total porosity under dry agricultural compare to those under irrigated management whereas bulk density under pasture was significant different ( $p < 0.05$ ) than all other managements. Soil particle density was not significantly different under any land use. Therefore, soil bulk density might be one of the few factors affecting porosity in these fields but not particle density.

Soils of these fields have strong acid and neutral pH conditions. Soil pH must be considered for fertilization. Agricultural practices that cause soil pH to drop should be avoided (Sezen, 2002). Considering the lime content of soils, lime application may be recommended to increase the pH since lime contents were also found to be low to provide sufficient nutrient availability for sustainable plant production under dry, irrigated, and pasture lands. Soil lime content ranged from 1.09% under dry management to 3.47% under pastureland. Therefore, lime fertilization to increase the lime content of these soils is recommended especially for those under irrigated conditions.

**Table 3.** Infiltration rates measured in soil samples ( $\text{cm h}^{-1}$ )

Time (min)	D1	D2	D3	D avrg	I1	I2	I3	I avrg	P1	P2	P3	P avrg
1	39.00	34.00	36.00	36.33	35.80	30.00	24.00	29.93	33.00	35.50	34.00	34.17
4	24.00	23.25	20.55	22.60	16.30	14.75	13.75	14.93	20.47	21.22	21.97	21.22
9	15.00	18.00	10.97	14.66	11.80	8.33	8.33	9.49	14.30	14.64	13.05	14.00
14	8.57	9.21	5.87	7.89	6.44	5.57	4.93	5.65	9.54	9.11	6.86	8.51
19	5.37	5.84	3.62	4.94	4.69	3.32	3.32	3.78	6.97	6.97	5.35	6.43
24	4.13	4.38	2.80	3.77	3.55	2.75	2.00	2.77	5.85	5.72	4.22	5.26
29	3.41	3.41	2.16	3.00	2.70	2.28	1.97	2.31	4.69	4.59	3.69	4.32
34	2.47	2.65	1.62	2.25	2.36	1.85	1.41	1.87	4.32	4.23	3.31	3.96
39	2.92	2.38	1.53	2.28	1.95	1.46	1.15	1.52	3.82	3.59	3.10	3.50
44	2.39	1.91	1.25	1.85	1.64	1.36	0.95	1.32	3.42	3.36	2.88	3.22
49	1.96	1.71	1.16	1.61	1.39	1.16	0.86	1.14	3.17	3.11	2.76	3.02
54	1.78	1.61	1.13	1.51	1.30	1.06	0.78	1.04	2.97	2.97	2.61	2.85
59	1.63	1.42	0.91	1.32	1.17	0.92	0.76	0.95	2.85	2.75	2.58	2.73
64	1.50	1.08	0.91	1.16	0.97	0.75	0.75	0.82	2.70	2.61	2.47	2.60
69	1.39	1.00	0.87	1.09	0.97	0.78	0.57	0.77	2.49	2.45	2.24	2.39
74	1.22	1.01	0.83	1.02	0.85	0.73	0.65	0.74	2.51	2.43	2.21	2.38
79	1.29	0.87	0.79	0.99	0.75	0.65	0.53	0.64	2.34	2.34	2.37	2.35
84	1.11	0.86	0.73	0.90	0.73	0.57	0.46	0.59	2.26	2.36	2.33	2.32
89	1.08	0.74	0.70	0.84	0.61	0.54	0.44	0.53	2.22	2.25	2.23	2.23
94	1.02	0.70	0.68	0.80	0.63	0.51	0.41	0.52	2.15	2.21	2.23	2.20
99	0.94	0.64	0.69	0.76	0.56	0.48	0.33	0.46	2.09	2.21	2.17	2.16
104	0.89	0.63	0.68	0.73	0.52	0.46	0.35	0.44	2.04	2.15	2.18	2.12

The lowest water stable aggregate value (16.86%) was determined under irrigated management (I2) in comparison those under pastureland (P2: 88.28%) (Table 1). Water stable aggregates were significantly influenced by land use (Table 2). The highest aggregate stability was measured under pasture. SOM in these fields can explain differences in aggregate stability (Canbolat and Demiralay, 1995). Barik (2011) stated that organic materials mixed into the soil significantly improve soil properties such as soil aggregate stability ( $p < 0.05$ ). Soil, if not processed in the structure of the naturally occurring secondary structures will not dissipate to ensure maintained stability (Six et al., 2002). Aggregate stability under dry and irrigated agricultural fields with tillage applied, was higher

than those under irrigated-no-till agricultural land. This can be interpreted as the aggregate stability is weakened due to increased mineralization and the intensive tillage applications under irrigated agricultural lands (Aksakal, 2004). In addition, wetting and drying with low intensification of tillage applications may improve stability compared to aqueous conditions. There was no significant difference in soil aggregate stability between the dry and irrigated fields whereas those under pasture areas showed significant difference (Table 2).

Hydraulic conductivity is a measure of soil conductivity and depends on soil and water properties. Soil hydraulic conductivity is particularly affected by soil properties such as

texture, structure, volume weight, SOM and bulk density (Lake, 2002).

According to tabulated detail values of soil water infiltration on Table 3, the highest readings for soil water infiltration was observed under pastureland (P2) and the lowest under irrigated management (I3). Soil water uptake

rates differed depending on the land use, whereas infiltration gradually decreased after the first 20 minutes. Infiltration measurements in the pasture area were measured higher than those used for tillage (Figure 1). It can be said that tillage has an adverse effect on infiltration. The high infiltration rate in unprocessed soils is due to the continuous and macro-pore networks of these soils (Erşahin, 2001).

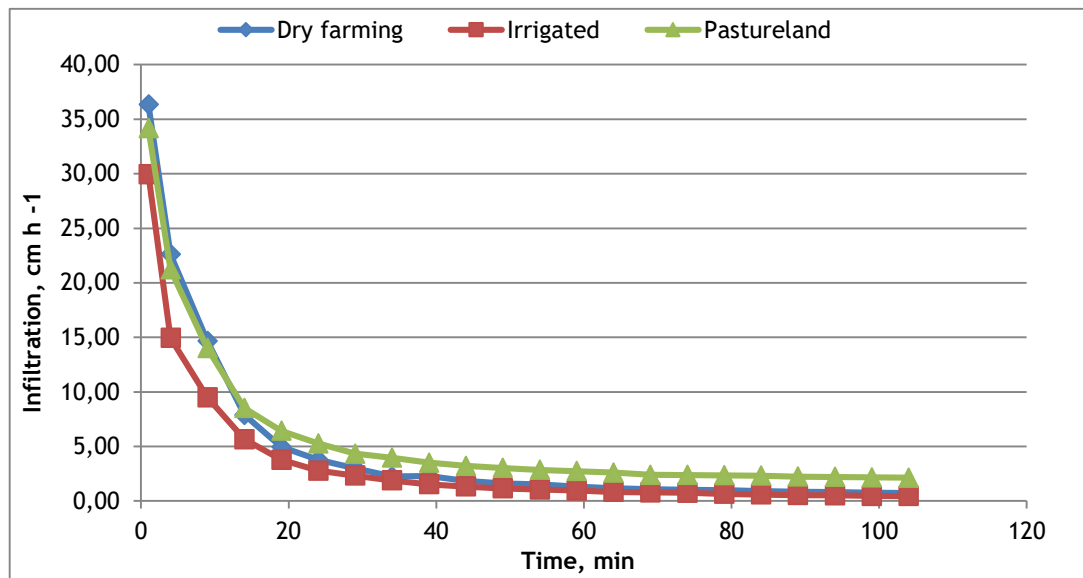


Figure 1. Infiltration curves of soils according to different land use conditions

The contribution of these surface-associated macropores to the infiltration rate is determined by their hydraulic properties, origins, shapes and bendability (Edwards, 1982). The decrease in soil infiltration over time is a result of changes in soil properties. The average infiltration values measured in

the pastureland were higher than the readings in dry and irrigated areas where embroidered agriculture was carried out over time. This can be explained by the fact that the permeability of soils in pasture areas as dispersions is lower than that of embroidered areas (Erşahin, 2001).

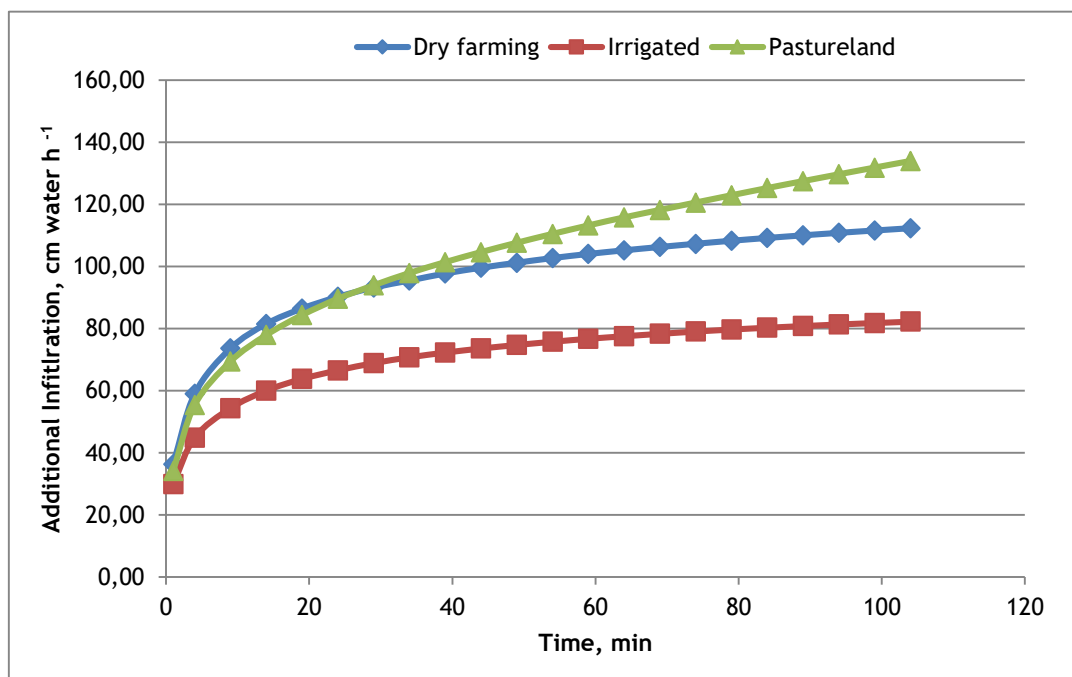


Figure 2. Additive infiltration graphs according to the use of soils

**Table 4.** Additive infiltration values measured in soil samples (cm water h<sup>-1</sup>)

Time (minute)	Dry farming Average	Irrigation Average	Pastureland Average
1	36.33	29.93	34.17
4	58.93	44.87	55.39
9	73.59	54.36	69.38
14	81.47	60.00	77.89
19	86.42	63.78	84.32
24	90.18	66.55	89.58
29	93.18	68.86	93.91
34	95.43	70.73	97.86
39	97.71	72.26	101.36
44	99.56	73.58	104.58
49	101.17	74.71	107.60
54	102.67	75.76	110.45
59	103.99	76.71	113.18
64	105.16	77.53	115.77
69	106.24	78.31	118.17
74	107.26	79.05	120.55
79	108.25	79.69	122.89
84	109.15	80.28	125.21
89	109.99	80.81	127.44
94	110.79	81.33	129.64
99	111.55	81.79	131.80
104	112.28	82.23	133.92

Infiltration is influenced by soil organic matter content, aggregate stability, pore, and thus bulk density, and texture and hydraulic conductivity. In the correlation test, significant positive relationships were determined between infiltration and aggregate stability ( $r=0.937$ ), hydraulic conductivity ( $r=0.818$ ) and organic matter ( $r=0.787$ ), whereas a negative correlation was found for infiltration and bulk density ( $r=-0.428$ ). When the total amount of water passing from the soil samples was evaluated, the highest water passed through pasture soils with 133.92 cm water and the lowest under irrigated agriculture (82.23 cm). This situation reveals the negative impact of embroidered agriculture (Edwards, 1982). Traffic on land, animal grazing, plant roots, soil management, soil processing and so on. As a result of the activities surface soil compacts, volume weight increases, infiltration decreases (Dao, 1993). This sequence of events is also in seasonal change in a cultivated soil.

According to Table 5, calculated infiltration rates, the highest infiltration rate (21.59) was calculated for the irrigated areas and the lowest infiltration rate (6.60) was calculated for the pasture lands. The low infiltration rate is a measure of the degree of degradation of soils over time. Accordingly, in the pasture infiltration measurements, soils were exposed to less distribution than dry and irrigated lands and as a result, they provided higher incremental infiltration value. In general, the main indicators of infiltration rate are soil properties (Arshad and Martin, 2002). The effect of organic matter on physical properties in soils rich in organic matter also increased the infiltration rate (Hawkes, 1984). The hydraulic conductivity of the soils was found highest in the pasture lands. This is an indication that pasture soils have

higher water transmission capabilities than embroidered agricultural areas. In this case, it is not correct to claim that there is no problem in pasture areas. Pasture areas usually consist of sloping lands may adversely affect the movement of water in the soil in these areas. For this reason, pastureland grazing in a plan will help to keep it covered. While the coarse structure of the soils initially leads to high infiltration, the decrease in the rate of infiltration over time may be due to the soil's easy dispersion depending on the organic matter content and the clogging of the pores rapidly. In this case, since the development of soil organic matter in the tilled areas will affect the stability positively, it will also decrease the tendency to erosion by reducing surface flow (Erşahin, 2001).

**Table 5.** Permeation rates determined in soil samples

Usage	Permeation Rate	Average
D1	16.77	
D2	28.36	20.46
D3	16.25	
I1	22.64	
I2	18.06	21.59
I3	24.07	
P1	7.02	
P2	6.80	6.60
P3	5.98	

Results from the principle component analysis showed that all three landuse were statistically different when we consider the total effect of all soil properties ude in the present study. Soil bulk density and clay content was key determinance for K,



whereas, lime content, SOM, and porosity showed to be key factors for M.

### Conclusion

Soils of the present study were identified as rough texture. There was a high variability in soil pH. Irrigated lands provide strong acidic conditions, while non-irrigated dry lands showed moderate acidity, and pasture areas neutral conditions. Soils of these fields were classified as lime-free. Calcification in dry and irrigated areas both for raising the pH and for insufficient lime addition in soils will affect production and soil properties positively. According to the infiltration curves of the soils, it is inevitable that the surface flow will occur as a result of the rapid fall of the infiltration in the tilled areas and as a result of this, negative impacts due to soil erosion may increase. Usage areas where this situation is felt the least are pasture areas. This is directly related to SOM content and the high stability of soil aggregate. Therefore, increasing SOM content in the soils of tilled agriculture will significantly reduce the tendency of soils to erosion. Therefore, increasing the organic matter content of the local soils will make significant contributions to sustainable soil management.

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