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Research Article

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The antioxidant and antialzhemier activities of the *Diplotaenia turcica* with phytochemical analysis

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Abstract

Diplotaenia turcica is an endemic plant that grows in eastern Turkey. This herb is used in herbal cheese, in meals and in traditional therapies. In this study, we aimed to examine some of the biochemical activities of this plant. Liquid chromatography-mass spectrometry (LC-MS) analysis was conducted on hydro alcohol extract of aerial part of DT. This analysis was applied to determine the total phenolic and flavonoid content, antioxidant and anti-alzheimer activities. LC-MS analysis showed that malic acid and quinic acid were found to be major compounds. The key flavonoids detected were hesperidine and rutine. The end of examination, the total amount of phenolic compound of extract was measured as 27.54 µg PEs/mg. And the total flavonoid amount was measured as 7.31 µg KEs/mg. β-carotene-linoleic acid test, DPPH free radical scavenging method, and ABTS cation radical scavenging outcomes were determined as IC50 of 169.71 µg/mL, 164.42 and 68.74 µg/mL, respectively. Cholinesterase inhibition test which indicate of alzheimer's did not inhibit acetylcholinesterase (AChE), but butyrylcholinesterase (BCHE) enzyme inhibition was 76.57%. As a result, further studies are needed in order to use Diplotaenia turcica plant for treatment or support purposes in the health field.

Keywords: Antialzhemier, Antioxidant, Activities, *Diplotaenia turcica*

Introduction

Antioxidants reduce or eliminate the harmful effects of free radicals in metabolism. It is also used as a preservative in the food industry. The antioxidant properties of many plants that used as food are examined and their health effects are investigated (Meydan, 2019). In this respect, very little work has been done on *Diplotaenia turcica* plant. Earlier studies with the root part of *Diplotaenia turcica* plants was determined to be nontoxic and to have good antioxidant content (Özdek Yıldırım et al., 2020).

The most important feature of *Diplotaenia turcica* plant is that it can be used in herbs, in meals and in traditional treatments. *Diplotaenia turcica* plant is used for protection from the bites of snake and other poisonous animals, as well as the root part has been used by the public since ancient times as rheumatism, diabetes and blood pressure balancer (Kaval et al., 2014; Uce and Tunçtürk, 2014). *Diplotaenia turcica* is an endemic plant and a new plant species introduced to the world of science in 2011. It is known as "Siyabo" in the region (Özdek et al., 2018).

Oxidative stress is a condition in which the balance between oxidant formation and antioxidant defense is disturbed in favor of oxidants (Koçak et al., 2020). This balance shifts towards pro-oxidants due to either increased production of reactive oxygen species or a reduction in the amount of antioxidants (Yuksek et al., 2017).

The most sensitive molecule to reactive oxygen species is thought to be lipids, the main component of the cell membrane.

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A sufficient amount of a reactive agent in the living organism may initiate lipid peroxidation. The reactive agent forms a radical by breaking one of the hydrogens of the fatty acid. This radical, which comes to the forehead, breaks off the proton of one of the neighboring fatty acids and leads to the formation of a new radical. As a result of the ongoing reactions, the radical concentration in the medium increases and consequently the lipid peroxidation takes place (Nordberg and Arner, 2001).

The effect of proteins on free radical damage is mainly the formation of carbonyl groups in amino acids such as histidine, tyrosine, phenylalanine. As a result of fragmentation and cross-linking with protein oxidation, degradation of protein functions (catalysis, transport, receptor, etc.) and antigenic changes that can stimulate the immunity system can occur (Nordberg and Arner, 2001).

Antioxidants are molecules that generally inhibit the formation of free radicals, or sweep up existing radicals, and which generally have phenolic function in their structure (Kähkönen et al., 1999). Under normal physiological conditions, cells are protected by antioxidant defense systems against oxidative damage caused by free radical products and molecules such as peroxides. Antioxidants have a complex structure and act in two types of mechanisms. These are defined as direct antioxidants and indirect antioxidants. Direct antioxidants (such as glutathione, phenolic compounds, tocopherols, ascorbic acid, and carotenoids) take part in physiological, biochemical, or cellular processes to inactivate free radicals or prevent chemical reactions initiated by free radicals (Rice-Evans et al., 1997). Indirect antioxidants do not play a role in preventing free radical or redox reactions. They strengthen the antioxidant capacity of the cell. This is because a group of enzymes (glutathione transferase, quinone reductase, epoxide hydrolase) in the human body cause detoxification of electrophilic species (Papetti et al., 2006).

Antioxidants are divided into two groups as enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants Glutathione peroxidase, Glutathione-S-transferase, glutathione reductase, superoxide dismutase, peroxidase and catalase. Non-enzymatic antioxidants include glutathione, flavonoids, ascorbate (Vit.C), β-Carotene (Vit.A), α-Tocopherol (Vit.E), urea, bilirubin, melatonin, ceruloplasmin, transferin, ferritin, lactoferrin, albumin and lipoic acid (Scandalios, 2002).

In this study, phytochemical profile, determination of total phenolic and flavonoid contents, antioxidant and anticholinesterase activities of aerial part of *Diplotaenia turcica* were investigated.

Materials and Methods

Plant material

The aerial part of *Diplotaenia turcica* was collected from Hakkari in June. Identification of the plant was carried out by Mehmet Fırat (Herbarium no: *32858 VANF)*, Department of Biology of the Faculty of Education Van Yüzüncü Yıl University.

Preparation of the extract

The aerial part of plant was dried up and powdered by using an electrical mill. A 100 g powdered sample was added to 1000 ml of 96% ethanol. Initially, 96% ethanol was utilized and after 24 hours of time period, the solution was filtered. A mixture of 70% ethanol 30% water was added to the pulp obtained after filtration. After 24 hours, the solution was filtered and then both filtered solutions were mixed together and then evaporated repeatedly by rotary evaporator at 50 °C and 70 rpm. 5.7% w/w dry extract were obtained from Concentrated extract by lyophilizing and stored at -20 °C (Özdek Seçkin et al., 2020).

LC–MS/MS analysis

LC–MS/MS analyses of the phenolic compounds were performed by using a Nexera model Shimadzu UHPLC coupled to a tandem MS instrument. MS detection was performed using Shimadzu LC–MS 8040 model triple quadrupole mass spectrometer equipped with an ESI source operating in both positive and negative ionization modes. The liquid chromatograph was equipped with LC-30AD binary pumps, DGU-20A3R degasser, CTO-10ASvp column oven and SIL-30AC autosampler. The chromatographic separation was performed on a C18 reversed phase Inertsil ODS-4 (100 mm × 2.1 mm, 2 µm) analytical column. The column temperature was fixed at 35 °C. The elution gradient consisted of mobile phase A (water, 10 mM ammonium formate and 0.1% formic acid) and mobile phase B (acetonitrile). The gradient program with the following proportions of solvent B was applied t (min), 0-10 minutes %B (5-20), 10-22 minutes (20), 22-36 minutes (20-50), 36-40 minutes (95), 40-5- minutes (5). The solvent flow rate was maintained at 0.25 mL/min and injection volume was settled as 4 µL. Subsequent to several combinations of trials, a gradient of acetonitrile and water (10 mM ammonium formate and 0.1% formic acid) system was concluded to be the best mobile phase solution. For rich ionization and the separation of the molecules, the mentioned mobile phase was proved to be the best of all. ESI source was chosen instead of APCI (Atmospheric Pressure Chemical Ionization) and APPI (Atmospheric Pressure Photoionization) sources as the phenolic compounds were small and relatively polar molecules. Tandem mass spectrometry was used for the current study since this system is commonly used for its fragmented ion stability. The working conditions were as follows interface temperature; 350 °C, DL temperature; 250 °C, heat block temperature; 400 °C, nebulizing gas flow (Nitrogen); 3 L/min and drying gas flow (Nitrogen); 15 L/min.

Determination of total phenolic and flavonoid content

Phenolic content was determined using the Folin-Ciocalteu colorimetric method (Slinkard and Singleton, 1977) with some modifications. Phenolic amounts were expressed as micrograms pyrocatecol (µg PEs/mg extract) per milligram of sample, and were calculated according to the following equations.

Absorbance = 0.0351 pyrocatechol (µg) + 0.0466 (R^2: 0.9952)

Flavonoid content was determined according to the aluminum chloride method (Moreno et al., 2000) with some modifications. Quantities of flavonoid content in the extract were expressed as quercetin equivalents (QEs) in micrograms per milligram of sample (µg QEs/mg extract) and were calculated according to the following equations.

Absorbance = 0.0353 quercetin (µg) + 0.0477 (R^2: 0.9914)

Antioxidant activities

Antioxidant activities of *Diplotaenia turcica* plant were investigated using β-carotene-linoleic acid test (total antioxidant activity test) (Kosanić et al., 2012), DPPH free radical scavenging method (Kosanić Ranković, Dašić, 2012), ABTS cation radical scavenging method (Re et al., 1999) and copper II ion reducing method (CUPRAC) (Apak et al., 2004).

Enzyme inhibitory activities

A spectrophotometric method was used to demonstrate acetyl- and butyryl-cholinesterase inhibitor activity by the method developed by Ellman et al. (Boğa et al., 2011).

Statistical analysis

The mean of 3 parallel measurements obtained from the results of antioxidant and anticholinesterase activities assays were taken as ± SD (n=3). Significant differences between means were determined by student's-t test, p values <0.05 were regarded as significant.

Results and Discussion

It is known that about 13000 plant species are used worldwide for therapeutic purposes (Pattanayak et al., 2010). Plants protect the cells against natural oxidation reactions due to the antioxidant substances they contain (Kähkönen Hopia Vuorela Rauha Pihlaja Kujala, Heinonen, 1999).

DT is an endemic plant species growing in eastern Turkey Van-Bitlis-Hakkari (Özdek Yıldırım, Değer, 2020).

Phenolic compounds carry an aromatic hydroxyl nucleus. There are about 8000 different compounds in nature. Phenolic compounds found in plants are free radical terminators and are known as important antioxidants. Flavonoids are important phenolics. Flavonoids have more than 4000 species found in the roots, flowers and leaves of plants. (Ertaş et al., 2014). According to the LC-MS analysis results, hesperidin (27.71µg/g extract), p-coumaric acid (31.18 µg/g extract), gallic acid (238.86 µg/g extract), caffeic acid (7.68 µg/g extract), quinic acid (3505.57 µg/g extract), 4-OH-benzoic acid (40.83 µg/g extract), tr-ferulic acid (69.38 µg/g extract), chlorogenic acid (1011.51 µg/g extract), protocatechuic acid (27.26 µg/g extract), malic acid (15641.23 µg/g extract) and rutin (89.02 µg/g extract) molecules were detected (Table 1).

Table 1. Analytical parameters and results of LC-MS/MS analysis of the aerial part of *Diplotenia turcica* extract

No	Analytes	RT[a]	M-H$^+$ (m/z)[b]	Linearity Range (µg/L)	LOD/LOQ (µg/L)[c]	U[d]	Quantification (µg analyte / g extract)[e] *Diplotaenia turcica*
1	Hesperidin	20.118	610.90	25-1000	3.4/4.2	0.0262	27.71±0.007
2	p-Coumaric acid	15.675	162.95	25-1000	7.3/9.1	0.0516	31.18±0.016
3	Gallic acid	4.427	168.85	250-10000	95.5/106.9	0.0282	238.86±0.067
4	Caffeic acid	12.182	178.95	25-1000	18.4/22.4	0.0354	7.68±0.003
5	Quinic acid	1.27	190.95	250-10000	75.8/79.4	0.0082	3505.57±0.287
6	4-OH-benzoic acid	10.1	136.95	250-10000	33.2/38.1	0.0289	40.83±0.012
7	tr-Ferulic acid	17.113	192.95	250-10000	36.6/42.0	0.0494	69.38±0.034
8	Chlorogenic acid	10.189	353.15	25-1000	6.2/8.1	0.0069	1011.51±0.070
9	Protocatechuic acid	7.16	152.95	100-5000	28.2/31.4	0.0411	27.26±0.011
10	Malic acid	1.45	133.00	250-10000	55.3/67.5	0.0113	15641.23±1.767
11	Rutin	17.486	609.05	25-1000	5.5/6.5	0.0159	89.02±0.014

aRT: Retention time, bM-H+(m/z): Molecular ions of the standard compounds (mass to charge ratio), eLOD/LOQ (µg/L): Limit of detection/ Limit of quantification, dU (%): Percent relative uncertainty at 95% confidence level (k=2), eValues in µg/g (w/w) of plant extract

The total amount of phenolic compounds (27.54 µg PEs/ mg extract) and total flavonoid amounts of DT were found to be low (7.31 µg QEs/mg extract) (Table 2). In our study, β-carotene-linoleic acid test, DPPH free radical scavenging and ABTS cation radical scavenging activities results were determined as 169.71 µg/mL, 164.42 µg/mL and 68.74 µg/mL in terms of IC_{50}, respectively (Table 3).

Table 2. Total phenolic and flavonoid amounts of extract of Diplotaenia turcica aerial part (DT)[a]

	Phenolic content (µg PEs/mg extract)[b]	Flavonoid content (µgQEs/mg extract)[c]
DT	27.54 ± 1.87	7.31 ± 0.60

a: Values expressed are means ± standard deviation of three parallel measurements (p<0.05)
b: PEs, pyrocatechol equivalents (y=0.0351x + 0.046 R^2=0.9952)
c: QEs, quercetin equivalents (y=0.0353x + 0.0477 R^2=0.9914)

Table 3. Antioxidant activities results of *Diplotaenia turcica* aerial part extract (DT), BHT, BHA and α-TOC [a]

	IC_{50} values (µg/mL)		
Samples	β-Carotene-Linoleic acid	DPPH Free Radical	ABTS Cation Radical
DT	169.71±2.56	164.42±3.02	68.74±0.94
BHA	1.5±0.01	7.88±0.20	17.59±0.10
α-TOC	2.1±0.10	16.30±0.79	9.74±0.42
BHT	1.3±0.03	58.86±0.50	13.25±0.27

a: Values expressed are means ± standard deviation of three parallel measurements (p<0.05)

In the study, β-carotene-linoleic acid test, DPPH free radical scavenging and ABTS cation radical scavenging activities results can be said to be moderate active. On the contrary, in the CUPRAC results were found to be low in all concentrations compared to the same standards (Table 4).

Table 4. CUPRAC results of *Diplotaenia turcica* aerial part extract (DT), BHT, BHA and α-TOC [a]

	10 µg/mL	25 µg/mL	50 µg/mL	100µg/mL
DT	0.106±0.015	0.153±0.006	0.216±0.023	0.384±0.042
BHT	0.605±0.086	1.344±0.035	2.256±0.042	3.987±0.007
BHA	0.205±0.014	0.365±0.027	0.616±0.029	1.171±0.110
α-TOC	0.305±0.023	0.746±0.057	1.528±0.068	2.551±0.066

a: Values expressed are means ± standard deviation of three parallel measurements (p<0.05)

In antioxidant activity studies, it is stated that it is necessary to use different methods because the reaction conditions such as pH, temperature, working sensitivity and solvent affect the results (Frankel et al., 1994; Koleva et al., 2002). These different results may be due to them.

The *Diplotaenia turcica* extract used in the study was prepared using ethanol-water as a non-toxic solvent. The vegetal samples exhibit structural differences in their content and therefore different solvents may be used for each sample in extraction methods (Boğa Hacıbekiroğlu, Kolak, 2011). In other studies, the most suitable solvent for the plant can be selected by working with different solvents. Thus, accurate and high results can be obtained about the antioxidant capacity of plants.

In recent years, Alzheimer's disease has increased significantly. Researching new and useful strategies for the treatment of Alzheimer's disease is one of the most important issues. The enzyme inhibition method has been one of the research subjects. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes are enzymes that play an important role in Alzheimer's disease. It is known that this disease increases as acetylcholine deficiency increases. Thus, Alzheimer's disease has been associated with these enzymes. Therefore, inhibition of acetylcholinesterase and butyrylcholinesterase enzymes is thought to have a positive effect on the course of the disease (Raskind et al., 2004).

In the study, the effect of DT on inhibition of BChE enzyme was 76.57% as inhibition, while the inhibition value of galantamine used as standard was 84.3. It was observed that DT did not have AChE enzyme inhibition effect (Table 5).

Table 5. Anticholinesterase activity results of *Diplotaenia turcica* aerial part extract (DT) [a]

Samples	AChE(%inhibition)	BChE (%inhibition)
DT	N.A.	76.57±0.67
Galantamine[b]	84.04±1.13	84.30±0.99

Values expressed are means ± standard deviation of three parallel measurements (p<0.05).
[a] 200µg/mL
[b] Standard substance.
N.A.: Not active.

Conclusion

The present study shows that the total phenolic content is more than flavonoid content of the *Diplotaenia turcica* aerail part extract and has moderate antioxidant properties and strong anti-butyrylcholinesterase activity. Phytochemical studies are required to characterize the active components of the *Diplotaenia turcica*. To better understand the antioxidant and anticholinesterase potential, more laboratory and clinical trials of the active compounds found in the *Diplotaenia turcica* plant extract are required.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Photosynthetic performance responses in different phyiological development stages of some *Brassica juncea* genotypes in field condition

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Abstract

In this study *Brassica juncea* genotypes were planted as fall sowing at the experimental fields located at Yenimahalle-Ankara location based on randomized block design. In order to investigate photosynthetic performances of three *B. juncea* genotypes; net photosynthetic rate = P_N [µmol(CO_2) $m^{-2}s^{-1}$], transpiration = E (mmol $m^{-2}s^{-1}$), stomatal conductance = g_s (mmol $m^{-2}s^{-1}$), intercellular/ambient CO_2 air= C_i [µmol (CO_2) mol^{-1}(hava)], mesophyll conductance= g_m [mmol(CO_2)/m^2s^1] and photosynthetic water use efficiency = PWUE [µmol(CO_2)/mol(H_2O)] parameters (model LCi Photosynthesis System, ADC Bioscientific Ltd., Hertfordshire, UK) were measured and phenological aspects were recorded at different physiological stages as booting, anthesis and grain filling during experiment. Photosynthetic rate and stomatal conductance were associated with seed yield in *B. juncea* genotypes and the selection of genotypes with high gas exchange may provide development of mustard (*B. juncea*) genotypes with high yield.

Keywords: *Brassica juncea*, Gas exchange, Stomatal conductance, Yield

Introduction

Brassicaceae family includes economically important industrial oilseed, spice, vegetable, and fodder crops and exhibits extreme morphological diversity and many crop species (Li et al., 2017). This family, comprises a number of plant species. The dicotyledonous family Brassicaceae has 338 genera and 3709 species (Warwick et al., 2006). The species which belong to the family *Brassica* L., Turkish natural flora of *Sinapis alba* (white mustard), *S. arvensis* (wild mustard), *B. juncea* (brown mustard), *B. rapa* syn. *B. campestris* (field mustard) and *B. nigra* (black mustard) are the most important among them (Babac, 2004; Guner et al., 2012). Brown mustard belonging to the family *Brassicaceae* are cultivated for different usage, especially for spice and energy industries. Brown mustard is tap rooted, herbaceous, upright growing, multi-branched, yellow-flowered and seed and grow as an annual plant cultivated worldwide for the industrial oilseed, spice, vegetable and fodder crop species (Kayacetin 2019; Mulligan and Bailey, 1975). In India, *B. juncea* is dominant, whereas in Europe and Canada, *B. juncea* is planted in minor areas just for condiment use (Sovero 1993). Fertilization of ovules generally stem from self-pollination, with interplant outcrossing rates

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of 20-30% (Rakow and Woods, 1987). *B. rapa* [AA (n:10)] and *B. nigra* [BB (n:8)] are two basic diploid species and their natural interspecific cross lead to amphidiploid species, *B. juncea,* [AABB (n:18)] (Nagaharu, 1935). Brown mustard, which is among the genetic resources of our country, is one of the important non-edible that can be cultivated for industrial oil purposes on marginal areas (Kayacetin, 2019).

Photosynthesis measurements have been succesfully used to demonstrate genetic diversity in performance and to explain physiological responses to environmental effects and crop inputs. Direct mesurements of photosynthesis from gas exchange are performed with an infrared gas analyzer (IRGA) which measures the carbon dioxide flux within a sealed chamber containing a leaf sample. Measurements of gas exchange have become increasingly valuable in presicion phenotyping studies. The eficiency of utilazing the absorbed photosynthetically active radiation (PAR) for biomass production can change with variation in plant growth stage, field management practices and environmental stress intensity (Orange and Ebadi, 2012). In present paper photosynthetic performance responses and phenological aspects in different phyiological development stages of some *B. juncea* genotypes in field condition were compared.

Materials and Methods

The field experiment was carried out during fall season of 2017-2018 at Central Research Institute for Field Crop at the experimental fields under fall sowing condition. The study made use of three brown mustard genotypes as research material which were selected from among a large number of genotypes belonging to different origin obtained from the USA gene bank.

Table 1. The long-term and 2017-2018 monthly agro-climatic meteorological data pertaining to vegetation period (September to August) conditions of Yenimahalle location (925 m altitude)

Climatic factors	Years	Months										Total or mean
		S	O	N	D	J	F	M	A	Ma	Ju	
Precipitation (mm)	Long years	17.5	31.8	34.2	42.0	40.2	33.0	36.7	46.7	49.9	34.2	366.2
	2017-2018	3.2	10.0	37.2	41.5	48.0	43.5	62.0	2.6	86.2	37.4	371.6
Relative humidity (%)	Long years	49.1	60.5	69.7	76.5	76.4	70.7	63.2	59.0	56.5	52.1	63.4
	2017-2018	34.2	55.1	70.0	78.3	77.0	73.3	63.2	44.4	60.0	53.1	60.9
Mean temp. (ºC)	Long years	19.0	13.1	6.8	2.3	0.4	2.3	6.4	11.5	16.2	20.3	9.8
	2017-2018	22.6	12.4	7.2	4.8	3.1	6.6	10.2	15.4	18.0	21.4	12.2
Maximum temp. (ºC)	Long years	32.6	27.6	19.7	13.9	11.9	14.7	21.4	25.7	29.3	33.6	33.6
	2017-2018	37.7	23.7	18.6	17.0	11.4	15.6	22.7	28.1	28.9	33.9	37.7
Minimum temp. (ºC)	Long years	6.6	1.1	-3.8	-8.2	-11.5	-9.9	-5.9	-0.8	4.1	8.1	-11.5
	2017-2018	7.7	3.2	-1.9	-4.6	-3.9	-2.7	-2.8	0.6	9.1	12.4	-4.6

(S, September; O, October; N, November; D, December; J, January; F, February; M, March; A, April; Ma, May; Ju, June)
The data were obtained from Yenimahalle Meteorology Station of Central Field Crops Research Institute, Ankara Turkey

Table 2. The soil analysis during 2017, performed out of the soil taken at a depth of 0-20, 21-40 cm of Yenimahalle location

Depth (cm)	Texture	Saturation content (%)	Total salt (%)	pH	Lime (%)	Phosphorus (P)	Potassium (K)	Organic Substance (%)
0-20	Clay loamy	56.0	0.025	7.81	5.3	9.3	126.0	1.35
21-40	Clay loamy	56.0	0.025	7.81	5.2	10.5	240.0	1.28
Mean		56.0	0.025	7.81	5.3	9.9	183.0	1.32

Data were obtained from Soil Fertilizer and Water Resources Institute

Genotypes were planted as fall sowing at the experimental fields located at Yenimahalle location 39°12′ - 43°6′ N, 35°58′ - 37°44′ E, and 925 m altitude, rainfed conditions.

The montly meteorological data pertaining to vegetation period (September to June) of long term and 2017-2018 agro climatic conditions of Yenimahalle, Ankara are given in Table 1. There was total precipitation of 366.2 and 371.6 mm, mean temperature of 9.8 and 12.2 °C, and an mean humidity of 63.4% and 60.9%, respectively at Yenimahalle location.

The soil analysis during 2017, performed out of the soil taken at a depth of 0-20, 21-40 cm showed low organic matter (1.35% and 1.28% respectively), in alkaline (pH 7.81), limey (5.3% and 5.2%, respectively), and clay-loamy soils (Table 2).

Each genotype was planted as two rows, 3 m plots with 30 cm row spacing and three replicates. In this study, the thousand seed weight and seed yield were determined as described by Kayacetin (2019).

The crude oil content was determined by grinding 10 g of powdered mustard seed samples and extracting by hexane that were used with Gerhardt 2000 soxhlet apparatus (Singh et al., 2014).

Genotypes were grown under natural conditions without using any fertilizer or pesticide to measure their potential under natural conditions. The seeds of these genotypes were considered mature and harvested on achieving 8.5% moisture content (CFIA, 1999).

At Yenimahalle location sowing date was 31th October 2017, emergence date was 12th November 2017, harvest date 13th-17th June 2018, days to maturity 225-229 d. Phenological aspects were (days to emergence, days to 50% flowering and days to maturity, plant height, number of branches) recorded

Statistical analysis: All data were statistically analyzed using AVCI's analysis of variance technique and the treatment means were compared using LSD test at 0.01 probability level (Steel & Torrie 1984).

In order to investigate photosynthetic performances of three brown mustard genotypes; net photosynthetic rate = P_N ***[µmol(CO_2)*** $m^{-2}s^{-1}$], transpiration = E (mmol $m^{-2}s^{-1}$), stomatal conductance = g_s (mmol $m^{-2}s^{-1}$), intercellular/ambient CO_2 air= C_i [µmol (CO_2) mol^{-1}(hava)], mesophyll conductance= g_m [mmol (CO_2)/ m^2s^1] ve photosynthetic water use efficiency = PWUE [µmol(CO_2)/mol(H_2O)] parameters (model LCi Photosynthesis System, ADC Bioscientific Ltd., Hertfordshire, UK) were measured at different physiological stages as booting, anthesis and grain filling during experiment.

Measurements were made from full developed youngest leaves at PAR of 892 to 2055 [µmol (foton) m^2s^1] and environmental CO_2 concentration value of 272-368 ppm. Mesophyll conductance was calculated by dividing Pn by Ci (Fischer et al., 1998). Photosynthetic water use efficiency (PWUE) was calculated by dividing Pn by gs (Ahmadi and Siosemardeh, 2005).

Results and Discussion

Significant (p<0.05) differences were noted among different genotypes regarding some phenological aspects and yield components characters of *Brassica juncea* genotypes in different growth stages. The Izmir genotypes showed higher plant height (132.90 cm), number of branches (12.60 branch $plant^{-1}$), number of capsules (282.71 capsule $plant^{-1}$), thousand seed weight (2.88 g), seed yield (290.76 g m^{-2}) and crude oil content (29.06%) than the other genotypes. India genotypes showed lower plant height (100.27 cm), number of branches (8.27 branch $plant^{-1}$), number of capsules (215.07 capsule $plant^{-1}$), thousand seed weight (2.53 g), seed yield (213.17 g m^{-2}) and crude oil content (25.65%) than other genotypes. Days to emergence, days to 50% flowering and days to maturity did not change among the genotypes (Table 3. and 4).

Net photosynthetic rate (A) was higher at anthesis stage than booting and grain filling stages in all *Brassica juncea* genotypes. Transpiration rate (E) was different among all stages and genotypes. Transpiration (E) increased in the same way in Izmir and Tekirdag genotypes while it increased through late stages in India genotype. Photosynthesis and transpiration are both physiologically complex processes. Plants are thought to optimize water use efficiency by adjusting the rate of photosynthesis in relation to the rate of transpiration (Farqual et al., 2002). Intercellular CO_2 concentrations (Ci) was highest at booting stage in Izmir and Tekirdag genotypes and was highest in grain fillling stage in India genotype. Intercellular CO_2 concentrations (C_i) was almost inversely related with photosynthesis rate in India genotype. Stomatal conductance (Gs) was highest at booting stage in all genotypes and decreased through later stages.

Mesophyll conductance (MC) was highest at anthesis stage in all *Brassica juncea* genotypes. Photosynthetic water use efficiency (PWUE) was highest at grain filling period in Izmir and Tekirdag genotypes and at booting stage in India genotype. PWUE (photosynthetic water used efficiency) gives the information about the assimilation of dry mass produced per unity of lost water through transpiration process. This parameter is important for indicating water management

to agricultural productivity. PWUE increased from booting through grain filling period in the genotypes. A geat value of PWUE parameter does not mean the increased assimilation of plant. At ripening stage, a higher value means a higher water consumer (Acatrinei, 2010). The resulted relationships in different growth stages with decreasing slope toward the end of growing season show the possibility to save water with withdrowing water application during last growth stages (Azizian and Sepaskhah, 2014). Similar findings were also reported for wheat (Abbate et al., 2004) and potato (Ahmedi et al., 2010). Increased yields have been achieved by (i) increased or extended photosynthesis per unit land area and (ii) increased patitioning of crop biomass to the harvested product. The first has mainly been achieved by irrigation schemes and improved agronomic practices, in particular the use of inorganic fertilizers, but also to elevated atmosphere CO_2 concentrations, whereas the second has largely been due to plant breeding (Richards 2000). It has been showed positive correlations between grain yield, photosynthetic rate, and stomatal conductance in irrigated short spring wheats (Fischer et al., 1998; Shimshi and Ephrat, 1975), *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. (Rodriguez & Estrada 2005). Higher stomatal conductance in plants is known to increase CO_2 diffusion into leaves thereby favouring higher photosynthetic rates. Higher net assimilation rates could in turn favour a higher biomass and higher crop yields (Taiz and Zeiger, 1998). Recent studies have shown possitive correlation between yield increases and increases in stomatal conductance (Rodriguez and Estrada, 2005).

Traits related to stomatal conductance may prove useful for improving selection for yield potential. Higher yield-potential wheats had greater stomatal conductance and, therefore, cooler canopies than older, lower yield potential releases (Fischer et al., 1998).

Table 3. Variation of some photosynthetic parameters of *Brassica juncea* genotypes in different growth stages

Genotypes	Net photosynthetic rate µmol CO_2 m^{-2},s^{-1}				Transpiration mmol m^{-2},s^{-1}				Intercellular/ambient air CO_2 (Ci/Ca) µmol mol^{-1}			
	Anthesis	Grain filling period	Booting	Mean	Anthesis	Grain filling period	Booting	Mean	Anthesis	Grain filling period	Booting	Mean
Izmir	26.35	12.82	4.78	14.65	3.55	2.10	163.83	56.49	120.00c	164.50b	0.28d	94.93b
Tekirdag	21.20	17.13	4.10	14.14	4.56	3.41	183.25	63.74	160.00b	147.00bc	0.21d	102.40ab
India	21.93	9.15	3.78	11.62	5.40	6.33	142.17	51.30	144.67bc	223.67a	0.18d	122.84a
Mean	23.16a	13.03b	4.22c		4.50b	3.94b	163.08a		141.56b	178.39a	0.22c	
F $value_G$				2.81				1.38				3.77*
F $value_S$				95.91*				298.01*				159.69*
F $value_{GxS}$				2.87				1.84				4.20*
CV (%)				21.55				27.87				20.92

Table 4. Variation of some photosynthetic parameters of *Brassica juncea* genotypes in different growth stages

Genotypes	Stomatal resistance mmol m^{-2},s^{-1}				Mesophyll conductance(A/Ci) mmol m^{-2},s^{-1}				Photosynthetic water use efficiency (A/gs) µmol CO_2 / mol H_2O			
	Anthesis	Grain filling period	Booting	Mean	Anthesis	Grain filling period	Booting	Mean	Anthesis	Grain filling period	Booting	Mean
Izmir	0.22c	0.11c	30.57b	10.30b	0.22	0.08	17.83	6.04	125.15a	109.05ab	0.16d	81.23a
Tekirdag	0.20c	0.14c	30.90b	10.41b	0.13	0.11	19.49	6.58	124.82a	88.08bc	0.13d	78.11a
India	0.25c	0.15c	34.87a	11.75a	0.15	0.04	20.38	6.86	118.71a	64.39c	0.11d	50.86b
Mean	0.22b	0.13b	32.11a		0.17b	0.08b	19.23a		105.28a	104.79a	0.13b	
F $value_G$				22.33*				0.48				38.10*
F $value_S$				2324.73*				343.18*				278.63*
F $value_{GxS}$				42.75*				0.54				48.54*
CV (%)				3.89				27.51				19.75

Table 5. Variation of some phenological aspects and yield components of *Brassica juncea* genotypes in different growth stages

Genotypes	Days to 50% flowering (d)	Days to maturity (d)	Plant height (cm)	Number of branches (branch plant^{-1})
Izmir	198	261	132.90a	13.60a
Tekirdag	198	261	112.87ab	11.87ab
India	198	261	100.27b	8.27b
F value$_G$			26.83*	16.35*
CV (%)			4.77	10.36

Table 6. Variation of some phenological aspects and yield components of *Brassica juncea* genotypes in different growth stages

Genotypes	Number of capsules (capsul plant^{-1})	Thousand seed weight (g)	Seed yield (g m^{-2})	Crude oil content (%)
Izmir	282.71	2.88a	290.79a	29.06a
Tekirdag	238.67	2.69ab	246.99a	25.82b
India	215.07	2.53b	213.17b	25.65b
F value$_G$	3.05	7.60*	31.31*	8.83*
CV (%)	13.92	4.15	8.01	4.17

Conclusion

Higher photosynthetic rates could in turn favor a high crop yield and higher stomatal conductance appears to favor higher yields. These results suggest that Izmir genotype, which is the highest yielding genotype, has substantial reserve capacity for photosynthesis. In conclusion photosynthetic rate and stomatal conductance were associated with seed yield in *Brassica juncea* genotypes and the selection of genotypes with high gas exchange may provide development of brown mustard genotypes with high yield.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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The effect of soy flour and carob flour addition on the physicochemical, quality, and sensory properties of pasta formulations

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Abstract

In the present study, soy flour (SF) and carob flour (CF) were used as a substitute for wheat flour (WF) in 6 different pasta formulations. The effect of SF and CF on the quality properties of the enriched pasta formulations was investigated. With the increase in SF and CF, ash, protein, dietary fiber content of the pasta increased whereas moisture, fat, and carbohydrate contents decreased. With the increase in CF, a significant decrease was observed in the *L** (brightness) value and an increase in *b** value with an addition of SF. The addition of SF and CF reduced the amount of substance passed to the water, improving the quality of the pasta. According to the results of sensory analysis, the highest values in terms of the overall evaluation were determined in the D (80 WF: 0 SF: 20 CF) and the E (80 WF: 20 SF: 0 CF) samples, and it was determined that up to 20% SF and CF can be recommended. According to the study results, it was thought that SF and CF can be used as functional food additives in different food formulations to improve the functional and nutritional properties of food products.

Keywords: Pasta, Carob Flour, Soy Flour, Total Dietary Fiber, Functional Food

Introduction

Pasta, which is known and loved in every part of the world, is a cereal-based food produced from durum wheat (Cárdenas-Hernández et al., 2016; Tazrart et al., 2016). Pasta is an economical product, easy to prepare with a long shelf-life, and due to its nutritional properties, especially for low glycemic index (GI), it is a source of carbohydrates consumed by all ages (Bernard, 1988; Petitot et al. 2009). Pasta contains 11-15% protein, and low levels of lysine and threonine (Abdel-Aal and Hucl, 2002). Pasta quality is related to the quality of wheat used, the pasta production process, and the amount and the quality of protein effective during cooking (Feillet and Dexter, 1996; Del Nobile et al., 2005). Pasta is high in starch whereas low in dietary fiber, minerals, vitamins, and phenolic compounds (Gull et al., 2018). The growing demand for healthy foods has increased researchers and food manufacturers' interest in developing pasta products rich in minerals, vitamins, dietary fiber, and with a low glycemic index. In studies carried out to increase the nutritional value of pasta, different functional components, color pigments, high protein, and dietary fiber sources were added to the formulations (Nilusha et al., 2019; Menon et al., 2012; Bustos et al., 2013; Kaur et al., 2012; Adegunwa et al., 2012). In new functional pasta products enriched with bioactive compounds, vegetables (bean flour, sweet potato flour), fruits (carrot powder, apple peel powder, grape powder, green banana flour), legume flours (chickpea flour, fava protein), plant seeds (quinoa flour, amarant flour) (Goñi and Valentín-Gamazo, 2003; Zandonadi et al., 2012; Ginting and Yulifianti, 2015; Torres et al. 2007; Laleg et al., 2017; Bouasla et al., 2017; Lorusso et al. 2017),

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enriched durum wheat, cereal bran, cereal flours (wheat, rice, barley, oats) (Hussein et al., 2011; Kaur et al., 2012; Fuad and Prabhasankar, 2012; Fiorda et al., 2013; Chanu and Jena, 2015), for high protein content; milk and dairy products, whey protein, egg white powder, fish protein concentrates, etc. (Nielsen et al., 1980; Devi et al., 2013; Cappa and Alamprese, 2017; Xie et al., 2020) were used and the vitamin, mineral, essential amino acid, fatty acid and antioxidant contents of the pasta were enriched.

Soybean (*Glycine max* L.) is a plant species belonging to the *Leguminosae* family and is one of the legume products that are economically important and widely consumed worldwide (Vagadia et al., 2017). Soybean seeds contain over 40% protein, 30-35% carbohydrates, 20% fat, and it is rich in terms of dietary fiber sources, vitamins (A, B, C, and D), minerals (Ca, P), and unsaturated fatty acids (linoleic acid) (Nishinari et al., 2014; Ma et al., 2019; Rani et al., 2019; Mohajan, et al., 2018; Wang et al., 2004). It has been reported that soy protein has good components in reducing the risk of cardiovascular diseases, lowering cholesterol, in addition to having emulsifying, gelling, water, and oil holding capacity (Nishinari et al., 2014; Wietrzyk et al., 2005). Soybean and its products are used as additives, emulsifiers, vegetable oil sources, product-enrichers in the food industry and are also used as pulp in animal nutrition and as industrial substances in the manufacturing industry (Liu, 2004). Soy products, isoflavones, linolenic acid, also known as omega-3 fatty acids, and dietary fiber content are very important functional food components (Riaz, 2006; Nilüfer and Boyacıoğlu, 2008; Liu, 2004). Soy and soy products are an important source of nutrients and are preferred for increasing the nutritional and health properties of foods in product enrichment due to their seed's high content of soy protein, cholesterol-lowering properties, phytoestrogen properties, and also for reducing the risk of heart diseases and their positive effects on calcium metabolism (Jones, 2002; Wylie-Rosett et al., 2002; Garcia et al., 1997; Roccia et al., 2009; Shih et al., 2015; Zhang et al., 2020). There are studies on different cereal products in which soybean flour is used as a source of protein, dietary fiber, antioxidant, and polyphenol. It has been reported that soy flour is used in many foods including healthy drinks, soup, biscuit, bread, pasta, etc. to provide nutritional supplements and to extend the shelf-life (Mohajan et al., 2018). Several studies have been reported on the determination of the cooking properties and sensory acceptability of spaghetti, the use of soy flour with rice flour (Sereewat et al., 2015), determination of the sensory and nutritional composition properties of spaghetti enriched with soy flour (Shogren et al., 2006), effect on nutritional properties (Park et al., 2015), the effect of germinated, steamed and roasted soy flour on the physicochemical and sensory properties of bread (Shin et al., 2013), determination of structural and quality characteristics of soy-enriched functional noodles (Rani et al., 2019), lean soy flour, the effect of other flour ingredients on the quality properties of pasta (Jalgaonkar et al., 2018), lean soy flour enriched biscuits (Serrem et al., 2011; Singh et al., 2000), the use of soy flour in gluten-free bread making (Ribotta et al., 2004), use of soybean meal as a source of protein in trout feed (Bilgüven, 2006).

Carob (*Ceratonia siliqua* L.), a perennial plant belonging to the *Leguminosae* family grows widely in the Mediterranean countries (Durazzo et al., 2014). Fruit and peels of the plant are used as raw materials in different branches of the industry. It has been reported that carob is of importance for health in terms of cholesterol-lowering, blood sugar level regulating, and antimicrobial and antioxidant properties. (Chait et al., 2020; Ruiz-Roso et al., 2010; Kumazawa et al., 2002). Its milled flour form and seeds are used as a flavoring, stabilizer, thickener, and cocoa substitute in various food products (Seczyk et al., 2016; Durazzo et al., 2014; Bengoechea et al., 2008; Fadel et al., 2006; Hajaji et al., 2011). Carob flour is rich in dietary fiber, roasted in different degrees, and used in products. Carob flour attracts attention with its low-fat content, high carbohydrate, dietary fiber content (39.80%), and phenolic content (Román et al., 2017; Ortega et al., 2011). Carob flour was used for enrichment in different grain-based products. Various studies were carried out on various subjects including gluten-free bread (Tsatsaragkou et al., 2014), wheat bread enriched with lentil or carob flour (Turfani et al., 2017), pasta enriched with carob flour for improving antioxidant properties (Seczyk et al., 2016), soy and banana flour substituted cake where carob flour was substituted with cocoa (Rosa et al., 2015), pasta enriched with carob fiber (Biernacka et al., 2017), the effect of carob flour on gluten-free cakes and cookies (Roman et al., 2017), and the effect of carob powder on the sensory and physicochemical properties of the muffins (Pawlowska et al., 2018).

The present study aimed to develop pasta formulations enriched with protein-rich soy flour and dietary fiber-rich carob flour. Accordingly, pasta was produced using six different formulations (WF: SF: CF mixtures) and some physicochemical properties (moisture, ash, protein, fat, total dietary fiber, acidity, carbohydrate, energy, and color properties), quality properties (cooking properties) and sensory properties were determined in the pasta produced using these mixtures.

Materials and Method

Material

In the present study, soy flour was obtained from Doğasan Ltd. (Ankara) while carob flour was obtained from Global Gıda-Haşhaşcızade (Afyonkarahisar). Wheat flour was obtained from Bandırma Has Un (Toru Un) Factory and salt was obtained from local markets. Moisture, ash, fat, protein, titratable acidity, and total dietary fiber values in durum wheat flour (WF) were determined to be 14.8%, 1.15%, 2.04%, 13.9%, 0.03%, 2.93%, respectively. The durum wheat flour farinograph analysis was carried out at the Bandırma Toru Flour factory to determine the water-holding properties. In soy flour (SF) and carob flour (CF), moisture, ash, oil, protein, titratable acidity and total dietary fiber values were SF: 7.04%, 7.61%, 2.04%, 59.16%, 0.36%, 18.38%, CF: 7.57%, 3.09%, 0.31%, 6.03%, 0.27%, 32.87%, respectively.

Pasta Production

In the production of home-type pasta, laboratory-type pasta dough forming machine was used to prepare and shape the pasta dough. The formulations used in pasta production are given, respectively: (A: 100 WF: 0 SF: 0 CF), (B: 90 WF: 5 SF: 5 CF), (C: 80 WF: 10 SF: 10 CF), (D: 80 WF: 0 SF: 20 CF), (E: 80 WF: 20 SF: 0 CF), (F: 60 WF: 20 SF: 20 CF).

According to six different formulations to be used in pasta production, mixtures of durum wheat flour (WF), soy flour (SF), and carob flour (CF) (WF: SF: CF) were formed. Also, 0.5% salt was added to the mixtures. An average of 50-55 ml of water was added to the mixture at the rate determined by farinograph (Brabender, Germany) preliminary tests (ICC, 1992) according to the water holding capacity of the flour and the mixture was kneaded for10 minutes. After kneading, the dough was left to rest for 30 minutes. The pasta was passed through a thinning roller to be shaped as spaghetti (Ampia Machine, Italy). Spaghetti-shaped pasta was dried in the oven (Binder) at 50-55 ^{o}C for 5-6 hours. After the moisture content of the pasta samples decreased to 11%, they were preserved in polyethylene packages until they were analyzed. Pasta samples were passed through a grinder (IKA A 11 Basic) and prepared for analysis.

Physicochemical Analyses

In pasta samples, moisture (AACC Method No: 44-15A), ash (AACC Method No: 08-01), protein (using the Kjeldahl method AACC Method No: 46-11A (AACC, 1990), fat (using the Soxhlet system, AACC Method No: 30-25.01) were made according to AACC (1990). Titratable acidity (in terms of acetic acid) AACC Method No. was determined according to 02-31 (AACC, 2000).

The total dietary fiber amount was determined enzymatically (with alpha-amylase, amyloglucosidase, and protease enzymes) by the AOAC 985.29 method. Velp raw cellulose analyzer was used in the filtration processes. (Anonymous, 2007).

Carbohydrate and energy values in pasta samples were determined according to FAO (2003) (Anonymous 2003). It was calculated using the Atwater general factor system. It was calculated by the formula given below (the relevant model is given in Equations 1 and 2).

$$Carbohydrate\ \% = 100 - (Moisture\% + Ash\% + Protein\% + Fat\%) \quad (1)$$

$$Energy\ (kcal) = (9 \times Fat\%) + (4 \times Protein\%) + 4 \times (Carbohydrate\% - Dietary\ Fiber\%) \quad (2)$$

The colors of pasta samples were determined by using the Minolta CM 3600d model color measurement device. In the triple scale consisting of CIE Color Values (L^*, a^*, b^*) L^*=100 was evaluated as white, L^*=0 as black; high positive a^* as red, high negative a^* as green; high positive b^* as yellow, and high negative b^* as blue.

Quality Analyses

Pasta samples were examined in terms of cooking quality, optimum cooking time, the amount of substance passing to the water (cooking loss-CL), weight gain (water absorption-WA), and volume expansion (VE).

The optimum cooking time of pasta samples was determined according to AACC (2000). Cooked pasta samples obtained from the analysis of "cooking duration" were filtered and weighed. The weight value of the same amount of uncooked pasta was subtracted from the weight value of cooked pasta and the difference after cooking was determined as % (AACC, 2000). Similar to the weight gain, the water level in cooked and uncooked pasta during the applied processes was determined using the formula for determining the percentile overrun in the water level (AACC, 2000). 250 ml distilled water was placed in a 400 ml beaker and boiled. Then 10 g pasta sample was added and cooked. The filtrate obtained at the end of cooking was placed in glass Petri dishes which were previously tared at a fixed weight. The sample was kept at 98 ^{0}C for 24 hours in an oven. It was then transferred to a desiccator, cooled, and weighed. It was calculated according to the formulation and stated in %. (AACC, 2000).

Sensory Analyses

The sensory analysis of pasta was carried out by 20 panelists aged 21-60. All the sensory evaluations were carried out using a 1-5 hedonic scale (5 points) by Aydın (2009) with modifications, on a tasting form containing the sensory quality criteria of the pasta samples (5 points: Very Good, 4 Points: Good, 3 Points: Acceptable, 2 Points: Non-satisfactory, 1 Point: Poor). The sorting test was applied to evaluate the appreciation and acceptability of the pasta containing soy flour and carob flour. In the evaluation of sensory properties of cooked pasta samples, the evaluations were carried out in terms of color, odor, aroma, appearance, flavor, hardness, stickiness, chewiness, mouthfeel, and overall acceptability. Statistical analyzes were carried out by taking the average of the scores given by each panelist for different pasta formulations.

Statistical Analysis

As a substitute to wheat flour in pasta production, carob and soy flour were added in different proportions in 6 different formulations and all the analyzes were carried out in 3 replications. The data obtained as a result of the analyzes were statistically analyzed using the SAS Enterprise 5.1 software and the differences from the addition of carob flour and soy flour to pasta were evaluated. Significant differences in mean values, the LSD (Least Significant Difference) test was used to determine the statistical difference between the mean values ($P<0.05$).

Result and Discussion

Physicochemical properties of pasta formulations

The effect of the addition of soy flour (SF) and carob flour (CF) at different ratios to durum wheat flour (WF) flour on some chemical properties of pasta formulations is given in

Table 1a and Table 1b. Compared to the control samples, a significant decrease ($P < 0.05$) was observed in the moisture content of the pasta whereas a significant increase was observed in the ash values of the samples due to the increase in the soy flour and carob flour ratio in the formulations. The highest ash value was determined in the F example in which SF and CF were substituted with WF in equal ratios (60 WF: 20 SF: 20 CF) with 3.41% ($P < 0.05$). The highest ash values in pasta containing SF and CF positively affect the mineral content (Baysal, 2009). Similar results were reported in the studies on wheat bread with added chickpea flour (Sabanis et al., 2006), wheat bread with lentil, and carob flour (Turfani, 2017).

The fat value determined in the C example in which SF and CF were substituted with WF at equal ratios (80 WF: 10 SF: 10 CF) was found to be significantly higher than those of other samples ($P < 0.05$). In the E sample in which only SF was used as the additive (80 WF: 20 SF: 0 CF), protein value (25.56%) was statistically higher ($P<0.05$) compared to that of the control sample. This was associated with the fact that the amount of soy protein (53.16%) in soy flour was quite high. Similar study results were found in studies on enriching different foods with soy protein and soy flour (Tang et al., 2006; Li et al., 2013; Rinaldoni et al., 2014; Lamacchia et al., 2010; Limroongreungrat and Huang, 2007; Baiano et al., 2011). In a study, in which 25-35-50%soybean flour was added in pasta production, it has been reported that the protein values of pasta increased (Shogren et al., 2006). In pasta studies with different legume flours, when working with pea flour, bean flour, and bean flour, an increase in protein amounts were observed, similar to that determined in the present study (Petitot et al., 2010). Soy flour addition to wheat flour has been reported to increase the protein content and digestibility of biscuit (Vitali et al., 2009).

There were no statistically significant differences between the titratable acidity of the pasta samples ($P > 0.05$). The highest carbohydrate value was determined in the D (80 WF: 0 SF: 20 CF) sample whereas the lowest was determined in the E (80 WF: 20 SF: 0 CF) sample, and the differences were statistically significant ($P < 0.05$). When only CF (the D sample) was used in the flour mixture and as the additive rate increases, fat and energy values decrease compared to the control sample whereas acidity, total carbohydrate, ash, and total dietary fiber values (TDF) increased. In the formulation in which SF was solely used (the E sample), ash, protein, total dietary fiber increased whereas fat, total carbohydrates, and energy values decreased significantly. In the F sample in which SF and CF were used equally at 20% (60 WF: 20 SF: 20 CF), compared to the control sample, moisture, fat total carbohydrate, and energy values decreased whereas ash, protein, total dietary fiber values increased significantly ($P<0.05$). Similar to the present study, it has been reported that the dietary fiber value increased whereas the energy value decreased significantly compared to the control sample in pasta samples in which carob fruit pulp flour was added at 5%, 7.5%, or 10% ratios (Umay, 2019).

Dietary fibers are the edible parts of plants that can be partially or completely fermented in the large intestine by resisting digestion in the small intestine and defined as carbohydrate polymers consisting of components with many health benefits including hemicellulose, cellulose, lignin, pectin, gums, etc. (Dülger and Sahan, 2011; Jakobek and Matic, 2019; Li et al., 2017). According to Table 1b, CF and SF additions were found to cause a significant increase in TDF values compared to the control sample. The highest TDF value was determined in the F (60 WF: 20 SF: 20 CF) sample. Since SF (TDF:18.38% and CF (TDF: 32.87%) additions were rich in TDF, TDF values increased in the pasta samples. Evaluating the results in terms of energy values, all formulations were found to be significantly lower than the control sample. Production of formulations with high total dietary fiber and low fat resulted in a decrease in energy values, and therefore, a healthier product. Zunft et al. (2003) have reported that carob pulp with high water-insoluble fiber decreased the amount of LDL (serum cholesterol) in humans and the consumption of functional foods prepared with this fiber will be effective in the treatment of diseases. Ortega et al. (2011) have reported that the soluble nutrient fibers in carob flour increase the biological acceptability of phenols. Sebecic et al. (2007), in the study examining the dietary fiber content of biscuits enriched with different flour additives, carob flour has been reported to yield the highest TDF value by increasing the TDF value of biscuits by 42%. Similarly, some studies use the gum of the carob seed to increase the dietary fiber ratio in bread (Ktenioudaki and Gallagher, 2012).

Color properties are one of the important factors affecting consumer preferences (Mamat et al., 2010). The color values of the pasta samples are given in Table 2. Pasta formulations (SF and CF ratios) were found to have a significant ($P<0.05$) effect on the color values. The highest L* brightness value was in the control sample containing only wheat flour (L^*: 68.71), followed by the E sample containing 20% SF (L^*: 60.56). The L^* (brightness) value (37.11) of the F formulation prepared with the addition of 20% CF showed a significantly lower value ($P<0.05$) than those of the other samples. The unique dark color of the CF ad an effect on this result. It has been reported that roasted carob flour yielded a darker color value than cocoa color (Yousif and Alghzawi, 2000). Also, it has been stated that as the amount of protein and ash increases in food products, the L^* value decreases (Feillet et al., 2000). With the increase in SF, a significant decrease was observed in the L^* (brightness) value, and the darkening of the color was observed whereas yellow color was dominant due to the significant increase in b^* value with an increase in SF addition. Increasing the ratio of CF and SF in the pasta samples increases a^* (redness) values significantly ($P < 0.05$). In b^*, which is another color property of pasta, E formulation containing 20% SF yielded the highest b^* value (b^*: 20.00). Sereewat et al. (2015) have reported that using 15% non-fat soy flour in the production of rice pasta where they used lean soy flour, a^* and b^* values increased whereas L^* value decreased (62.3-49.6). It has been reported that the increase in protein and ash decreases the L^* value (Feillet et al., 2000). Similar results were obtained in the present study.

Table 1a. The effect of soy flour and carob flour addition on some chemical properties of pasta formulations*

Sample	Formulation	Moisture (%)	Ash (%)	Fat (%)	Protein (%)
A	100 WF: 0 SF: 0 CF	11.50±0.5^{a}	1.71±0.24^{e}	0.41±0.01^{b}	18.98±0.11^{d}
B	90 WF: 5 SF: 5 CF	10.05±0.35^{b}	2.24±0.28^{d}	0.39±0.01^{c}	19.51±0.07^{d}
C	80 WF: 10 SF: 10 CF	10.35±0.21^{b}	2.66±0.02^{c}	0.55±0.01^{a}	21.37±0.50^{c}
D	80 WF: 0 SF: 20 CF	10.00±0.00^{b}	2.14±0.01^{d}	0.34±0.01^{d}	14.71±0.13^{e}
E	80 WF: 20 SF: 0 CF	9.70±0.40^{b}	3.08±0.01^{b}	0.09±0.01^{f}	25.56±0.40^{a}
F	60 WF: 20 SF: 20 CF	8.94±0.13^{c}	3.41±0.03^{a}	0.10±0.01^{e}	24.01±0.10^{b}
Min-Max		8.94-11.50	1.71-3.41	0.09-0.55	14.71-25.56

*Calculated using the dry matter value.
* The mean values indicated with different letters in the same column are significantly different (P <0.05).

Table 1b. The effect of soy flour and carob flour addition on some chemical properties of pasta formulations*

Sample	Formulation	Titratable acidity (TA)	Total Carbohydrates (%)	Total Diet Fiber (%)	Energy (kcal)
A	100 WF: 0 SF: 0 CF	0.02±0.00^{a}	69.97±0.16^{b}	2.74±0.18^{e}	339.61±1.64^{a}
B	90 WF: 5 SF: 5 CF	0.09±0.55^{a}	69.99±0.25^{b}	5.40±0.42^{d}	332.07±0.17^{a}
C	80 WF: 10 SF: 10 CF	0.09±0.01^{a}	67.65±0.28^{c}	9.28±0.09^{b}	314.59±0.57^{b}
D	80 WF: 0 SF: 20 CF	0.12±0.01^{a}	74.50±0.12^{a}	9.35±0.97^{b}	316.58±3.90^{b}
E	80 WF: 20 SF: 0 CF	0.12±0.02^{a}	64.35±0.70^{e}	7.45±0.34^{c}	320.75±3.03^{b}
F	60 WF: 20 SF: 20 CF	0.18±0.01^{a}	65.92±0.18^{d}	11.09±0.15^{a}	313.84±7.38^{b}
Min-Max		0.02-0.18	64.35-74.50	2.74-11.09	313.84-339.61

*Calculated using the dry matter value.
* The mean values indicated with different letters in the same column are significantly different (P <0.05).

Table 2. The effect of soy flour and carob flour addition on the color properties of pasta formulations*

Sample	Formulation	*L**	*a**	*b**
A	100 WF: 0 SF: 0 CF	68.71±1.18^{a}	-0.57±0.13^{d}	17.95±0.14^{b}
B	90 WF: 5 SF: 5 CF	54.02±1.40^{c}	4.6±0.42^{b}	16.42±0.42^{c}
C	80 WF: 10 SF: 10 CF	39.16±1.47^{e}	7.61±0.20^{a}	17.01±0.13^{c}
D	80 WF: 0 SF: 20 CF	42.73±1.38^{d}	7.30±0.12^{a}	17.92±0.36^{b}
E	80 WF: 20 SF: 0 CF	60.56±0.25^{b}	2.66±0.25^{c}	20.00±0.84^{a}
F	60 WF: 20 SF: 20 CF	37.11±1.08^{e}	7.30±0.48^{a}	16.73±0.31^{c}
Min-Max		37.11-68.71	-0.57-7.61	16.42-20.00

* The mean values indicated with different letters in the same column are significantly different (P<0.05).

Cooking properties of pasta formulations

The effect of soy flour and carob flour addition on the cooking quality characteristics of pasta formulations is given in Table 3. The water absorption (WA), volume expansion (VE), and the amount of the substance passing to the water (CL) were found to be significantly higher (P <0.05) in the control sample compared to other samples. Similar to the present study, it has been reported that as the additive ratio increased in pasta samples produced with different leguminous flours such as broad bean flour and pea flour, water holding capacity increased (Petitot et al., 2010). In a study in which carob fiber and pea fiber were used in bread production, fiber addition

changed the water absorption values and the highest water absorption capacity was determined in the samples containing pea fiber and carob fiber (Wang et al. 2002). Cooking weight values in the present study were similar to those reported by Grant et al. (2004) for pasta (284% -305%) and to those by Edwards et al. (1993) for weight gain in cooked pasta (261% -280%). Hotsa (2012) in noodle samples with 30% pea flour and 30% chickpea flour additions have reported the amount of substance passing to water, water absorption, VE values as 8.70-10.63%, 169.26-181.66%, and 140-190%, respectively. In the present study, CL values were found lower, whereas VE and WA values were higher. Examining the level of substances passing to the water values, this value was found higher in the control sample compared to other samples. The addition of SF and CF to pasta formulations has increased the quality by decreasing the amount of substance passing to the water. Cook loss <6 = good; 6-8 = moderate; > 10 = poor pasta quality (Hummel, 1966; AACC, 2000).

Table 3. The effect of soy flour and carob flour addition on the cooking properties of pasta formulations*

Sample	Water absorption (WA) (%)	Volume expansion (VE) (%)	Optimum Cooking Time (Min.)	Cooking loss (CL) (%)
A	281.62±1.91^{a}	368.63±0.18^{a}	7	11.15±0.21^{a}
B	223.10±0.10^{b}	275.30±0.42^{c}	7	5.40±0.03^{b}
C	223.75 ±0.20^{b}	275.15±0.21^{c}	7	3.50±0.28^{d}
D	218.50±0.28^{c}	275.45±0.65^{c}	7	5.38±0.20^{b}
E	212.30±0.42^{d}	300.20±0.28^{b}	7	3.15±0.35^{d}
F	183.25±0.4^{e}	275.25±0.35^{c}	6	4.31±0.27^{c}
Min-Max	183.25-281.62	275.15-368.63	6-7	3.15-11.15

* The mean values indicated with different letters in the same column are significantly different (P<0.05).

Sensory properties of pasta formulations

Cooked pasta formulations with the addition of SF and CF are given in Figure 1 and the sensory properties of cooked pasta formulations are given in Figure 2 with a radar chart. Cooked pasta samples were evaluated by sensory analysis by 20 panelists. Evaluating the overall sensory analysis results, significant differences (P <0.05) were observed in the sensory properties of the pasta compared to the control sample. The most favored sample in terms of aroma and odor was the D sample with 20% CF addition while the most favored sample in terms of appearance was the E sample with 20% SF addition, which received the closest score of the control group (P <0.05). Mouthfeel score of SF and CF-added pasta formulations were determined to be higher compared to the control sample, and the highest score was in the F sample. The highest values in terms of overall evaluation scores were observed in pasta formulations D (4.80) and E (4.75) (P <0.05). The panelists gave higher sensory scores to the samples containing 20% sole soy flour or carob. All SF and CF-added formulations received scores 4 and above in terms of overall acceptability scores and were found to be acceptable in terms of aroma and taste. Similar to the present study, according to the results of sensory analysis, there are studies in which 20% carob flour addition was recommended in biscuits and the carob fiber improves the sensory properties of pasta (Aydın, 2012; Sęczyk et al., 2016). It has been reported that the soybean flour also changed the acceptable additive value ratios between 10-15% in terms of the sensory properties of products such as bread and biscuits (Islam et al., 2007; Awasthi et al., 2012; Taghdir et al., 2017).

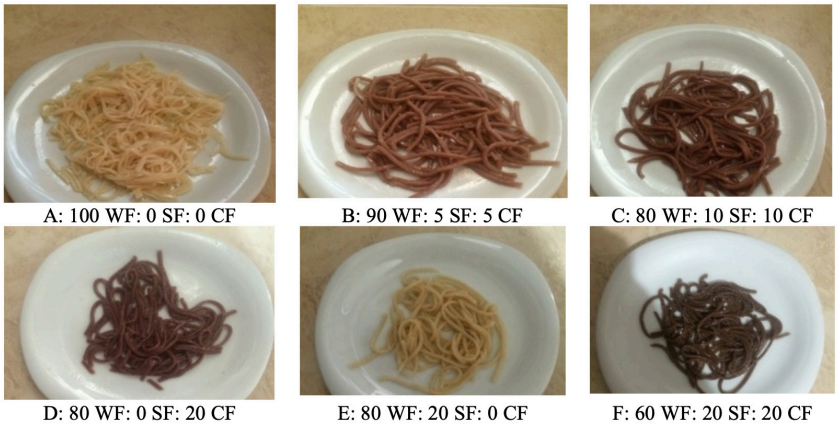

Figure 1. Cooked pasta formulations containing SF and CF

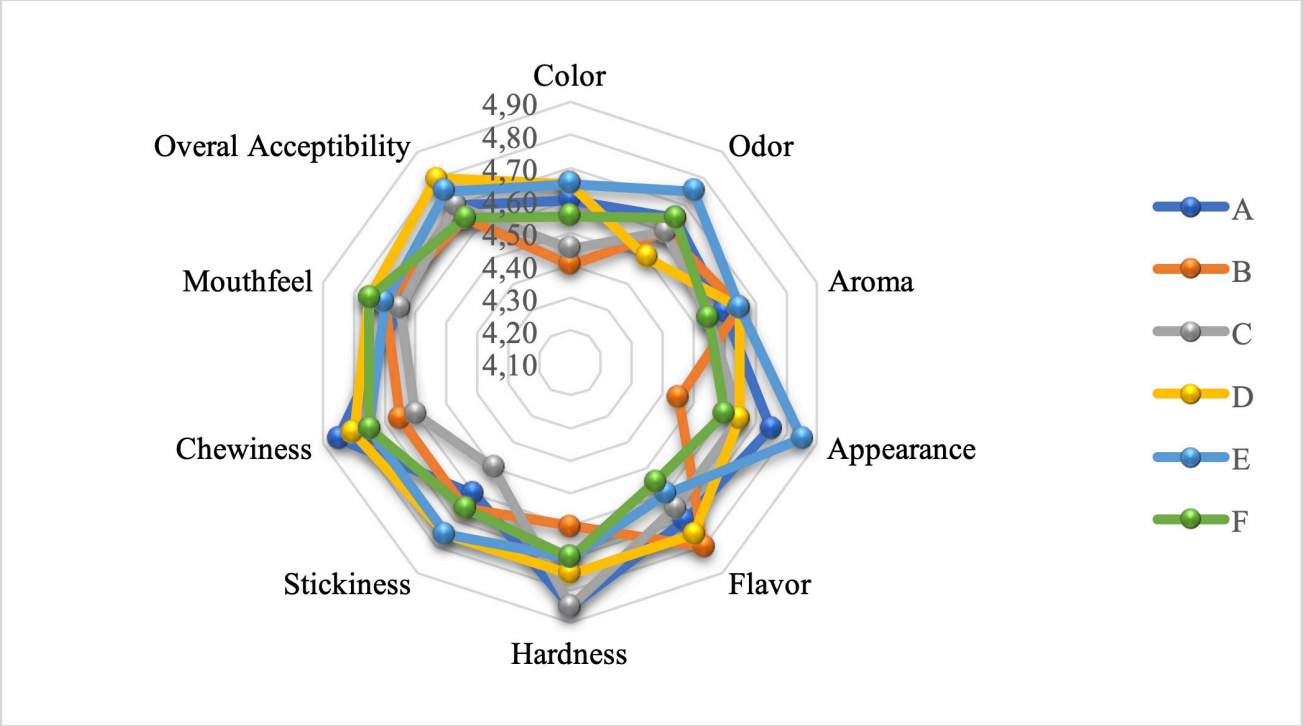

Figure 2. The effect of SF and CF addition on the sensory properties of pasta formulations

Conclusion

In the present study, physicochemical properties, cooking properties, and sensory properties of the pasta containing SF and CF at different substitution rates to wheat flour, and it was determined that the addition of SF and CF increased protein, ash, total dietary fiber whereas decreased the energy values. Functional pasta formulations with low fat and calorie content and high fiber and protein content were produced. According to the results obtained, the use of both additives has reduced the amount of the substance passing to the water and positively affected the cooking quality. There was a significant decrease in L^* (brightness) value of the pasta formulations in the samples with higher CF addition ratios. The dominant dark color of CF compared to CF has affected the color values of pasta formulations. According to the results of sensory analysis, the pasta formulations scored 4 and above in terms of the overall evaluation were the pasta formulations with 20% CF (D) and 20% SF (E), 10% CF + 10% SF (C) and these samples yielded the highest values, respectively. According to the sensory analysis results, it can be suggested that CF and SF can be evaluated by adding appropriately up to 20% in pasta as a substitute for wheat flour. Evaluating the results in general, carob flour, and soy flour with functional and nutritious properties can be evaluated in the food industry in the development of new and healthy food formulations as an alternative functional component.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

Dilek Dülger Altıner contributed as the thesis supervisor in conducting analyzes, statistical analyses of data, writing the article, and writing-review-proofreading-publishing procedures. The Master's thesis student Şeyma Hallaç carried out the preparation of samples, analyses, reporting, and writing and correction of literature sources. The authors have read and approved the final version of the article. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Not applicable.

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Data availability

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Consent for publication

Not applicable.

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Assessment of heavy metal levels and fatty acid compositions of some krill oil capsules marketed in Turkey

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Abstract

Krill oil has many positive health effects since it is rich in omega-3, phospholipids and astaxanthin. In recent years, as a result of rapid population growth and industrialization, the toxic heavy metal levels have increased especially in aquatic environments. Therefore, it is also possible to take up heavy metals to the human body through food supplements such as krill oil. This study aims to determine the fatty acid composition and the amounts of lead, mercury and cadmium in five different krill oil capsules. A total of 35 fatty acids were analysed. It was determined that the five different krill oil capsules met a proportion of daily recommended omega-3 fatty acid (EPA + DHA) and omega-6 fatty acid intake. Mercury levels were <0.2 µg/kg, cadmium levels <3 µg/kg and lead levels <3 µg/kg in all samples. It was observed that the heavy metal amount was below the limits specified by the Turkish Food Codex Contaminants Regulation. Based on the evaluation of the data obtained from our study, reliable krill oil capsules may be recommended for those who consume less than the recommended daily intake of omega-3 fatty acids, and for the vegetarians.

Keywords: Heavy metal, Krill oil, Omega-3, Omega-6, Fatty acid

Introduction

"Krill" is the general name given to the small crustaceans in the order Euphausiacea and means "young fish" in Norwegian. Krills are shrimp-like marine species usually found in cold oceans, feeding on planktons and algae. Although it is generally used in aquaculture, sport fishing and used as fish feed, interest in krill has increased recently due to its positive health effects. Krill contains essential fatty acids such as α-linolenic acid (18:3, ω-3) and linoleic acid (18:2, ω-6). Also, while krill has a low level of saturated fatty acids and monounsaturated fatty acids (MUFAs), its polyunsaturated fatty acid (PUFA) content is high. Krill's fatty acid pattern is similar to that of shrimp and fish. However, while most fatty acids in fishes are in the form of triglycerides, 65% of the fatty acids in crustaceans are in the form of phospholipids (Tou, Jaczynski, & Chen, 2007).

Krill oil extracted from Euphausia superba Dana, 1850 (Euphausiacea), a key crustacean species in the Southern Ocean, has been used as a food supplement across the world since 2001 due to its high content of essential fatty acids. NASA has included krill oil in the nutrition program of astronauts since 2002 (Sanlıer & Bolukbası, 2016). Krill oil has many positive health effects since it is rich in omega-3, its lipid type is in the form of phospholipids (high water solubility) and it contains astaxanthin, an antioxidant (Tou et al., 2007).

Omega-3 fatty acids, which are polyunsaturated fatty acids, are metabolized in the human body as eicosapentaenoic acid (EPA; 20:5 ω-3) and docosahexaenoic acid (DHA; 22:6 ω-6). EPA and DHA are known to play an important role in the prevention or treatment of cardiovascular diseases, cancer, stroke and certain inflammatory diseases. Also, since DHA is an

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important component of the brain and retina, it is also crucial for brain development, learning ability and visual acuity (Calder, 2014). It has been shown that there is a positive correlation between low levels of DHA in brain cells and neurological diseases such as depression, memory loss, Alzheimer's disease and schizophrenia (Grosso et al., 2016; Hur et al., 2018; Joy, Mumby-Croft, & Joy, 2003). Since krill oil is rich in omega-3 fatty acids, many studies have been conducted considering its relationship with the abovementioned chronic diseases (Berge et al., 2015; Bunea, El Farrah, & Deutsch, 2004; Gamoh et al., 2011; Konagai et al., 2013; Lobraico et al., 2015). Krill oil shows significantly lower plasma lipid levels and improves endothelial function compared to fish oil and olive oil (Berge et al., 2015; Bunea et al., 2004; Lobraico et al., 2015). In addition, it has been reported that krill oil increases brain function and affects memory positively with its phospholipid content (Gamoh et al., 2011; Konagai et al., 2013). Krill oil is also effective in the prevention and treatment of some chronic diseases, as it is rich in astaxanthin, a powerful antioxidant, and neutralizes free radicals that cause cell damage. Astaxanthin also increases the resistance of krill oil to oxidation (Sanlıer & Bolukbası, 2016). As a result, the fact that krill oil is rich in EPA, DHA, phospholipids and anti-oxidants shows that it is suitable for human consumption.

In last several decades, the toxic heavy metal levels have rapidly increased especially in the aquatic environments as a result of rapid population growth and industrialization. Heavy metals have serious negative impacts on environment and life forms since they are used widely and do not undergo biological degradation. Organisms in the aquatic ecosystem accumulate these metals at higher rates in their bodies compared to their density in the water. The metals entering the food chain through this accumulation begin to create harmful effects as their amount increase in every level starting from the lowest level of the chain to the organism at the highest level (Tekeli, Yipel, & Sakin, 2016). Lead (Pb), mercury (Hg) and cadmium (Cd) are among the toxic heavy metals included in the top 10 dangerous substances specified by the Agency for Toxic Substances and Disease Registry ((ATSDR), 2019). Fish and other aquatic organisms living in the ecosystems contaminated by mercury, are the most important source of methyl mercury. Methyl mercury passes through the blood-brain barrier, placenta, and lactiferous ducts, and has toxic effects especially on liver, kidney, brain and immune system (Ozkan, Taslipinar, & Yesilkaya, 2018). Since the wastewater generated as a result of industrial activities is released to the streams, lakes, dams and seas without being treated, particularly Cd is taken by aquatic organisms and creates harmful toxic effects at different levels. High Cd intake can cause kidney diseases, osteoporosis, anemia, lung and prostate cancers in humans (Asri, Sonmez, & Citak, 2007; Türk Gıda Kodeksi Bulaşanlar Yönetmeliği, 2011). Lead is another heavy metal found in high amounts in aquatic organisms as a result of water contamination. Lead accumulates in the brain, lung, spleen, kidney cortex, erythrocytes, teeth, and mostly in the bones. Lead exposure in the fetal period may lead to mental retardation, movement disorder and kidney dysfunction. It may also cause a decrease in neurotransmitters such as acetylcholine, dopamine, and glutamate. Lead is also an endocrine disruptor that disrupts the synthesis, secretion and elimination of hormones in the body. Depending on Pb toxicity, disorders, behavior changes, attention deficit, comprehension difficulties and a decrease in school success can be observed in specific cognitive tests (Ozkan et al., 2018). The food supplements heading, for the first time, was added in the Turkish Food Codex Regulation on Contaminants issued in 2011 in Turkey, and the maximum limits were specified in line with the values of the European Union Directive EC 629/2008 (Türk Gıda Kodeksi Bulaşanlar Yönetmeliği, 2011). Accordingly, the maximum amount of Hg allowed in food supplements is 0.1 mg/kg and the amount of Pb is 3 mg/kg. The Cd limit was specified as 3 mg/kg for food supplements produced from dried seaweed, seaweed products or dried shelled molluscs, or those which contain these as their main components, and 1 mg/kg in others (Asri et al., 2007).

Here we investigate the fatty acid composition of five different krill oil capsules on the market and also determine the amounts of Pb, Hg and Cd they contained to find out whether these products are compatible with the maximum limits specified for the food supplements by the Turkish Food Codex Regulation on Contaminants.

Materials and Methods

Within the scope of this research, five different brands of krill oil capsules which are used as food supplements and available in the market were sampled. Brands were kept confidential for ethical concerns and examples were named 1 - 2 - 3 - 4 - 5. The specified contents of krill oil samples used in the study are taken from the product leaflet and summarized in Table 1.

Table 1. Prospectus contents of krill oil samples

Contents of samples	Sample1	Sample 2	Sample 3	Sample 4	Sample 5
Krill Oil (mg)	500	700	500	500	500
Phospholipids (mg)	360	294	280	202	230
Omega 3 (mg)	260	175	145	86	135
EPA (mg)	125	102	85	40	72.5
DHA (mg)	62	60	45	25	44
Choline (mg)	76	-	35	-	28
Astaxanthin (µg)	200	70	50	101	640 (mg)

Sampling was performed in a single run, 1-gram samples were taken from each product and placed in sample containers. To protect the samples from oxidizing, the sample containers were covered with aluminum foil. Krill oil samples were taken to Akdeniz University Food Safety and Agricultural Research Center to determine their fatty acid composition and heavy metal levels. Fatty acid composition analysis was done according to Ozogul et al. (Ozogul, Ozogul, & Alagoz, 2007). Accordingly, 2 M methanolic KOH and n-heptane were added to the krill oil samples to form methyl esters and analysis was performed using Gas Chromatography-Mass spectrometry (GC-MS Thermo Scientific, ISQ series).

Results and Discussion

A total of 35 fatty acids were analysed in five different brands of krill oil capsule samples. Of which 15 were saturated fatty acids (SFAs), 9 were monounsaturated fatty acids (MUFAs) and 11 were polyunsaturated fatty acids (PUFAs). The types and quantities of these fatty acids were presented in Table 2. The highest level of SFA and MUFA was in the sample 5 (67.048 g/100g and 16.800 g/100g, respectively) and the highest level of PUFA was in the sample 1 (20.494 g/100g). The lowest level of SFA and MUFA was in sample 1 (64.297 g/100g and 15.209 g/100g respectively), while the lowest level of PUFA was in sample 5 (15.579 g/100g). When the krill oil samples were analysed for their EPA and DHA contents, the following results were observed. The highest EPA content was found in sample 3 (10.870 g/100g), the lowest EPA content in sample 2 (7.877 g/100g); the highest DHA content in sample 1 (3.054 g/100g), and the lowest DHA content in sample 5 (1.693 g/100g).

Figure 1 represents comparison of EPA; DHA; EPA+ DHA, omega-3 (ω-3), omega-6 (ω-6) fatty acid amounts obtained from krill oil capsule samples. It was observed that the highest ω-3 level and the lowest ω-6 content were in sample 3; the highest ω-6 level was in sample 1 and the lowest ω-3 content was in sample 2.

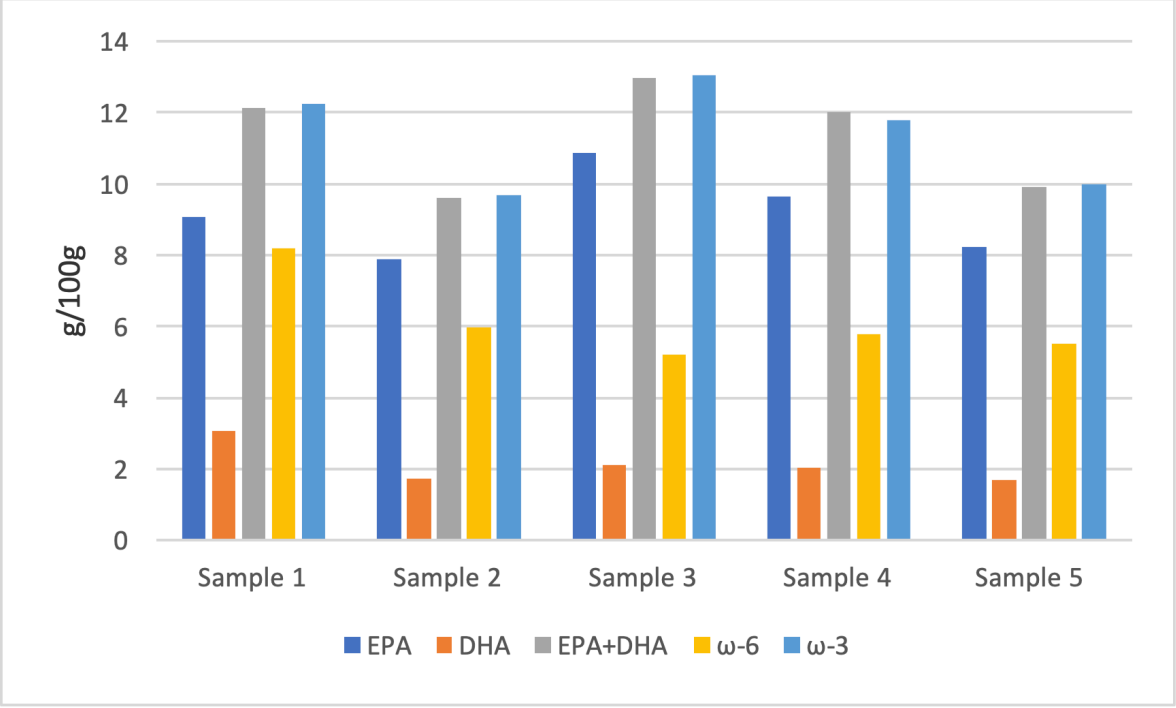

Figure 1. Comparison of EPA, DHA, ω-3 and ω-6 levels of krill oil capsules

Table 2. Fatty acids composition in the samples of krill oil capsules

Fatty Acids	(g/100g)				
Saturated Fatty Acid (SFA)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**
C8:0 Caprylic acid	N.F.D.L	N.F.D.L	N.F.D.L.	N.F.D.L	N.F.D.L
C10:0 Capric acid	0.546	N.F.D.L	0.212	N.F.D.L	N.F.D.L
C11:0 Undecanoic acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
C12:0 Lauric acid	0.159	0.131	0.135	0.088	0.123
C13:0 Tridecanoic acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
C14:0 Myristic acid	13.734	19.141	16.653	17.067	18.357
C15:0 Pentadecanoic acid	0.531	0.365	0.329	0.495	0.506
C16:0 Palmitic acid	44.586	44.320	44.375	45.791	45.141
C17:0 Heptadecanoic acid	0.237	0.095	0.090	0.140	0.087
C18:0 Stearic acid	4.324	3.599	4.065	3.301	3.242
C20:0 Arachidic acid	0.098	0.087	0.073	0.085	0.082
C22:0 Henicosanoic acid	0.017	0.009	0.008	0.011	0.012
C22:0 Behenic acid	0.066	0.044	0.058	0.070	0.072
C23:0 Tricosanoic acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
C24:0 Lignoceric acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
ΣSFA	**64.297**	**67.790**	**65.996**	**67.048**	**67.621**
Monounsaturated Fatty Acids (MUFA)					
C14:1 Myristoleic acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
C15:1 cis-10-Pentadecenoic acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
C16:1 Palmitoleic acid	2.910	4.672	5.283	5.401	6.631
C17:1 cis-10-Heptadecenoic acid	N.F.D.L	N.F.D.L.	N.F.D.L	N.F.D.L	N.F.D.L
C18:1n9c Oleic acid	11.895	11.314	9.954	9.396	9.701
C18:1n9t Elaidic acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
C20:1n9 cis-11-Eicosenoic acid	0.199	0.238	0.184	0.204	0.222
C22:1n9 Erucic acid	0.204	0.333	0.254	0.279	0.246
C24:1n9 Nervonic acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
ΣMUFA	**15.209**	**16.558**	**15.675**	**15.279**	**16.800**
Polyunsaturated Fatty Acids (PUFA)					
C18:2n6c Linoleic acid	3.280	2.162	1.846	2.340	2.227
C18:2n6t Linolelaidic acid	1.845	1.751	1.594	1.688	1.655
C18:3n3 γ-Linolenic acid	0.130	0.069	0.095	0.111	0.070
C8:3n6 α-Linolenik asit	3.000	1.972	1.714	1.709	1.601
C20:2 cis-11.14-Eicosadienoic acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
C20:3n6 cis-8.11.14- Eicosatrienoic acid	0.067	0.090	0.037	0.034	0.036
C20:3n3 cis-11.14.17- Eicosatrienoic acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
C20:4n6 Arachidonic acid	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L	N.F.D.L
C20:5n3 cis-5.8.11.14.17- Eicosapentaenoic acid	9.061	7.877	10.870	9.661	8.216
C22:2 cis.13.16-Docosadienoic acid	0.058	N.F.D.L	0.083	0.098	0.080
C22:6n3 cis-4.7.10.13.16.19- Docosahexaenoic acid	3.054	1.732	2.090	2.031	1.693
ΣEPA	**9.061**	**7.877**	**10.870**	**9.661**	**8.216**
ΣDHA	**3.054**	**1.732**	**2.090**	**2.031**	**1.693**
ΣEPA+DHA	**12.115**	**9.609**	**12.960**	**11.692**	**9.909**
ΣPUFA	**20.494**	**15.652**	**18.329**	**17.672**	**15.579**

N.F.D.L; Not found in detectable level

The amount of heavy metal found in krill oil capsule samples are given in Table 3. The analyses revealed that the level of mercury was <0.2 µg/kg. the cadmium <3 µg/kg and lead <3 µg/kg in all samples.

Table 3. The amount of heavy metal in krill oil capsule samples solution

Samples	Mercury (Hg)	Cadmium (Cd)	Lead (Pb)
Sample 1	<0.2 µg/kg	<3 µg/kg	<3 µg/kg
Sample 2	<0.2 µg/kg	<3 µg/kg	<3 µg/kg
Sample 3	<0.2 µg/kg	<3 µg/kg	<3 µg/kg
Sample 4	<0.2 µg/kg	<3 µg/kg	<3 µg/kg
Sample 5	<0.2 µg/kg	<3 µg/kg	<3 µg/kg

Seafood has potential effects on health as it contains high-quality protein and minerals such as selenium and iodine, as well as high amounts of omega-3 fatty acids (Dahl, Mæland, & Bjørkkjær, 2011). While fatty fish (> 5% lipid) is considered as a good source of omega-3, the krill oil obtained from Euphausia superba is stated to be a good source of long-chain omega-3 fatty acids (Adıguzel, Isgin, & Pekcan, 2015; Dahl et al., 2011). There are also vitamin A, vitamin E, phospholipids, and astaxanthin, an oil-soluble antioxidant carotenoid, in krill oil. Omega-3 fatty acids in krill oil are attached to phospholipids. Unlike triglycerides, phospholipids are amphiphilic and therefore their absorption is easier due to their emulsifying properties. This structural difference shows that krill oil provides better bioavailability and oxidative stability than oils extracted from other seafoods (Adıguzel et al., 2015; Lu, Bruheim, Haugsgjerd, & Jacobsen, 2014). It has been reported that krill oil has antithrombotic, antiarrhythmic, antiatherosclerotic and anti-inflammatory effects due to its rich EPA, DHA and anti-oxidant content (Ambati, Phang, Ravi, & Aswathanarayana, 2014; Calder, 2015; Casula, Soranna, Catapano, & Corrao, 2013).

The Food and Agriculture Organization of the United Nations recommends at least 250 mg/day EPA and DHA intake for male adults and non-pregnant/non-lactating females. It is emphasized that pregnant/lactating females should take 300 mg/day EPA and DHA, at least 200 mg of which should be DHA (FAO, 2010). The European Food Safety Authority (EFSA) states that it is sufficient to take 250 mg/day EPA and DHA, and an additional 100-200 mg/day DHA in pregnancy (EFSA Panel on Dietetic Products, 2010). Although the toxic dose of krill oil is unknown, The United States Food and Drug Administration (FDA) has reported that over 3g/day omega-3 fatty acids consumption can lead to gastrointestinal problems such as bleeding, gas, bloating and diarrhoea, and that the therapeutic dose ranges from 1-3 g/day and it is appropriate to recommend 500 mg/day of it (Cunningham, 2012).

Evaluation of EPA and DHA content of krill oil samples investigated here showed that 100 g krill oil contained a minimum of 9.609 g and a maximum of 12.960 g EPA and DHA. The analysed samples were used in accordance with the EFSA's intake recommendations (250 mg/day) and they met EFSA's daily intake of EPA and DHA. The krill oil content in a single capsule of the analysed products varies between 500-700 mg and 1-2 capsule(s)/day intake is recommended.

Omega-3 and omega-6 fatty acids are essential fatty acids that are not synthesized in the human body and must be taken from external sources. While anti-aggregator, anti-inflammatory and vasodilating eicosanoids are synthesized from EPA and DHA, which are formed as a result of desaturation of omega-3 fatty acids, eicosanoids having the opposite effect are synthesized from arachidonic acid formed by omega-6 fatty acids. Currently, many chronic diseases are characterized by the overproduction of eicosanoids derived from omega-6 fatty acids. Therefore in order to protect health, it is essential to follow a balanced diet of omega-3 and omega-6 (Saini & Keum, 2018). Turkey Dietary Guidelines recommends a 1.6 g/day omega-3 and 17 g/day omega-6 intake for males between the ages of 19-50, and 1.6 g/day omega-3 and 14 g/day omega-6 for those between the ages of 51-65. A 1.1 g/day omega-3 and 12 g/day omega-6 intake is recommended for the females between the ages of 19-50, and 1.1 g/day omega-3 and 11 g/day omega-6 for those between the ages of 51-65. Therefore, at least 2-3 servings (approximately 300-500 g) of fish should be consumed per week to obtain the required fatty acids. Unfortunately, fish consumption is very low in Turkey, and 18.8% of the individuals living in the urban areas and 12.4% of the individuals living in the rural areas consume fish 1-2 times a week, according to the data obtained from Turkey Nutrition and Health Survey in 2010 (Guzelsoy & Izgi, 2015).

The data obtained here suggest that the highest n-3 content (13.055 g/100 g) and the lowest n-6 content (5.191 g/100 g) were in sample 3. On the other hand, the highest n-6 content (8.192 g/100 g) was in sample 1 while the lowest n-3 content (9.678 g/100 g) was in sample 2. Provided that all samples analysed were used in accordance with the FDA's intake recommendations (1-3 g), they met a portion of the daily intake of omega-3 and omega-6 recommended by the Dietary Guidelines for Turkey. The most important limitation of this study is that fatty acid analysis has not been repeated due to financial reasons.

In recent years, due to the rise of environmental and water pollution, the heavy metal levels (Cd, Pb, Hg) in the aquatic environment have increased dramatically. These heavy metals are accumulated in marine organisms, including fishes, through food chain. Therefore, these heavy metals may transmit to fish oils or krill oils as they are extracted from marine organisms. Here, it was determined that the heavy metals found in krill oil samples were below the maximum values specified for the food supplements by the Turkish Food Codex Regulation on Contaminants.

Conclusion

Based on the evaluation of the data obtained from our study, reliable krill oil capsules may be recommended for those who consume less than the recommended daily intake of omega-3

fatty acids, and for the vegetarians. However, considering the increase in the use of fish oils and other herbal/animal food supplements, it is thought that further studies should be carried out for their long-term use in terms of other contaminants and dosage values.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Insects on lavender in Isparta province, Turkey

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Abstract

Since ancient times, people have been interested in parfumed and spicy plants and used them in many areas such as cosmetics, flavors, foodstuffs and dyes. The effects of plant extracts and essential oils derived from these plants on plant diseases and pests have been studied in detail for many years. The lavender is becoming one of the most important aromatic plants in Isparta province and there has recently been an increase in lavender plantations. However, there is limited or no any study on pests of lavender in Turkey. This study aimed at identifying insect that attack to lavender plants. The surveys were performed in two locations of Isparta province in Turkey between 2015-2016. The Insects on lavander plants were sampled by three different methods (sweep net, pitfall and direct plant sampling). The collected insects' samples were brought to the laboratory in the plastic containers. Once morphologically identified, the insects were sent to the authorities for species identification.

As a result, some insects belonging to different orders (Orthoptera, Hemiptera, Coleoptera, Diptera, Lepidoptera, Hymenoptera) were determined. *Poecilimon glandifer* Karabağ (Orthoptera: Tettigoniidae), *Cercopis vulnerata* Rossi (Hemiptera: Cercopidae), *Lepyronia coleoprata* (L.), *Neophilaenus lineatus* (L.), *Philaenus spumarius* (L.), *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae), *Atrococcus achillae* (Kiritchenko) (Hemiptera: Pseudococcidae) were found to be the most common pest species in sampled lavender palants.

Keywords: Aromatic plants, *Lavandula angustifolia*, Insect

Introduction

Since ancient times, perfumed and spicy plants have been used in many areas such as cosmetics, flavors, foodstuffs and dyes. In the early 20th century, before the development of the chemical industry, more than 40% of all drugs were originated from herbal plants (Bayram et al., 2010). Because of the side effects of synthetic chemicals, interest in medicinal and aromatic plants has been increased in recent years. Effects of extracts and essential oils derived from these plants on plant diseases and pests were studied in detail (Mansour et al., 1986; Regnault-Roger and Hamraoui, 1994; Regnault-Roger and Hamraoui, 1995; Regnault-Roger, 1997; Shaaya et al., 1997; Thorsell et al., 1998; Karakoç et al., 2006; Kara et al., 2014; Kurşuncu Şahin and Karaca, 2019; Şanlı et al., 2020).

The origin of lavender is known from the Mediterranean basin, especially rocky and calcareous areas. It also grows in North Africa, Europe and Western India. Lavender was cultivated by the ancient Greeks and Romans in England. The name of the plant comes from the Latin verb "lavare", meaning "to wash" or "to bathe" (Anonymous, 2009). Lavender plant is one of the most common aromatic plants used in pharmacy and cosmetic area. Recently, it is becoming an important herbal plant for Isparta province and cultivation area of lavender are increasing day by day. According to Turkish Statistical Institute

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(TUİK)'s data in 2019, lavender production was about 1462 tons in Turkey and Isparta province provides 93% of Turkey's total lavender production (Başaran, 2017). The aim of this study is to identifiy harmful and beneficial insects on lavender plant in Isparta province, Turkey.

Materials and Methods

Field surveys were performed in 2015-2016 in all lavender cultivation areas in Isparta province, Turkey. Three sampling methods, sweep net, pitfall trap and direct plant sampling, designed to sample insects on lavender plants. All sampled insects were brought to the laboratory with plastic containers and sent to the authorities for species identification. Insect samples belonging to Cercopidae, Aphrophoridae and Cicadellidae families of Hemiptera were identified by Prof. Dr. Ünal ZEYBEKOĞLU (Second author of this manuscript). The other insect samples, Pseudococcidae family by Prof. Dr. Bora KAYDAN (Çukurova Üniversitesi, İmamoğlu Vocational School, Adana, Turkey) and Tettigoniidae family of Orthoptera by Prof. Dr. Battal ÇIPLAK (Akdeniz University, Science and Arts Fuculty, Biology Department, Antalya, Turkey) were identified. Insect specimens were deposited in Entomological Museum of Isparta, Turkey (EMIT) at the Plant Protection Department of the Agricultural Faculty, Isparta Applied Sciences University, Isparta, Turkey.

Results and Discussion

Insects collected from lavender plants grown in Isparta province in 2015 and 2016 are given at Table 1.

Table 1. Insects collected from lavender plants grown in Isparta province

Species	Family	Order
Pests		
Cercopis vulnerata Rossi, 1807	Cercopidae	Hemiptera
Lepyronia coleoprata (L.)	Aphrophoridae	Hemiptera
Neophilaenus lineatus (L.)	Aphrophoridae	Hemiptera
Philaenus spumarius (L.)	Aphrophoridae	Hemiptera
Cechenotettix martini (Lethierry, 1883)	Cicadellidae	Hemiptera
Platymetopius major (Kirschbaum,1868)	Cicadellidae	Hemiptera
Atrococcus achilleae (Kiritchenko, 1936)	Pseudococcidae	Hemiptera
Eurydema ornatum (Linnaeus, 1758)	Pentatomidae	Hemiptera
Dolicorus baccarum (Linnaeus, 1758)	Pentatomidae	Hemiptera
Nezara viridula (Linnaeus, 1758)	Pentatomidae	Hemiptera
Pyrrhocoris apterus (Linnaeus, 1758)	Pyrrhocoridae	Hemiptera
Lygaeus equestris (Linnaeus, 1758)	Lygaeidae	Hemiptera
Poecilimon glandifer Karabağ, 1950	Tettigoniidae	Orthoptera
Natural Enemies		
Coccinella septempunctata	Coccinellidae	Coleoptera
Hyppodamia variegata	Coccinellidae	Coleoptera
Anisochrysa carnea	Chrysopidae	Neuroptera
Eupeodes ochrostoma (Zetterstedt)	Syrphidae	Diptera

***Cercopis vulnerata* Rossi, 1807**

Length of adults are 8.2-10.5 mm. Head, thorax and abdomen of the adults are black. Front wings have red bands. Legs are black.

This species is distributed in some parts of Europe and Asia and found in Japan, ABD, Canada, Albania, Austria, Czech Republic, England, France, Germany, Greece, Hungaria, Italy, The Netherlands, Norvay, Poland, Romania, Spain, Switzerland and Yugoslavia (Lodos and Kalkandelen, 1981).

Host plants: *Beta vulgaris*, *Fragaria vesca*, *Pyrus communis, Malus domestica*, *Medicago sativa*, *Ribes rubrum*, *Prunus avium*, *Prunus domestica*, *Corylus avellana*, *Rubus caesius*, *Humulus lupulus*, *Salix alba* and some weeds (Hill, 1987).

This species was found in lavender areas during April and May.

Lepyronia coleoprata

Adults are 3-5 mm in length, wings are light brown and have bold brownish, black spots. Scutellum is brown. Legs are dark brown.

This species is distributed in Afghanistan, Algeria, Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Uruguay, Iraq, Italy, Mongolia, the Netherlands, Norway, Poland, Portugal, Romania, Spain, Switzerland, Swiss, Syria, Turkey, Yugoslavia (Lodos and Kalkandelen, 1981).

Host plants: *Medicago sativa*, *Populus tremula*, *Salix alba*, *Rubus caesius*, *Juglans regia*, *Acarus calamus*, *Galium aparine*, *Cirsium arvense*, Rumex acetosella and *Lepidium draba* (Lodos and Kalkandelen, 1981).

This species was collected in this study during summer.

***Neophilaenus lineatus* (Linneaus, 1758)**

Adults are 5-7 mm in length. Front wings have pale brownish spots. Scutellum is dark. Legs are light brown.

This species is distributed in Albania, Algeria, Austria, Belgium, Bulgaria, Czech Republic, Denmark, England, Finland, France, Germany, Hungary, Italy, Ireland, Mongolia, the Netherlands, Norway, Poland, Portugal, Romania, Spain, Switzerland, Swiss, Tunusia, Turkey, Yugoslavia (Lodos and Kalkandelen, 1981).

Host plants: *Luzula sylvatica, Eriophorum vaginatum, Scirpus cespitosus, Carex nigra, C. rostrata, Poa nemoralis, Phalaris arundinacea, Calamagrostis canescens, C. villosa* and *Nardus stricta* (Novotny, 1995). *Juncus squarrosus* (Brooks and Whittaker, 1999). Some weeds, woody plants and pasture plants are also hosts of this species (Biedermann, 2003).

This species was found in lavender areas of Isparta between June and July.

Philaenus spumarius

Adults are 5.8-6.7 mm in length. Its body has more than 20 different colors. Generally, it is yellowish, brownish and blackish. The pronotum is black and legs are light brown.

This species is distributed in Afghanistan, Algeria, Austria, Belgium, China, Cyprus, Greece, Czech Republic, Denmark, England, Finland, Hungary, Italy, Iraq, Ireland, Mongolia, Japan, Morocco, the Netherlands, Norway, Poland, Portugal, Romania, Spain, Switzerland, Swiss, Syria, Tunusia, Turkey, Yogoslavia (Lodos and Kalkandelen, 1981). It was also reported to be common and harmful all over the world (Whittaker, 1968; Raatikainen, 1971).

Host plants: Halkka et al. (1976) reported that there were more than 150 hosts of this species in Finland. *Anaphalis margaritacea, Medicago sariva* (Hofman and McEvoy, 1985), *Agrostis capillaris, Ammi visnaga, Argyrolobium biebersteinii, Avena barbata, Bromus hordeaceus, Calendula officinalis, Caucalis platycarpos, Cichorium inthybus, Clematis vitalba, Echium vulgare, Euonymus japonicas, Galega officinalis, Hordeum bulbosum, Hordeum murinum, Iris germanica, Melilotus officinalis, Populus nigra, Psorolea bituminosa, Salix babylonica, Taraxacum officinale* (Toper Kaygın and Elçi, 2017).

This species was found in July.

Cechenotettix martini

Adults are 4.2-5.4 mm in length. There are light and dark brown spots on the body. Scutellum is dark. Center and both sides of pronotum has brown spots. Legs are black.

This species is distributed in France (Della Giustina et al., 1989), Algeria, Morocco, Belgium, Portugal and Spain (Nast, 1987).

Host plants: Lavandula x intermedia *Trifolium hybridens, T. repens, Senecio vulgaris, Vinca rosea* (Valenta et al., 1961), *L. angustifolia* (Lis-Balchin, 2002). It was reported that this species was a vector of Stolbur disease in *Lavandula hybrida* (Moreau and Leclant, 1973; Boudon-Padieu and Cousin, 1999)

This species was found in July. This is the first record for Turkish fauna.

Platymetopius major

Adults are 5.11-5.62 mm in length and head is narrow than pronotum (Zeybekoğlu, 1991).

Distribution: Algeria, Armenia, Austria, Belgium, Czech Republic, Denmark, France, Germany, Greece, Iran, Italy, Jordan, The Netherlands, Poland, Siberia, Slovakia, Sweden, Turkey (Dlabola, 1967; Lodos and Kalkandelen, 1988; Zeybekoğlu, 1991; Bosco and Arzone, 1997).

Host plants: *Acer japonicum, A. palmatum, A. campestre* (Walczak et al., 2012).

This species was found in June and July.

Atrococcus achilleae

Females are elongate, 2.1-3.5 mm in length and light gray color and its body covered by wax secretion. Ovisac is not clear.

This species is distributed in Armenia, Kazakhistan, Moldova, Mongolia, Nort Korea, Ukrain (Ben-Dov, 1994), Bulgaria (Gavrilov, 2010), China (Tang, 1992), Hungaria, Russia (Danzig, 1998), Italy (Longo vd., 1995), Slovenia (Seljak, 2010), Swedish (Gertsson, 2001), Turkey (Kaydan et al., 2007) Turkey and Bulgaria, (Kaydan et al., 2015).

Host plants: Amaranthaceae, Asteraceae, Caryophyllaceae, Crassulaceae, Cyperaceae, Dipsacaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Liliaceae, Papaveraceae, Plantaginaceae, Plumbaginaceae, Ranunculaceae, Rosaceae, Scrophulariaceae (Ben-Dov, 1994; Danzig, 1998; Kaydan vd., 2004).

This species was found during the year and its population was higher from the end of June till middle of September.

Eurydema ornatum

Adults are 7-8 mm in length. Body is gray, shiny red and it has black line on the body. Sometimes color of the adults is changed from red to yellow or green.

This species is distributed in Palearctic Region, especially Europe and Mediterranean (Lodos, 1982), the Netherlands (Aukema, 1993) and Turkey (Özgen et al., 2005a)

Host plants: *Brassica oleracea, Calluna vulgaris, Echium vulgare, Erodium cicutarium, Gladiolus italicus, Raphanus raphanistrum, Ornithogalum sigmoideum, Salvia verticillata, Stipa bromoides, Salvia viridis, Rumex crispus, Hypericum perforatum, Melilotus officinalis, Picris strigosa, Sigesbeckia orientalis, Lavatera bryoniifolia, Malva neglecta, Sinapsis arvensis, Sideritis taurica, Verbascum sp., Vicia lutea* (Dursun and Fent, 2011).

This species was found during summer.

Dolicorus baccarum

Adults are 10-12 mm in length and brown color. Corners of scutellum are whitish yellow.

This species is distributed in Europe, Korea, China, Japan (Lodos et al., 1998) and Turkey (Fent and Aktaç, 1999; Özgen et al., 2005a)

Host plants: wheat, pistachio, apricot, cherry and olive (Özgen et al., 2005a,b).

This species was found in August, September and October.

Nezara viridula

It is generally green but edge of the head, pronotum and connexivum are yellowish. Body is flat and broad (Lodos, 1986). Antenna are yellow with 5 segments. Scutellum is triangular. Female adults are 14.6 mm and male adults are 13.6 mm long.

This species is cosmopolitan and common all over the world (Önder et al., 2006). *N. viridula* is distributed widely in the tropics, subtropics, and temperate zones of the world (Yukawa et al., 2007).

Host plants: *N. viridula* is a polyphagous species. Kiritani et al., reported that *N. viridula* has 145 host plant species. Corn, sorghum, soybean, castorbean, wildcrucifer, melilotus, thornapple, sunflower, lucerne, groundcherry, clover and soybean are hosts of *N. viridula* (Velasco and Walter, 1992)

This species was found in the lavender areas of Isparta province during spring, summer and autumn.

Pyrrhocoris apterus

It has black head and antenna, red pronotum and black scutellum (Awad and **Önder**, 1997). Adults are 10-12 mm long.

This species is distributed in Afganistan, Albania, Algeria, Austria, Balear Islands, Belgium, Bulgaria, Caucasus, Asia, Rusia, Corsica, Costa Rica, Cyprus, Czechoslovakia, Danmark, England, France, Germany, Greece, Hungary, Iran, Iraq, Israel, Italy, Kashmir, Mongolia, Morocco, Finland, Poland, Portugal, Romania, Sardinia, Siberia, Spain, Sweden, Switzerland, Syria, Tacikistan, Tunusia, Turkey, USA and Yogoslavia (Awad and **Önder**, 1997).

Host plants: The main host plant of *P. apterus* is mallow but it can also be seen on okra and other Malvaceae (Lodos, 1986). Kaya and Kovancı (2004) reported that this species was seen around raspberry growing areas in Bursa province of Turkey.

This species was found in Isparta during spring and summer months.

Lygaeus equestris

These bugs can reach about 11 to 12 millimetres in length. They have a characteristic red-black pattern, fully developed wings and long, powerful legs.

Host plants: *Astragalus spruneri* Boiss, *Cardaria draba, Centaurea sp*., *Chenopodium sp., Convolvulus sp., Elaeagnus orientalis* (L.), *Fragaria spp., Pistachio vera* (L.), *Platanus sp., Populus sp., Prunus armeniaca* (L.), *Prunus domestica* (L.), *Pyrus communis* (L.), *P. malus* (L.), *Rosa sp., Rubus sp., Rumex sp., Salix sp., Sinapis sp*., *Spinacia oleracea* (L.), *Tamarix sp*., *Verbascum sp*., *Vicia sp*. *Vitis vinifera* (L.) (Lodos et al., 1999; Yanık and Yücel, 2001; Özsaraç and Kıyak, 2001; Gençer et al., 2004).

This species is distributed in Albania, Austria, Belgium. Bosnia, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Italy, Macedonia, Montenegro, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland (Péricart, 2001), Holo-palearctic and Pakistan and India (Linnavuori, 2007; 2011) and Turkey (Lodos et al., 1999; Ulusoy et al., 1999; Özsaraç and Kıyak, 2001; Gençer et al., 2004; Kaya and Kovancı, 2004; Zobar and Kıvan, 2005; Önder et al., 2006; Demirözer and Karaca, 2011).

This species was found during summer.

Poecilimon glandifer

Adults are 5-9 mm long. Females are bigger than males and ovipositor is green. Adults have green and black bands on pronotum and legs are greenish. Antenna are as long as body length.

Host plants: fruit trees, grass and pastures and some ornamentals (İren, 1973).

It is reported that this species is endemic for Turkey and distributed inland (Mol et al., 2016).

This species was found during May and June. Growers apply pesticides on lavender only for this pest.

Conclusion

Our research demonstrated that 13 pest and 4 natural enemy insect species collected from lavender plants of Isparta province. This study is the first record for *Cechenotettix martini* in Turkish fauna. Among these species, *Poecilimon glandifer* was the only important pest determined in the study.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Analysis of Resource Use Efficiency and Elasticity of Production among Smallholder Broiler Producers in Ikwuano Local Government Area of Abia State, Nigeria

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Abstract

Resource use efficiency is the extent at which a firm or production unit makes proficient decisions by utilizing limited resources to maximize profit. Elasticity of production is a measure of a firm's success in producing maximum output from a set of input. This research analyzed resource use efficiency and elasticity of production among smallholder broiler producers in Ikwuano LGA of Abia State, Nigeria. Specific analytical techniques employed was the production function analysis involving the four functional forms of linear, double log (Cobb Douglas), semi-log and exponential. This was used to obtain the parameters for the measurement of resource use efficiency among the broiler producers. The results of the analysis shows that resource use efficiency (r) was 1.064, 0.018, –0.046 and 0.049 for quantity of broiler produced (stock size), labour expenses, cost of medicines/drugs and feed expenditure respectively. Stock size and feed expenses had the highest efficiency index of 1.064 and 0.049. The least efficiency index was recorded for medicines/drugs with a negative value of –0.046 which implied that the resource use efficiency for medicines/drugs was grossly inefficient. The resource use efficiency for stock size/quantity of day-old-chicks shows that the resource was completely over used. The resource use efficiency with which poultry feeds were utilized shows that the quantity of feeds was over utilized. The elasticity of production of the smallholder broiler producers was 1.016 ($\Sigma Ep>1$). This shows that, if all resources were to increase by 100%, output would increase by 101.6%. This implies that fixed resources (land, depreciated equipment) were abundant relative to variable resources (feed, day-old-chicks, medication and labour); yet, these fixed resources were not efficiently utilized due to lack of sufficient quantity of variable resources. There is a paramount need to increase variable resources in order to maximize profit.

Keywords: Resource Use Efficiency, Elasticity of Production, Production Function, Smallholder Broiler Producers, Abia State Nigeria

Introduction

The development and improvement of the poultry industry has been a major focus of Nigeria's Federal Government initiative; because, apart from the fact that poultry meat contribute tremendously to the protein intake of individuals, it also serves as a ready source of income to smallholder farmers (Afolabi, 2002). Poultry offer short-term investment opportunities and thus helps to increase meat and egg availability, thereby improving the health and standard of living of the people (Onyenweaku, Igwe and Mbanasor, 2004). Broilers are birds (male or female) reared for meat. They attain a market weight of between 1.5 kilogram and 2.5 kilogram in about 7 – 10 weeks (Ramrao *et al.,* 2008*)*.

Economic efficiency is the capacity of a firm or a production unit to maximize profit (Onyenweaku, Igwe and Mbanasor, 2004). Economic efficiency is tantamount to resource use

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efficiency. The extent to which small scale broiler farmers make efficient decisions by utilizing their low inputs (limited resources) up to the level at which their marginal contribution to the value of production is equal to the factor costs is a measure of her resource use efficiency.

Elasticity of production is the degree of responsiveness of output to changes in a variable input. It is the percentage change in output as a ratio of a percentage change in input. It is a measure of a firm's success in producing maximum output from a set of input (Farrel, 1957).

Efficiency in the use of farm resources is concerned with the relative performance of the processes used in transforming given inputs into outputs. There are basically three major types of efficiency, *viz*., technical, allocative and economic efficiency. Technical efficiency refers to the ability of firms to employ the best practices in the production processes so that not more than the necessary amount of a given set of inputs is employed in producing the "best" level of output (Carlson, 1989). Allocative efficiency denotes the choice of optimum combination of inputs consistent with the relative factor prices *(*Nwaru, 1993*)*. Economic efficiency on the other hand is the ability of a firm to fully maximize profits. Farrel (1957) and Iheke (2006) emphasized that technical efficiency tenders on the ability of production units to produce maximum outputs from a given set of inputs. Efficiency of resource use is acquiescent to technical research, thereby leading to the identification of opportunities for increased performance through appropriate adjustment in the pattern of resource use. (Mbanasor and Chidebelu, 2001).

Attainment of efficiency in small scale broiler production is a very important factor for its productivity and growth; most especially in developing agrarian economies such as Nigeria where resources are meager and opportunities for developing commercial poultry enterprise are capital intensive, which doesn't provide a measurable level of employment to greater percentage of the populace.

With over 65 percent of the population in Sub Saharan Africa suffering chronic food insecurity, the need for efficient resource utilization cannot be over emphasized. Efficient resource use remains a major challenge for proficient policy measures targeted at improving livelihoods of small scale broiler farmers (Kuriuki, Ritho and Muneik, 2008).

This research analyzed resource use efficiency and elasticity of production among smallholder broiler producers in Ikwuano Local Government Area of Abia State, Nigeria. The project recommends proficient policy measures that enables smallholder broiler farmers increase her efficiency.

Methodology

Study Area

The study was conducted in Ikwuano Local Government Area of Abia State, which is located in Umuahia Agricultural Zone of Abia State. Ikwuano Local Government is made up of four clans which comprises several autonomous communities and villages. These clans include: Ariam, Ibere, Oboro and Oloko. The Local Government area is situated in the south-eastern part of Abia State. Ikwuano has a population of 52,214 people, with male having 28,840 and females 23,374 (Federal Government of Nigeria - FGN, 2009). Ikwuano is found between latitudes 5^0 24' – 5^0 30' North and longitudes at 7^0 32'- 7^0 37'East. The people are mostly farmers which constitute about 70% of the rural population. The predominant soil of the area is sandy loam while the natural vegetation is the tropical rainforest (Iheke, 2006) and is characterized by two distinct seasons; dry and wet seasons. The dry season lasts from November to March while the wet season lasts from April to October. The settlement pattern is rural and farming is the predominant occupation of the inhabitants. Most families are involved in one farming activity or the other as a primary or secondary occupation. Livestock are also kept especially on a smallholder basis (Nwaru, 2005).

Sampling Procedure / Sampling Technique

Multi stage random sampling technique was employed in selecting the respondent. In the first stage, one (1) clan was randomly selected from the four clans that make up Ikwuano Local Government area of Abia State. In the second stage, four (4) autonomous communities were randomly selected from the clan. In stage three, one (1) village was randomly selected from each of the four (4) autonomous communities; making a total of four (4) villages. In the fifth stage, 21 smallholder broiler producers were randomly selected from each of the four (4) villages; making a total of 84 smallholder broiler producers for a comprehensive study.

From the four villages, each village was allocated 21 questionnaires which were randomly administered to the different smallholder broiler producers (21 x 4 = 84). However, only 80 copies of the questionnaires which were satisfactorily and appropriately filled were used for the analysis.

Method of Data Collection

Data was collected from primary source. The data collected include cost of broiler production, return from the sales of broiler produce (culled birds, droppings), labour employed, quantity of broiler output, price of output, amount spent on inputs, total revenue realized, etc. Well-structured questionnaires were employed in soliciting information from the randomly selected broiler farmers.

Analytical Techniques and Model Specification

Estimation of Resource Use Efficiency among Smallholder Broiler Producers

The analytical procedure employed was production function analysis. This was used to obtain the parameters for the measurement of resource use efficiency among the smallholder broiler farmers.

A multiple regression analysis was used to estimate the production function and violation of assumptions of Ordinary Least Squares (OLS) were checked before interpreting the results. The model used was illustrated as follows:

$Q = f(X_1, X_2, X_3, X_4, X_5, \mu)$ --------1

Where:

Q = Quantity of broiler produced (Kilogram)

X_1 = Number of birds housed (Farm size)
X_2 = Labour expended (man days)
X_3 = Cost of veterinary services and Medicine/drugs (Naira)
X_4 = Cost of feed (Naira)
X_5 = Years of experience in broiler production
μ = Stochastic error term

The linear, double log (Cobb Douglas), semi-log and exponential functions were employed to determine which of the functional forms would best fit the relationship between the quantity of broiler produced (dependent variable) and the exogenous variables. The final choice of the functional form was based on:

(I) highest value of adjusted R^2,
(ii) Significance of regression coefficients, and
(iii) High value of F-value.

Whichever model that has the highest adjusted R^2 and shows many statistical significant were adopted following (Koutsoyiannis, 1977 and Adinya *et al*., 2008).

The functional forms fitted were specified below:

(a) Linear production function: ------2

$$Q = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + \mu$$

(b) Cobb-Douglas Function (double log)

$$\ln Q = a + b_1\ln X_1 + b_2\ln X_2 + b_3\ln X_3 + b_4\ln X_4 + b_5\ln X_5 + \mu$$

(c) Semi-Log Production Function:

$$Q = a + b_1\ln X_1 + b_2\ln X_2 + b_3\ln X_3 + b_4\ln X_4 + b_5\ln X_5 + \mu$$

(d) Exponential Production Function:

$$\ln Q = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + \mu$$

Where:

X_1-X_5 = are defined in the implicit form
b_1-b_5 = Regression coefficients of variables X_1-X_5
a = Constant term
μ = Error term
ln = Natural Logarithm

The resource use efficiency was obtained from the estimated equation by comparing the Marginal Value Product (MVP) of a particular input with the Marginal Factor Cost (MFC) of that input.

The following ratio i.e. r = MVP/MFC, was used to estimate the relative efficiency of resource use.

Where:

MVP = Value added to broiler output due to the use of an additional unit of input, calculated by multiplying the MPP (Marginal Physical Product) by the price of mature broiler, i.e., $\{MPP \times P_Q = bi \times P_Q\}$

MPP x Price of broiler = Marginal Value Product (MVP). Where MPP = bi (slopes of the coefficients of resource input).

MFC = Cost of one unit of a particular resource: The marginal factor cost (MFC) of an input is the addition to cost as a result of using additional unit of the input; which for a firm operating in purely competitive industry is the market unit price.

In this study, the smallholder broiler farmers were assumed to operate in a purely competitive input market, thus, the market unit price of input was taken as the marginal factor cost.

As of the time of the analysis, the marginal factor cost of farm land was the tribute paid to the tenant farmer which was ₦2,650 on the average for the building per production cycle (one batch). That of labour was ₦950 per manday, that is, the average market rate of hired labour.

In order to evaluate the resource use efficiency of smallholder broiler farmers, the study adopted the method used by Oladeebo and Ezekiel (2006) thus: $MVP = MPP.Xi \times P_Q$. Depending on the functional form selected as lead equation for regression, the MPP and the corresponding values of MVP was obtained as follows:

Linear: $MPP = dQ/dX = bi$; $MVP = bi \times P_Q$
Double log: $MPP = bi \times Q/Xi$; $MVP = bi \times Q/Xi \times P_Q$
Exponential: $MPP = bi \times Q$; $MVP = bi \times Q \times P_Q$
Semi log: $MPP = bi / Xi$; $MVP = bi /Xi \times P_Q$
Therefore, Resource Use Efficiency (r) = MVP / MFC --3

Decision Rule for Resource Use Efficiency

If r = 1; it shows that resources is efficiently used, that is optimum utilization of resources, hence the point of profit maximization.

If r < 1; resources is excessively used or over utilized, hence decreasing the utilization of that resource increases profits.

If r > 1; resources is under used or underutilized, hence increasing its rate of use will increase the profit level.

Economic optimum takes place where MVP = MFC.

Thus, When Resource Use Efficiency (r) =1, resources are optimally utilized. When r<1, resources are over utilized. When r > 1, resources are underutilized

Where:

bi = regression coefficients, i=1,2,3,……
Q = mean output of broiler produce,
Xi = mean value of resource,
Pxi = price of resource per unit
P_Q = price of output per unit
MFC = marginal factor cost
Xi = of particular resource input.

Analysis of Elasticity of Production among Smallholder Broiler Farmers

Elasticity of production (Ep) is the percentage change in output as a ratio of a percentage change in input. The elasticity of various inputs was determined by this formula:

$Ep = dQ / dXi \times Q / Xi$ or $Ep = MPP / APP$ ----- 4
$APP = Q / Xi$ and $MPP = dQ / dXi$

Where:

Q is the broiler output in Kg
Xi's are the various input used in production,
X and Q are the averages of input and output respectively.
MPP = Marginal Physical Product and APP = Average Physical Product
ΣEP = Summation of Elasticity of Responds.

Since the Cobb-Douglas production function gave the best fit, the regression coefficient will still be the elasticities; and used to measure the rate of return; which is a measure of a firm's success in producing maximum output from a set of input. $[\Sigma EP = b_1+b_2+b_3+b_4+b_5]$

Criteria for return to scale estimation

ΣEP =1: constant return to scale
ΣEP <1: decreasing return to scale
ΣEP >1: increasing return to scale

Results and Discussion

Analysis of Resource Use Efficiency among Smallholder Broiler Producers

The analytical procedure employed was the production function analysis. This was used to obtain the parameters for the measurement of resource use efficiency among the smallholder broiler farmers.

Table 1. Production Function of Smallholder broiler farmers in Ikwuano LGA, Abia State, Nigeria

Variables	Linear	Semi log	Double log^{++}	Exponential
(a) Constant	53.017	–3735.816	3.085	5.305
	(2.527)**	(-0.402)	(1.715)*	(7.050)***
(X_1) Number of Birds	1.628	273.161	0.949	0.005
	(46.781)***	(18.498)***	(31.138)***	(19.029)***
(X_2) Expenditure on Labour	0.153	48.769	0.014	–0.002
	(0.802)	(2.628)**	(2.542)**	(-1.818)*
(X_3) Cost of Medicines/drugs	–0.005	73.645	–0.074	–7.422E-5
	(–0.672)	(1.781)*	(–2.003)*	(–0.919)
(X_4) Cost of Feed	0.003	162.582	0.108	–3.387E-6
	(–2.647)**	(0.683)	(5.320)***	(-0.397)
(X_5) Years of Experience	1.181	7.387	0.019	0.003
	(1.726)*	(1.824)*	(0.961)	(0.600)
R^2	0.949	0.829	0.969	0.835
R^2 Adjusted	0.938	0.818	0.967	0.824
F – ratio	381.176***	71.785***	475.177***	75.034***

Source: Field Survey Data, 2012; ( ) = t values computed, $^{++}$ = lead equation
***, **, * = Significant at 1 percent, 5 percent and 10 percent, respectively

Linear, semi – log, double log and exponential function of the production function were estimated. All models were significant (P < 0.05) with F – values of 381.176, 71.785, 475.177 and 75.034 respectively, with R^2 of 0.949, 0.829, 0.969 and 0.835 for linear, semi – log, double log and the exponential, in that order. The regression coefficients had the *a priori* expected signs. The Double log production function was chosen as the lead equation for the analysis based on conformity with *a priori* expectations of signs and magnitude of the coefficients, overall significance of the functional form (F-ratio) as well as the explanatory power of the variables (adjusted R^2). The F-value was statistically significant at 1.0%, which shows that the regression model gave a good fit, signifying that the exogenous variables (X_1, X_2, X_3, X_4 and X_5) included in the model best explain the dependent variable (Q).

In Table 1, the lead equation (double log) shows that R^2 is 0.969, which infers that about 96.9% of the variation in broiler output was explained by the variables (number of broilers, amount of labour, cost of medicine, cost of feed and years of experience), while the remaining 3.1% was the unexplained variation.

The estimated production function for the smallholder broiler producers in Ikwuano Local Government Area of Abia State is $Q = 3.085^* + 0.949^{***}X_1 + 0.014^{**}X_2 – 0.074^*X_3 + 0.108^{***}X_4 + 0.019X_5$. The equation showed that all other statistically significant variables will increase the output of broiler to the tune of 308.5kg. The stock size (number of birds), amount of labour and cost of feed were positive and statistically significant at 1.0%, 5.0% and 1.0% respectively. The implication of these positive signs is that an increase in each of these variables would lead to an increase in the level of broiler output produced, while the negative sign denote a decrease in the level of broiler output.

This result agrees with *a priori* expectation, suggesting that, as the quantity of consumed feed increases, output of broiler is likely to increase. This is true because broiler birds have a high feed intake to be able to justify their genetically endowed fast growing ability. Also, the more the stock size, the higher the profitability of an enterprise. This agrees with the findings of Ghadoliya (2000) who reported that larger flocks sizes (>20,000 birds) yielded higher returns per bird compared to smaller flocks.

The inverse relationship between cost of medicines/drugs and broiler output is consistent with *a priori* expectation, that is, as the cost of medicines/veterinary services decreases, output increases, and vice versa. The cost of labour was positively directly related to the output of broiler at 5.0% level of significant. This implied that the higher the labourers employed, the more specialized the enterprise becomes. As specialization increases, output also increases; due to an

efficient use of scarce productive resources. Years of experience was found to be insignificant in the study, denoting that it never affected the output of broiler production.

Table 2. Resource Use Efficiency Indicators among Smallholder Broiler Producers in the study area

Resources	MPP	MVP	MFC	MVP/MFC = r
(X_1) Number of Birds	1.064	2500.40	₦2350.0/bird	1.064
(X_2) Expenditure on Labour	0.018	9.55	₦530.53/manday	0.018
(X_3) Cost of Medicines/drugs	–0.046	–1.68	₦36.38/unit/bird	–0.046
(X_4) Expenditure on Feed	0.049	23.93	₦488.42/Kg of bird	0.049

Source: Field Survey Data, 2012

Given the level of technology and prices of both inputs and outputs, efficiency of resource use was ascertained by equating the Marginal Value Product (MVP) to the productive Marginal Factor Costs (MFC) of resources. A resource is said to be optimally allocated if there is no significant differences between the MVP and MFC *i.e.* if the ratio of MVP to MFC = 1 (unit). The Resource Use Efficiency (r) was 1.064, 0.018, – 0.046 and 0.049 for stock size, labour expenses, cost of medicines/drugs and feed expenses respectively. These results indicate that stock size and feed expenses had the highest efficiency index of 1.064 and 0.049. The least efficiency index was recorded for medicines/drugs, with a negative value of about – 0.046, which implied that resource use efficiency index of medicines/drugs was grossly inefficient.

With the ratio of MVP/MFC for stock size (number of broilers) being greater than unity, the broiler farmers appear to be underutilizing their resources. This is evident from the fact that the scales of their holding are small. These call for the expansion of their broiler scale of production and improved medication/drugs.

The resource use efficiency, with which feeds are utilized (0.049), shows that the amounts of feeds are over utilized. These results was supported by similar findings by earlier researchers (Salasya *et al*., 1986; and Nandwa *et al*., 1997) who identified input costs such as feeds as a key determinant to enterprise improvement. In order words, economic efficiency and productivity could be achieved if a farmer uses poultry feeds more efficiently.

In summary, the ratios of the MVP to the MFC were greater than unity (1) for stock size except labour, cost of medicines/drugs and feed expenses. This implies that within the limits of statistical error, none of the inputs was efficiently allocated by the broiler farmers.

Table 3. Elasticity of Production among Smallholder Broiler Farmers in the Study Area

Productive Inputs	Elasticity
(X_1) Quantity of Birds	0.949
(X_2) Expenditure on Labour	0.014
(X_3) Cost of Medicines/drugs	–0.074
(X_4) Cost of Feed	0.108
(X_5) Level of Experience (Years)	0.019
Returns to scale	1.016

Source: Field Survey Data, 2012

Table 3 shows the elasticity and returns to scale of the smallholder broiler farmers. The regression coefficients constituted the respective elasticities of production in the chosen lead equation (Cobb- Douglas production function). The summation of all the partial elasticities of production with respect to every input is 1.016 ($\Sigma Ep>1$), representing the total output elasticity/function coefficient; also referred to as returns to scale. If all factors are varied by the same proportion, the function coefficient indicates the percentage by which output would increase. In this case, it means, if all of the variables were to increase by 100%, output would increase by 101.6% representing increasing return to scale.

This implied that each additional unit of input adds more to total product than the preceding unit. In this stage, fixed resources (land, depreciated equipment) are abundant relative to variable resources (feed cost. cost of day-old-chicks, medication cost, labour cost, etc.), and these fixed resources were not efficiently utilized due to lack of sufficient quantity of variable resources.

This finding conform to similar study by Ramrao *et al*., (2008), who reported that farmers who maintained a flock size of 10,000 broilers were able to recover their fixed invested capital from production in about two years compared to those who keep smaller flock sizes.

Conclusion and Recommendations

Given the level of technology and prices of both inputs and outputs, efficiency of resource use was ascertained by equating the Marginal Value Product (MVP) to the productive Marginal Factor Costs (MFC) of resources. The Resource Use Efficiency (r) was 1.064, 0.0018, - 0.046 and 0.049 for stock size, labour expenses, cost of medicines/drugs and feed expenses respectively. The ratios of the MVP to the MFC were greater than unity for stock size, except labour, cost of medicines/drugs and feed expenses; which implies that within the limits of statistical error, none of the inputs was efficiently allocated by the broiler farmers.

The resource use efficiency with which poultry feeds were utilized shows that the quantities of feeds were over utilized. The efficiency analysis indicates underutilization of land and overuse of labour resources. The returns to scale of the smallholder broiler producers are 1.016. The implication of this in general is that, the broiler farmers in the study area are not yet operating at optimum scale of production. Hence, there is need for improvement such as better equipment and using more variable inputs to boost production.

It is therefore recommended that credit facilities be made available to the broiler farmers. There is a paramount need to increase variable resources in order to maximize profit. Livestock research centers and State Agricultural Developing Programmes (ADP) should develop genetically improved breeds of poultry which efficiently convert feed. Effectively harnessing the potentials in alternative but cheaper sources of poultry feed ingredients away from the conventional ones will lower the cost of production leading to more profits per broiler sold.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Experimental procedures were approved by Michaek Okpara University of Agriculture, Umudike, Abia State, Nigeria (July, 23, 2020).

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Data availability

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Consent for publication

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Comparative study on germination and seedling growth of wheat cultivars under salt stress regimes

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Abstract

Salinity interferes with germination and hampers the growth of wheat especially at seedling stage which necessitates determining of salt tolerant cultivars. Based upon the current situation a controlled experiment was carried out at the Laboratory of Horticulture, Faculty of Agriculture, Cukurova University, Turkey to comparatively evaluate wheat varieties response to imposed salt stress. Germination and seedling growth properties under salt stress were taken as response variables. The seeds of five bread wheat genotypes ('Wafia', 'Lucilla', 'Envoy', 'Lok1' and 'RSP- 561') were placed in Petri dishes with salinity doses (Control (0), 4, 8 and 12 $dS.m^{-1}$ NaCl) which were applied at germination and subsequent early seedling phases under laboratory conditions. The results revealed that root growth was highly sensitive to salt stress and the varieties of Envoy and Lucilla remained relatively tolerant to salt stress than other cultivars. The biochemical analysis revealed that proline content spiked with increasing salinity level, 'RSP-561' under 8 $dS.m^{-1}$and 12 $dS.m^{-1}$ recorded the maximum proline content. Salt stress boosted leaf proline content of salt sensitive wheat genotypes ('Wafia' and 'Lok1'), whereas declined proline level was observed for salt tolerant cultivars. In addition, salt-sensitive genotypes showed a reduction in chlorophyll content *a*, *b*, total chlorophyll and carotenoid while, 'Wafia' and 'RSP-561' recorded the minimum Chlorophylls and Carotenoid contents. Further investigations are needed, however, to enhance understanding of the salt stress effects during the whole growing cycle of wheat.

Keywords: Biochemical analysis, Proline content, Salt tolerance, Chlorophyll, Wheat seedling

Introduction

Globally, soil salinity has emerged as the leading challenge to modern farming systems which reduces crops yield by damaging the different cellular functions of plants. Gradually, accumulation of salt in upper soil horizons leads to destruction of soil structure and texture (Hassan et al., 2018). Among abiotic stresses, soil salinity has posed a serious threat all over the world to global food security by affecting over 7% of the land area across the globe (Hasan et al., 2015). Soil salinity has emerged as a drastic constraint that adversely affect the growth and development of the wheat crop globally (Kizilgeci and Yildirim, 2014). Exogenous salt accumulation drastically affects seed germination, lowers water retention and ion imbalance leading to ion toxicity and osmotic imbalance (Khan and Panda, 2008). Moreover, it inhibits the growth and development of seedling by reducing photosynthesis rate and negatively effecting other vital physiological processes such as respiration and protein synthesis (Pal et al., 2004).

Wheat (*Triticum avestivum* L.) is considered as a major cereal crop and staple food worldwide. Wheat meets the increasing food requirements of a lot of country. To ensure the food and nutritional security of the skyrocketing population,

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its yield needs to be multiplied as owing to the limitation in increasing its acerage (Hossain et al., 2018; Jahan et al., 2019). In addition, wheat becomes a source of providence to million of growers across the globe and thus contributes to national grass domestic production (GDP) of agrarian-based economies (Barutcular et al., 2017; Yildirim et al., 2018)

Seed germination along with early seedling growth phases are the most critical stages at which salt stress may impart drastic effects on growth of crop plants (Almansouri and Lutts, 2001; Oral et al., 2019; Kizilgeci et al., 2020) caused by salt ions toxicity. Wakeel et al. (2011) reported that some plants tend to be salt sensitive at shoot growth stage because of nascent salinity tolerance mechanism. Root and shoot length is one of the dominant factors against salt stress because roots are in direct touch in soil and they uptake the water and minerals from the soil and supply it to other parts of the plant (Bhutto et al., 2018). Root and shoots traits are important indicators for assessing plants tolerance or susceptibility to salt stress (Almodares et al., 2007). Moreover, these parameters provide valuable clues for selections of genotypes in future breeding programs (Khan et al., 2008).

The germination vigour and salt tolerance mechanism usually vary among different crops and even different cultivars respond differently to salt stress owing to varying genetic makeup (Shalhevet, 1995). Throughout the life cycle of a plant, its susceptibility to salinity at the time of seed germination as well as early seedling stages are much higher than the subsequent growth stages (Ashraf et al., 1986). Thus, it was hypothesized that wheat cultivars might respond differently to salt stress leading to screening and identification of the most salt tolerant cultivar. Therefore, the current trial was executed with dual objectives to evaluate germination and seedling response of five wheat varieties to induced levels of salinity and identify the superior cultivars for salt tolerance.

Material and Methods

Location and duration

The experiment was conducted at the Laboratory of Horticulture, Faculty of Agriculture, Cukurova University, Turkey.

Plant materials, experimental treatments and design

Factorial experiment using a completely randomized design (CRD) with three replications was used for current study. Five bread wheat genotypes viz. 'Wafia', 'Lucilla', 'Envoy', 'Lok1' and 'RSP-561' were used in this experiment. Four levels of salinity viz., control (0), 4, 8 and 12 $dS.m^{-1}$ NaCl were applied as salt treatments on wheat genotypes from germination to early seedling stage under laboratory conditions.

Experimental procedure

Seeds were subjected to surface sterilization using sodium hypochlorite (5% v/v) for 10 minutes and then washed thrice with distilled water. The seeds were placed Petri-dishes containing filter paper, each petri-dish consisted of 20 seeds. Day and night lengths were 18/6 h, with 24±1 °C at growth chamber. Salt solutions were prepared by using a calculated amount of NaCl for 4, 8 and 12 $dS.m^{-1}$concentration, and NaCl solution applied in each petri-dish according to the treatment specification.

Data collection

The experiment was complated by harvesting seedlings after 7 days and measured the seed vigour, shoot length, root length, coleoptile length, seedling fresh weight, root fresh weight. After measuring the fresh weight, the seedlings were then placed into an oven at 70 °C temperature for 48 h to measure root dry weight and seedling dry weight.

The relative water content (RWC) was determined by using the following formula

RWC = (FW - DW) x100 / (TW - DW)

Where, FW: Fresh weight, DW: Dry weight and TW: Fresh weight at full turgidity.

Proline content was determined as suggested by Bates et al. (1973). The solution's optical density was noted on a spectrophotometer using 528 nm wavelength, while proline's quantification was done with the help of a calibration curve which was obtained by plotting the intensity rate increment as a function of L-proline concentration (0-5µg ml^{-1}).

Chlorophyll *a, b* and total chlorophyll along with carotenoids were determined by following the methodology of Lichtenthaler and Wellburn (1983). The Absorbance was determined with the help of spectrophotometer using 470, 646 and 663 nm wavelengths.

Statistical analysis

Statistical analyses of recorded data were performed by employing LSD (Least Significant Difference) test at 5% level of probability using 'JMP 13.0'Command program.

Results and Discussion

Coleoptile length (cm): The coleoptile length of different wheat varieties showed different results under varying levels of salt stress (Figure 1). The highest coleoptile length was observed at 4 $dS.m^{-1}$ doses, followed by 8 $dS.m^{-1}$ and 12 $dS.m^{-1}$. The highest coleoptile length was produced in the 'RSP-561' variety (3.21 cm) at 8 $dS.m^{-1}$salt application. Similar to our studies, Kizilgeci et al. (2010) reported that the highest coleoptile length value had a high salt dose compared to control conditions. It was recorded that the decrease in root length in response to higher salt stress and noted that the response of roots was very low in both saline and sodic soils due to higher ionic toxicity (Leishman and Westob, 1994). Previously, it has been reported that salt stress drastically reduced germination rate, while seedling growth was also significantly reduced (Valadyani et al., 2007).

Root length (cm)

Wheat varieties recorded a significant reduction in the length of roots under varying levels of salinity (Figure 2). However, the highest decline in root length was recorded for salinity level of 12 dSm^{-1} for all the varieties. Among wheat cultivars, the highest root length was assessed for Lucilla followed by 'RSP-561' and 'Wafia'. Salinity adversely affects the shoot growth of plants by altering water relations due to salt accumulation in intercellular spaces (Khayatnezhad et al., 2010). The seedling establishment at early growth stages of plants is crucial for getting the maximum shoot length and consequently higher yield (Khatkar and Kuhad, 2000). The salinity stress induced root length reduction might be attributed to NaCl's growth inhibitory effect which drastically hampered root growth to a

greater extent compared to shoot growth (Rahman et al., 2001). Previously, root length and seedling growth of wheat cultivars were drastically reduced with increasing NaCl stress (Khatun et al., 2013). Yildirim et al. (2015) noted that root length could serve as reliable criteria for determining the salinity tolerance of crops genotypes, as roots have direct contact with soil and are responsible for absorption of nutrients and water.

Seedling length (cm)

Seedling length of wheat varieties significantly affected by different levels of NaCl concentration (Figure 3). The highest seedling length was produced by 'Lucilla'and 'RSP-561'. The reduction of seedling length is a common phenomenon of several crops grown under saline environments (Amin et al., 1996). High salinity may inhibit the root and shoot elongation due to slowing down the water uptake by the plant (Bayuelo-Jiménez et al., 2002). Similar findings were reported by Qu et al. (2012), who observed that salinity accelerated leaf abscission along with reducing inter-nodal development which resulted in stunted shoot growth. It was inferred that reduction in root and shoot growth might be attributed to sensitivity of cultivars to slight increment in salinity level in the growth medium (Hassan et al., 2018).

Root fresh weight (mg)

Root fresh weight (mg) was significantly influenced by genotype and salinity levels interaction (Figure 4). The root fresh weight was found significantly higher at control, However, The heaviest root fresh weight was obtained in Lucilla although the slightest was obtained in 'RSP-561'. It was recorded that the lowest root fresh weight (1 g) was noted at 16 dSm^{-1} NaCl level (Mansour & Salama, 1996). Shahzad et al. (2012) observed a decrease in root fresh weight as salt concentration increases and noted that this trait may be used for salt stress tolerance.

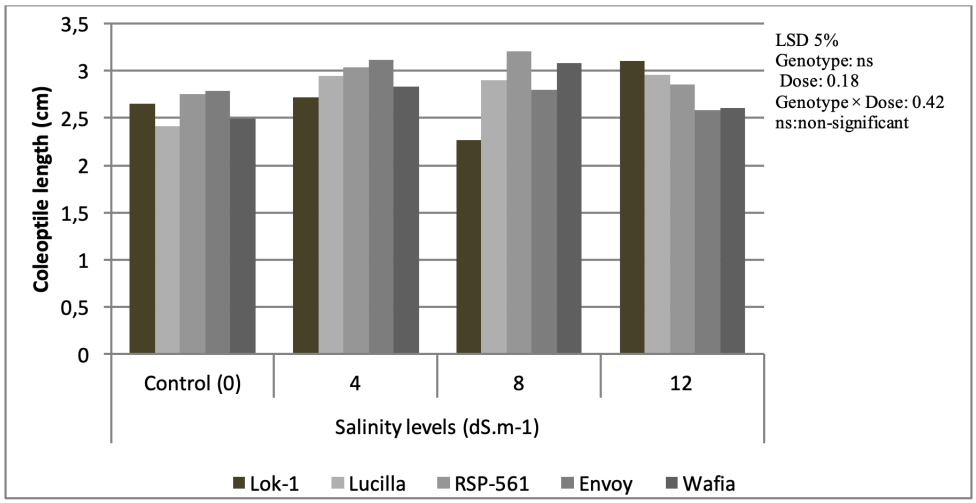

Figure 1. Coleoptile length (cm) of bread wheat genotypes grown under different salinity levels.

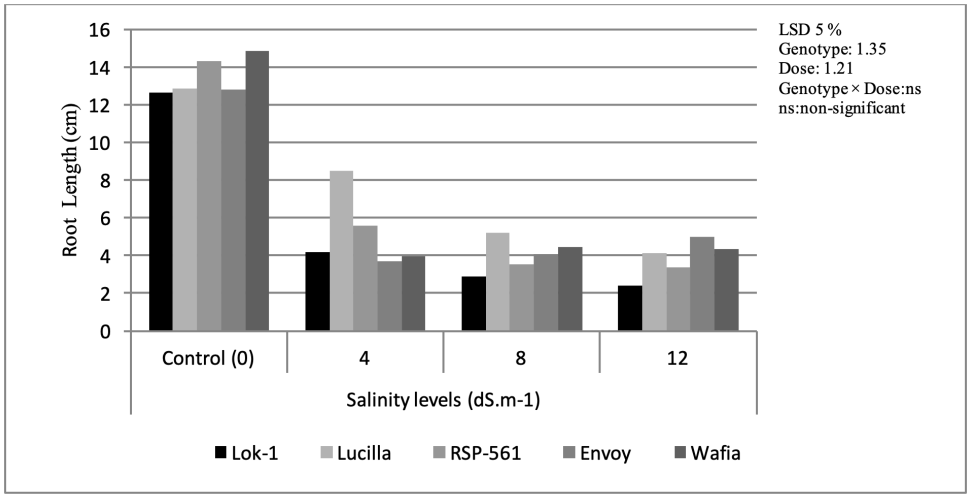

Figure 2. Root length (cm) of bread wheat genotypes grown at different salinity levels

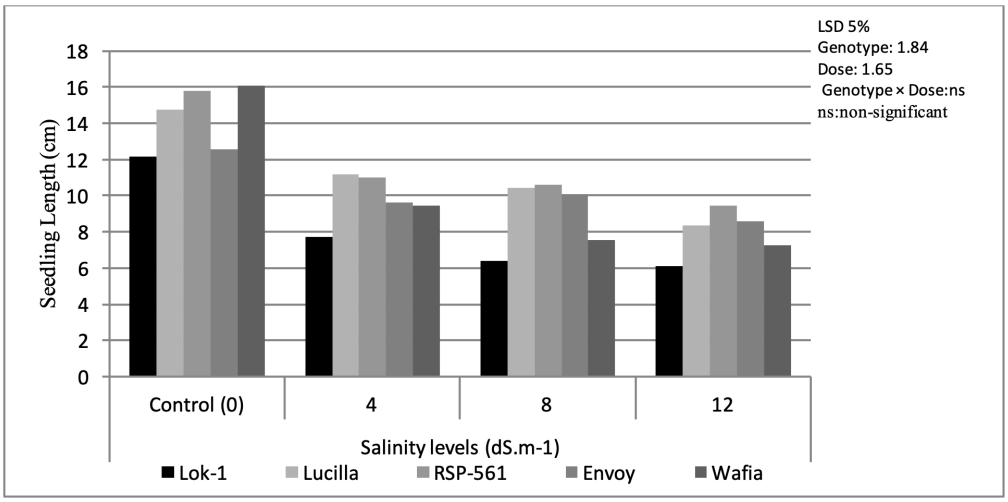

Figure 3. Seedling length (cm) of bread wheat genotypes grown at different salinity levels

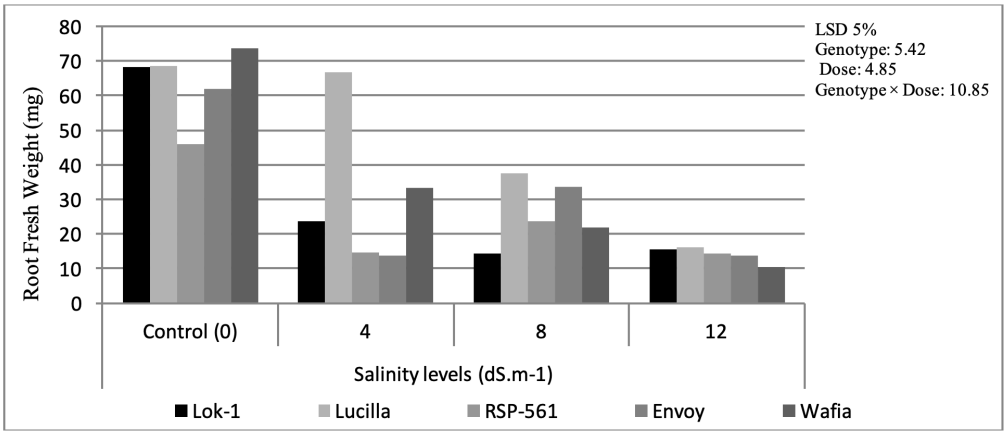

Figure 4. Root fresh weight (mg) of bread wheat genotypes grown at different salinity levels

Seedling fresh weight (mg)

The results related to seedling fresh weight indicated significant ($P<0.05$) response of wheat cultivars to salt stress. The decreased seedling fresh weight was recorded at an increasing level of NaCl concentration (Figure 5). The maximum seedling fresh weight was recorded in 'Lucilla' and 'RSP-561'. while the lowest was recorded in 'Envoy'. Studies have found that salt stress affects plant growth at different levels in some wheat varieties and causes a reduction in vegetative organs, in the fresh weights of plants in general. In their studies investigating the effects of salt stress on wheat, Kizilgeci et al. (2010) reported that salt stress caused losses in fresh weight of seedlings. The shoot fresh weight was induced by salinity stress Karaki (1998), also noted that the root fresh weight was not affected by salt stress. However, seedling fresh weight was significantly affected by salinity.

Root dry weight (mg)

Root dry weight was affected by the different concentrations of NaCl levels (Figure 6), among various NaCl levels, the maximum root dry weight was recorded under control condition, While, minimum dry weight was observed under highest salinity level of 12 dSm^{-1}. In the case of varieties, the highest root dry weight was obtained from 'Lucilla' and 'Envoy', followed by 'RSP-561' and 'Wafia', while the lowest value was observed in 'Lok-1'. It was found the salinity had a significant adverse effect on root dry weight (Baalbaki et al., 1999).This is in confirmation to previous reports which concluded that salinity level increment reduced growth and development of cereals including rice (Masood et al., 2005). It was also inferred that shoot dry weight remained prone to salinity than root dry weight (Essa, 2002). Similarly, different cultivars of bread wheat recorded a significant reduction in shoot dry weights under increasing levels of salt concentration (Kizilgeci et al., 2010).

Seedling dry weight (mg)

The results related to the seedling dry weight of wheat varieties was influenced by various NaCl levels (Figure 7). The highest seedling dry weight value was obtained under control conditions and the lowest was under 8 dSm^{-1} and 12 dSm^{-1} conditions. Whereas the highest seedling dry weight value was obtained in the wafia variety under control conditions, the lowest value was obtained in the 'Lok-1' varieties in the 12 dSm^{-1} salt application. These finding are in line with previously reported conclusions whereby varying seedling dry weights of wheat genotypes were taken as indication of varying salt sensitivity (Khatun et al., 2013).

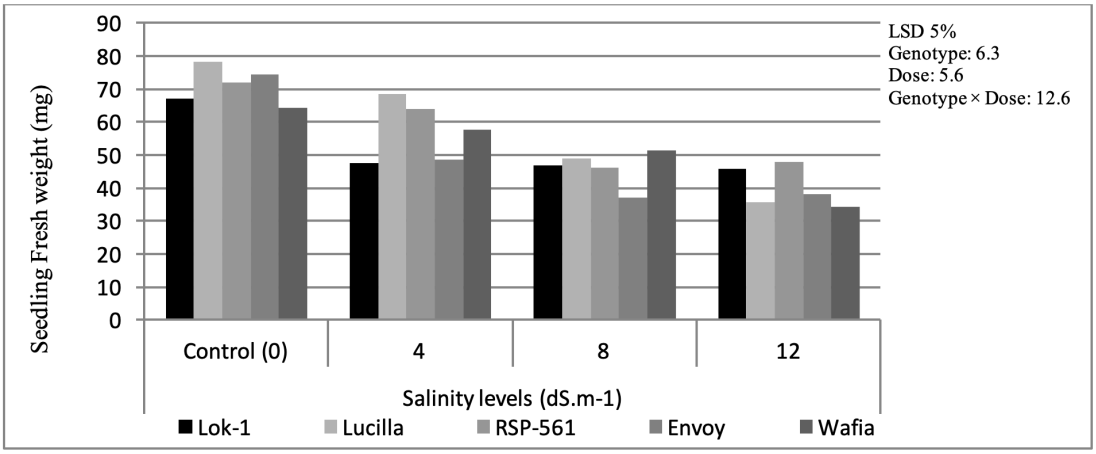

Figure 5. Seedling fresh weight (mg) of bread wheat genotypes grown at different salinity levels

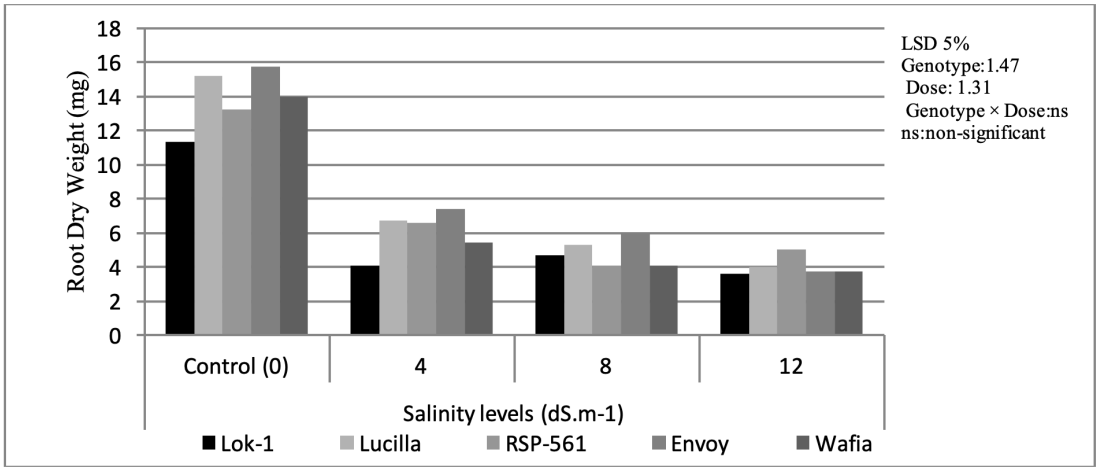

Figure 6. Root dry weight (mg) of bread wheat genotypes grown at different salinity levels

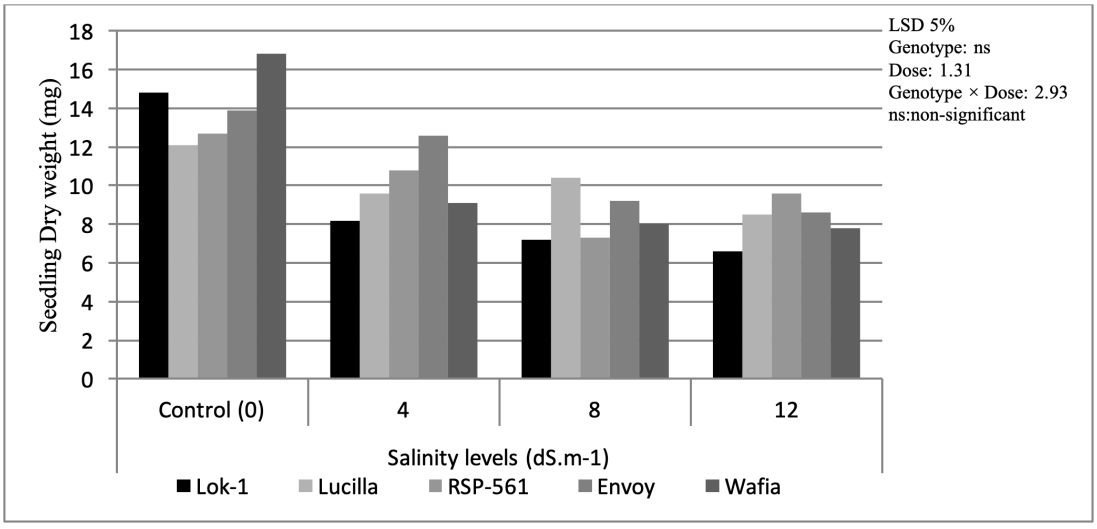

Figure 7. Seedling dry weight (mg) of bread wheat genotypes grown at different salinity levels

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Relative water content

The data related to relative water content revealed a highly significant response of wheat varieties to various salinity levels. The interaction between varieties and NaCl levels was also significant (Figure 8). The maximum relative water content was recorded under control conditions, while the lowest relative water content (RWC) was determined under 8 dSm^{-1} conditions.due to drought with PEG and salt-water content in the shoot reduced significantly (Bajji et al., 2000) and salt stress conditions lead to drought stress in root due to less uptake of water by roots.

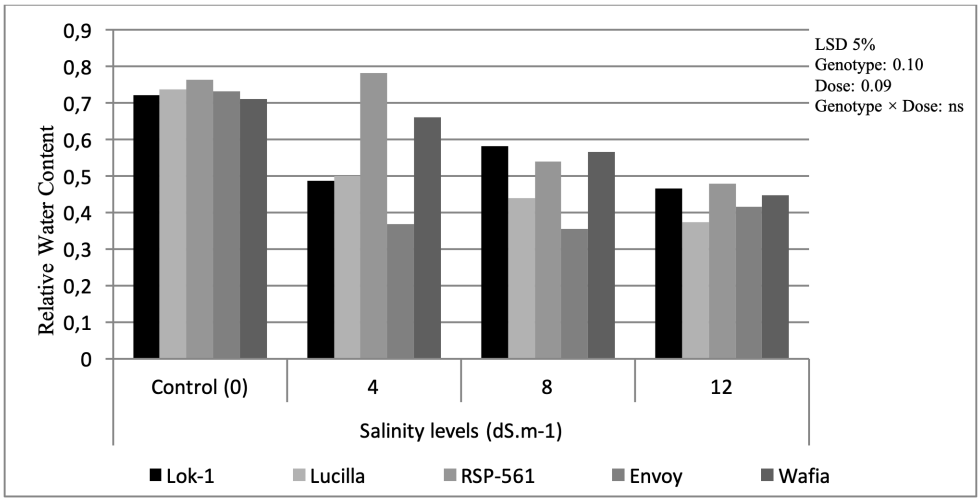

Figure 8: Relative water content of different wheat genotypes grown at different salinity levels

Seed vigour (%)

Salinity level interacted significantly wheat genotypes to influence seed vigour which expressed the speed of germination (Figure 9). Germination vigour index was found higher at control, with a range from 90.83% in 'Wafia' to 65.42% in 'Lok-1'. The drastic reduction in seed germination might be attributed viability loss caused by imposed salinity as reported by Gulzar et al. (2001).

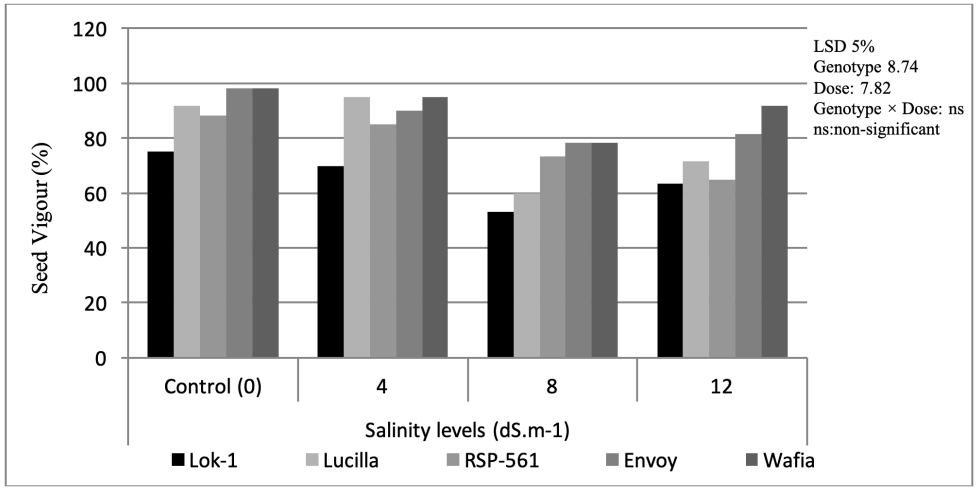

Figure 9. Seed vigour (%) bread wheat genotypes grown at different salinity levels

Proline content (micromol g^{-1})

Proline content varies significantly between varieties and salinity stress. Salinity stress enhanced proline accumulation in leaves of varieties, but the increase in 'Wafia' leaves was higher than that in other leaves under 4 $dS.m^{-1}$ treatment, compared to control (Figure10). The maximum proline was determined at 4 and 8 dSm^{-1}, while the minimum value was determined at 12 dSm^{-1}. Among the genotypes, 'Wafia' and 'RSP-561' had the maximum proline levels and the minimum was 'Lok-1'. The highest proline levels were recorded in wafia cultivars at 4 $dS.m^{-1}$ salt dose, while the lowest proline levels were recorded in 'Lok-1' and 'Lucilla' varieties at 12 $dS.m^{-1}$salt dose. Enhanced proline concentration in the leaves might be due to its rapid synthesis and the breakdown of proline rich proteins during stress (Greenway and Munns, 1980). It has also been described as an adaptive physiological characteristic of plants

for ensuring their survival under salt stress (Datta et al., 2009). These findings are in line with other studies which concluded that proline wasa physiological marker of increasing salinity level which got increased in wheat genotypes which were salt-tolerant compared to the salinity sensitive genotypes (Hasan et al., 2015). Accumulation of solutes (proline, glycinebetaine and sugars) have been reported as reliable indicators to quanitify and predict the drastic impact of salt stress (Qasim et al., 2003). There was some evidence which supported the role of proline accumulation in imparting salinity tolerance to crops under moderate to severe salt stress (Khan et al., 2009). It has been reported that in flag leaf of salt-tolerant wheat genotypes, ıncreased proline levels could have a protective function (Hasan et al., 2015).

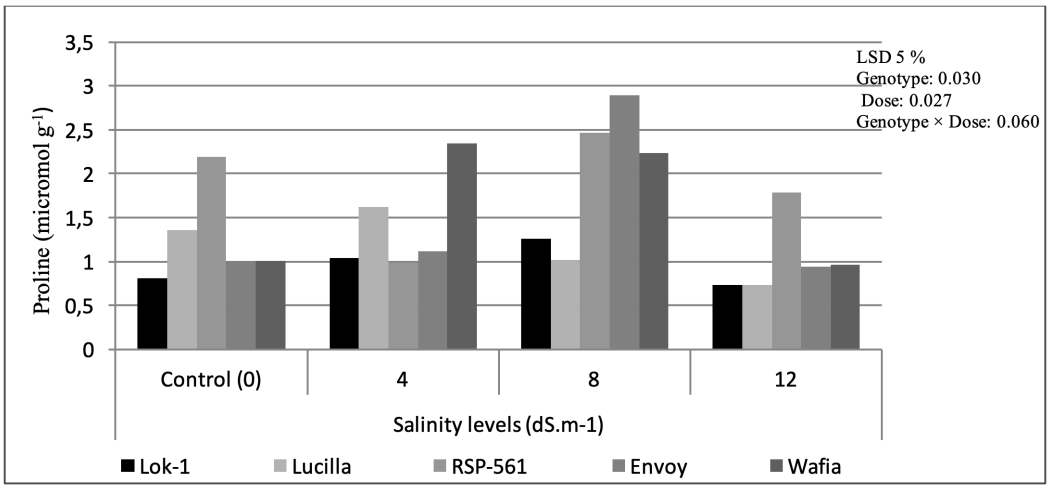

Figure10. Proline (micromol g^{-1}) of bread wheat genotypes grown at different salinity levels

Chlorophyll *a* (mg g^{-1})

The results revealed that salinity regimes reduced chlorophyll *a* of all measured wheat cultivars. However, these had varied responses to the increased levels of salinity (Figure 11). The maximum chlorophyll *a* was documented under control condition, whereas the minimum value was determined at 8 and 12 dSm^{-1}. Among wheat cultivars, 'Lok-1' had the maximum chlorophyll- while 'RSP-561' and 'Wafia' varieties at 12 dSm^{-1} salt concentration recorded the minimum values. The photosynthetic rates were drastically reduced under salt stress resulting in significant reduction of seedling dry weight (Netondo et al., 2004). The contrasting findings were also reported where six genotypes of rice had higher chlorophyll content under salt stress (Alamgir and Ali, 1999). However, contradictory results inferred that chlorophyll tend to decline significantly under salt stress (Hasan et al., 2015). It was also concluded that cholorophyll content declined under stress owing to membrane instability which led to degradation of pigment along with ceased its synthesis (Ashraf et al., 2005).

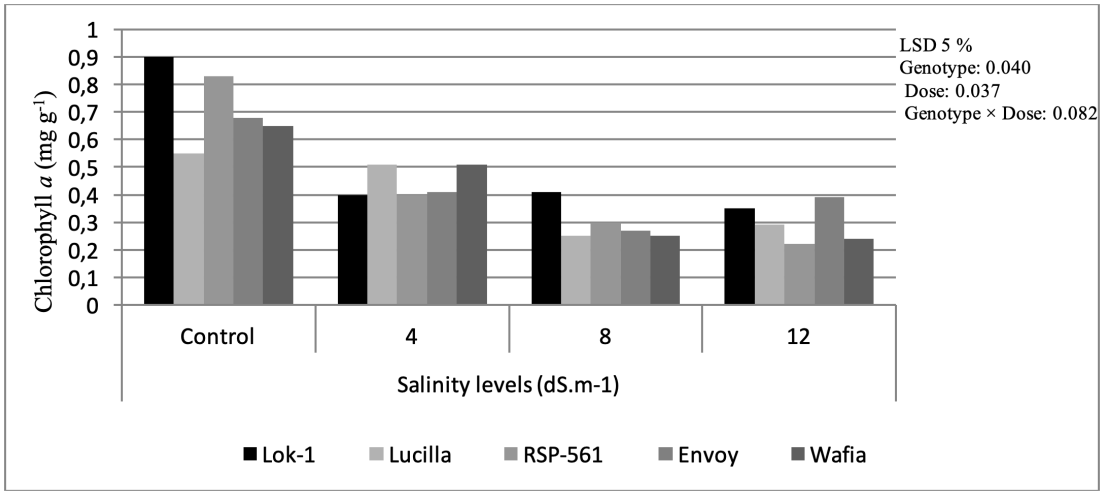

Figure 11. Chlorophyll *a* (mg g^{-1}) of bread wheat genotypes grown at different salinity levels

Chlorophyll *b* (mg g^{-1})

The chlorophyll *b* of wheat varieties was affected significantly by salinity (Figure 12). Statistically, significant differences were found between genotype, salt applications and Genotype × Dose applications for chlorophyll *b*. The highest chlorophyll *b* was determined under control condition,

while the minimum value was determined at 8 and 12 dSm^{-1}. 'Envoy' had the highest chlorophyll *b*, whereas 'Lucilla' and 'Wafia' had the lowest chlorophyll *b*. The highest chlorophyll *b* was recorded in 'Lok-1' cultivar at control, whereas the lowest chlorophyll *b* was recorded in 'Wafia' variety at 8 $dS.m^{-1}$salt dose. It might be inferred that salt ions toxicity ions coupled with physiological water deficit in the flag leaves tend to delay synthesis of chlorophyll along with triggering degradation of previously synthesized chlorophyll (Hasan et al., 2015; Zheng et al., 2008).

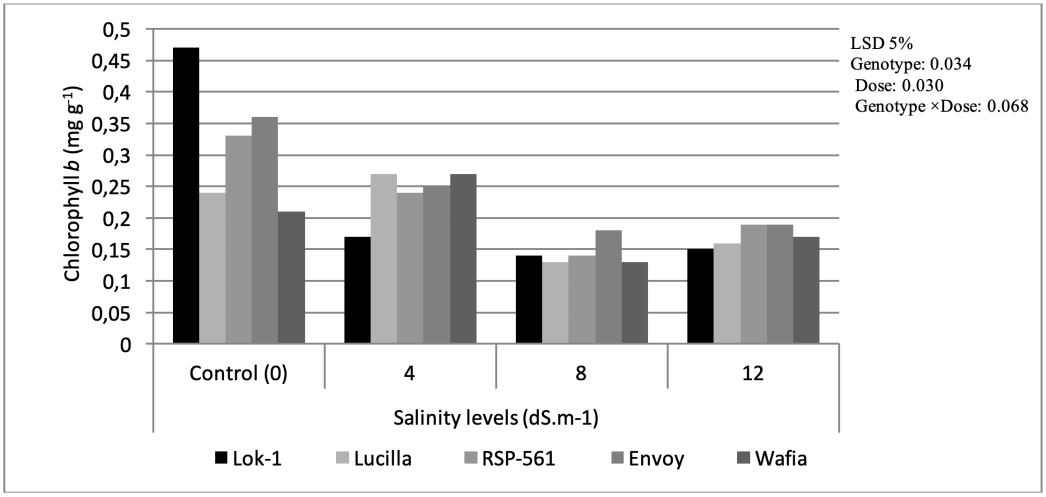

Figure 12. Chlorophyll-b (mg g^{-1}) of bread wheat genotypes grown at different salinity levels

Total chlorophyll (mg g^{-1})

With increasing NaCl concentration, total chlorophyll content decreased sharply of all wheat varieties. Statistically, there were significant differences between the genotype, salt applications and Genotype × Dose for total chlorophyll. The highest total chlorophyll was recorded under control condition, while the minimum value was recorded at 8 $dSm^{-1}$and 12 dSm^{-1}.'Lok-1' had the highest total chlorophyll, whereas 'Lucilla' and 'Wafia' had the lowest total chlorophyll (Figure 13). The highest total chlorophyll was recorded in 'Lok-1' cultivar at control, whereas the lowest total chlorophyll was recorded in 'Wafia' and 'Lucilla' varieties at 8 dSm^{-1} salt dose.

The chloroplastids cell membrane deterioration under salt stress might be responsible for significant reduction in total chlorophyll content leading to lesser photosynthetic efficiency (Seeman et al., 1985; Datta et al., 2009; Ashraf et al. 2005; Iqbal et al. 2006; Khan et al., 2009; Hasan et al., 2015). It has also been suggested that chlorophyll might serve as a key parameter to indicate salt tolerance or sensitivity in crop plants.

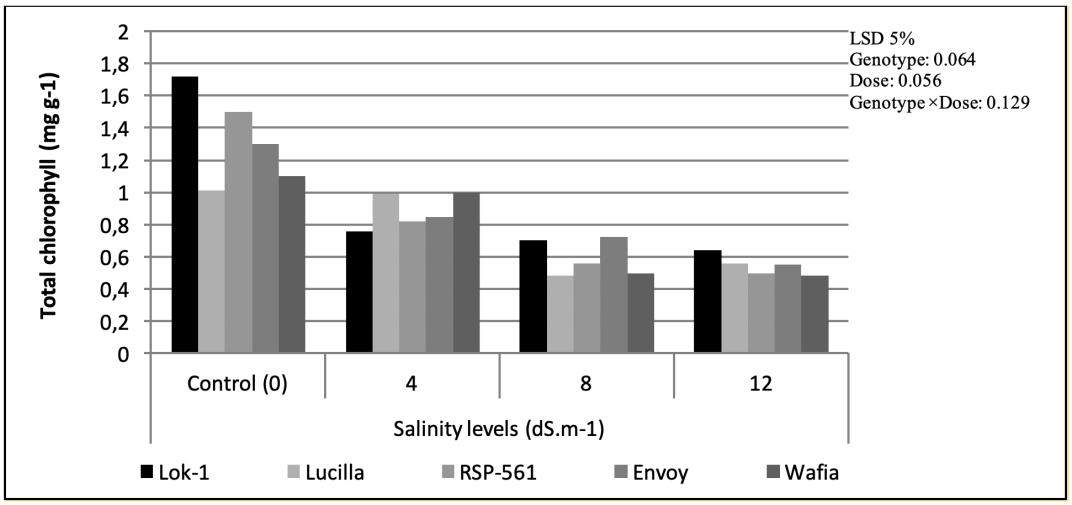

Figure 13. Total chlorophyll (mg g^{-1}) of bread wheat genotypes grown at different salinity levels

Carotenoid (mg g^{-1})

It was observed that a significant variation in carotenoid concentrationresulted in all wheat varieties under varying salinity regimes (Figure 14). The highest carotenoid was recorded under control condition, while the minimum value was recorded at 8 and 12 dSm^{-1}.'Lok-1'had the highest carotenoid, whereas 'Lucilla' had the lowest carotenoid. The highest carotenoid was recorded in 'Lok-1' cultivar at control,

whereas the lowest carotenoid was recorded in 'RSP-561' variety at 12 dSm^{-1} salt dose. These findings are in agreement to previous researches which inferred that salt stress altered and interfered with the vital morphological developments, physiological processes as well as metabolic activities within crop plants (Rhoades, 1993).

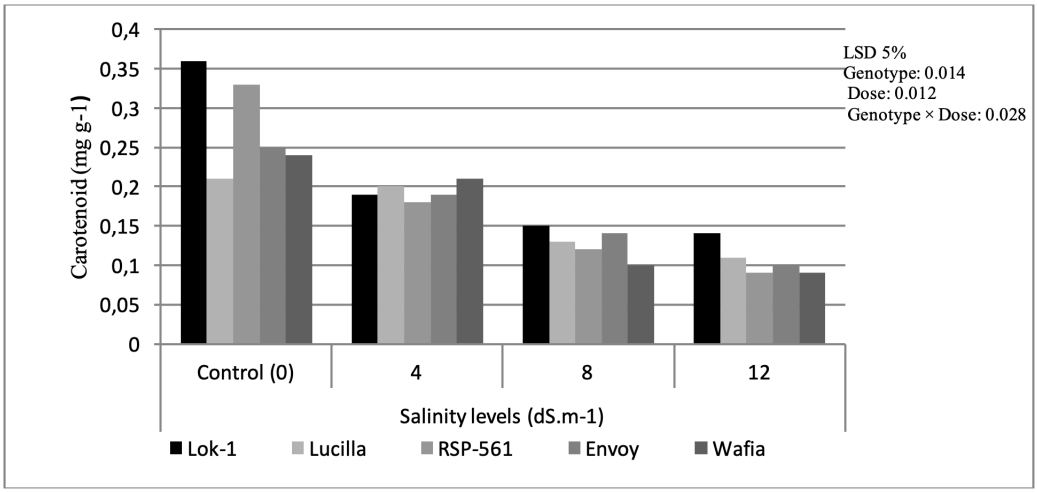

Figure 14. Carotenoid (mg g^{-1}) of bread wheat genotypes grown at different salinity levels

Conclusion

Result of current study indicated that wheat cultivars 'Lucilla' and 'Envoy' performed better based on seedling traits, while 'Envoy' and 'RSP-561' remained superior for biochemical characteristics under salt stress condtions. Accordingly, Envoy might be suggested as planting material to conduct further indepth investigations on drastic impacts imparted by salinity on growth and biochemical processes taking place at different phonological phases of crop plants. In addition, findings of this study might be used as reference point to conduct further field investigations in order to understand physiological and biochemical changes during vegetative and reproductive developmental processes of wheat under varying salinity regimes.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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Research Article

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Callus induction and antibacterial activity of various explants of *Indigofera zollingeriana*

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Abstract

The study explore antibacterial potential of *Indigofera zollingeriana,* a popular medicinal and forage plant of the tropics. Methanol extracts of calli from hypocotyl, cotyledon and leaf excised from *in vitro*-grown plants using different plant growth regulator and their combinations had significant reaction against Gram-positive *Staphylococcus aureus* ATTCC25923 and Gram-negative *Pseudomonas aeruginosa* ATCC25823 bacteria. The best antibacterial activity was noted on the callus extracts of hypocotyl explants with significant inhibition zones on MS medium containing 2 mg/L BAP + 0.1 mg/L NAA. The *P. aeruginosa* had maximum inhibition on 0.5 mg/L BAP + 0.1 mg/L NAA. This antibacterial activity (by the hypocotyl induced calli extracts) was higher compared to the antibacterial activity noted from the extracts of other two explants and non treated control treatments. It was concluded that antibacterial activities were affected by explants source and plant growth regulators. The extracts from *in vitro* induced callus from hypocotyl, cotyledon and leaf explants of *I.zollingeriana* could be used variably and effectively against both type of bacteria used in this study.

Keywords: Callus, Extract, Legume, Medicinical, Phytohormone

Introduction

A huge quantity of antibiotics are being introduced into pharmaceutical industry since last 40-50 years. Inspite all efforts to introduce new drugs, there are number of pouring reports that exhibit pogressive increased antibiotic resistance to the drugs due to natural genetic ability of bacteria to acquire increased resistance (Cohen, 1992). This has lead to the development of increased hospital deaths among people due to development of new types of infections (Nascimento et al., 2000).

A review of literature from 1970s to present times show a large number of researchers, who have reported increased incidence of resistant bacteria all over world (Jansen et al., 1987; Inouye et al., 2001; Chouhan et al., 2017). In view of the present circumstances, the outlook for use of these drugs is obscure. Therefore, there is need to carry out consistent efforts and research to reduce the problem for safe and effective use of antibiotics and understand the genetic and biological mechanisms that induce resistance in bacteria. The ultimate target for each research is to suggest the most appropriate and yielding new antibacterial drugs for the benefit of the people (Jansen et al., 1987; Chouhan et al., 2017).

Indonesia is home to more than 10 percent of the world's known plant species. The genus *Indigofera* among them, comprise around 750 species distributed in tropical regions (Bakasso et al., 2008). *Indigofera zollingeriana* is multiple use crop plant, used for extraction of natural blue dye, as forage crop and as a medicinal plant.

It is believed that *I. zollingeriana* exhibits antibacterial characteristics as they are widely used in folk medicines for

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treatment of many health disorders. However, there is no study that reports antibacterial activity/ies of *I. zollingeriana* showing it as a potential phytochemical agent against wide range of bacteria and microrganisms. Discovery of new antibacterial compounds or drug is always desirable.

Antioxidant and antibacterial studies have been carried out in many legume plant species including *Phyllanthus emblica* (Singh and Sharma, 2015), *Ziziphus mauritiana* Lam. (Annathurai et al., 2015), *Alkanna orientalis*'s roots (Petrosyan et al., 2015), *Daucus carota*'s root, stem and petiole explants (Arafa et al., 2016), *Salvia corrugata*'s leaf and stem explants (Bisio et al., 2016), *Pterocarpus santalinu*'s *leaf explants* extracts (Ashrafee et al., 2014), *Trigonella foenum-graecum*'s cotyledons and hypocotyls explants extracts etc. from the callus extracts (ElNour et al., 2015).

Except *I. zollingeriana*, antibacterial studies have been reported from direct leaves extracts of *I. dendroides, I. oblongifoli, I. suffruticosa* and *I. tinctoria* (Dahot, 1999; Esimone et al., 1999; Leite et al., 2006; Ranukadevi and Suhani Sultana, 2011; Santos et al., 2015), crude extract of *I. gerardiana* and *I. trita* (Nisar et al., 2009; Kumar et al., 2013), root extract of *I. lupatana* and *I. aspalathoides* (Ngoci et al., 2012; Rajaperumal et al., 2013), leaves and root extract of *I. glandulosa* (Prabakaran et al., 2011) and leaves and callus leaf extracts under *in vitro* cultures on *I. tinctoria* (Jisha and Benjamin, 2009). These researchers found either negative or positive expressions in response to treatment with extracts obtained from the explants using different solvents.

Therefore, this study aimed to find the antibacterial potential of *Staphylococcus aureus* ATTCC25923 Gram-positive and *Pseudomonas aeruginosa* ATCC25823 Gram-negative using respective callus methanol extracts of hypocotyl, cotyledon and leaf explants obtained after culturing them on 10 combinations of BAP + NAA through disc diffusion method.

Material and Methods

The seeds were collected from the Department of Nutrition Sciences and Feed Technology, Bogor Agricultural University, Indonesia.

Preparation of Plant Material

Germination and Micropopagation Studies

The seeds were treated with sandpaper, ensued by washing with 98% H_2SO_4 for 5 minutes. Subsequently, they were rinsed 3×5 minutes with autoclaved distilled water to remove the traces of H_2SO_4. The sterilized seeds were transferred to erlenmeyer flasks containing liquid 0.1 mg/L of GA_3 that was shaked at 120 rpm, at 24°C in the dark using a horizontal shaker. The 4 days old germinated seedlimgs were transferred to agar solidified sterile MS medium (Murashige and Skoog, 1962), pH 5.6-5.8 for 30 days to grow and develop of seedlings to appropriate stage. Thereafter, hypocotyl, cotyledon, leaf and explants were obtained from these seedlings and cultured on 10 different combinations of plant growth regulator (PGR) as BAP + NAA to induce callus on the respective explants for two months. These were dried and powdered to obtain respective methanol extractsand test their antibacterial activities against *Staphylococcus aureus* ATTCC25923 Gram-positive and *Pseudomonas aeruginosa* ATCC25823 Gram-negative bacteria.

The antibacterial tests were performed using agar-well diffusion assay (Perez et al., 1990). The agar plates were prepared using sterile Mueller-Hinton (MH) medium + 0.65 g/l agar. Each plate was inoculated with 100 µL of the bacterial strains with OD600= 0.8 followed by their even spread onto the surface of the agar plates. The 20 µL of each methanol extract (OD600 = 0.5) were added to the discs (5 mm diameter) from the cultured bacterial strains. The plates with bacterial strain and methanol extracts were incubated at 37°C for 24 h.

The liquid extracts obtained from the respective explants obtained after pretreatment with liquid GA_3 were used as control treatment 1, 2, 3, 4, 5 and 6. The Erythromycin (15µg), Gentamycin (30 µg), Penicillin (1IU) and Chloramphenicol (10 µg) containing discs were used as control treatment 7, 8, 9 and 10 respectively.

Determination of Antibacterial Activities

The inhibition of bacterial growth was indicated by a clear zone around the discs that was measured and expressed as an average diameter of the inhibition zone.

Antimicrobial Index

Antimicrobial index of extracts were calculated as formula below (Ghasemi et al., 2003).

Antimicrobial Index = (Extract inhibition zone/ Antibiotic inhibition zone) × 100

Statistical Analysis

The average fresh and dry callus weight and average inhibition zones induced due to methanol extracts of hypocotyl, cotyledon and leaf were measured in triplicate. Callus weight data was analyzed by comparing means using IBM SPSS 24 program for Windows. The significant differences among the means were determined by Duncan's Multiple Range Test.

Results and Discussion

Induction of Callus Fresh and Dry Weight

Fresh Weight

Compact and friable soft calli were induced on all hypocotyl, cotyledon and leaf explants (100%) irrespective of the BAP + NAA treatments excluding control treatments (Table 1). Callus fresh weights ranged 3.39-6.88 g on hypocotyl, 1.18-3.83 g on cotyledon and 1.23-4.91 g on leaf explants. The maximum fresh weight of 6.88 g, 3.83 g and 4.91 g of hypocotyl, cotyledon and leaf based calli was noted on MS medium containing 1.0 mg/L BAP + 0.10 mg/L NAA (Fig. 1a), 2.5 mg/L BAP + 0.10 mg/L NAA (Fig.1b) and 2.5 mg/L BAP + 0.15 mg/L NAA (Fig.1c) in the same order. Inhibited shoots and roots were also observed on callus induced on hypocotyl explants on all explants that were cultured on 1.0 mg/L BAP + any concentration of NAA. No callus was induced on hypocotyls, cotyledon and leaf explants of control 1, 2 and 3 treatments. Therefore, the extracts of the respective explants were obtained directly from the growing seedlings that were not treated with any concentrations of BAP+NAA.

Dry Weight

Callus dry weights ranged 0.24-0.42 g on hypocotyl, 0.11-0.27 g on cotyledon and 0.13-0.32 g on leaf explants.

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The maximum dry callus weight of 0.42 g, 0.27 g and 0.32 g was induced on each of hypocotyl, cotyledon and leaf based explants on MS medium containing 1.0 mg/L BAP + 0.10 mg/L NAA, 1.0 mg/L BAP + 0.15 mg/L NAA and 2.5 mg/L BAP + 0.10 mg/L NAA in the same order and 2.0 mg/L BAP + 0.15 mg/L NAA, respectively (Table 1).

Table 1. Effects of MS medium containing different concentrations of BAP + NAA on callus induction of fresh and dry weight of *I. zollingeriana* under *in vitro* conditions after two months of culture

Medium		Fresh weight (g)			Dry weight (g)		
BAP (mg/L)	NAA (mg/L)	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf
0.5	0.10	3.39dA	1.18dB	1.25eB	0.26cA	0.11cB	0.13cB
0.5	0.15	4.42cA	1.59dC	2.88dB	0.27cA	0.13cC	0.21bB
1.0	0.10	6.88aA	1.54dB	1.68eC	0.42aA	0.15cB	0.15cB
1.0	0.15	5.16bA	3.56aB	3.96bB	0.25cA	0.27aA	0.25bA
1.5	0.10	4.61cA	2.00cC	2.42dB	0.27cA	0.18cB	0.20bB
1.5	0.15	5.33bA	2.71cB	1.86eB	0.24cA	0.19cB	0.17cB
2.0	0.10	5.82abA	1.03dB	1.23eB	0.35bA	0.10cB	0.13cB
2.0	0.15	5.48bA	3.01bC	4.45aB	0.28cA	0.22bB	0.32aA
2.5	0.10	4.35cA	3.83aB	3.42cB	0.25cA	0.27aA	0.24bA
2.5	0.15	5.73bA	3.19bC	4.91aB	0.30bcA	0.21bB	0.31aA
Control treatments		1	2	3	1	2	3
		0.00	0.00	0.00	0.00	0.00	0.00

[1] Means not followed by the same small letter within a column differ significantly at $p < 0.05$ using Duncan test
[1] Means not followed by the same capital letter within a row differ significantly at $p < 0.05$ using LSD test.

In this case, the best results of fresh and dry callus weight were obtained from hypocotyl explants on MS medium using various combinations and concentrations of plant growth regulators. The results are in agreement with Soorni and Kahrizi (2015), who indicated that adding different plant growth regulators and their concentrations might support variable callus growth leading to variable fresh weight from different explants. This could be related to accumulation of antimicrobial (antibacterial) compounds that in turn activate diffrent responses to bacterial defence mechanisms. Furthermore Sari et al. (2018) showed that concentrations of plant growth regulators influence rapid cell division and stimulate cell division and enlargement of *Myrmecodia tuberosa*'s explants correlated to callus size and protein synthesis.

It is established that playing with the ratio and concentrations of plant gowth reulators, sugars, growth medium, culture environment affects the activity and induction of secondary metabolites in the regenerating explants/callus positively or negatively and accumulation of metablic compounds from them during *in vitro* cultures (Skoog and Miller 1957; Basra, 2000; Spartz and Gray, 2008; Saikia et al., 2013; Murthy et al., 2014). In addition, taking into account the obtained results, it can be stated that the composition of PGRs in the growth medium is important to optimize both extracts and callus induction. These conditions must be optimised to positively increase the biosynthesis of alkaloids and plant metabolites (Verpoorte et al., 1991).

Callus, shoot and root induction was noted on hypocotyl explants (Figure 1.). Other studies conducted by Shrikhande et al. (1993) and Vaezi et al. (2015) also approve influence of BAP and NAA treatments also lead to callusing and rhizogenesis in species like *Azadirachta indica* and *Trigonella foenum-graecum* L respectively. There were no shoot induction and root induction on callus derived from cotyledon and leaf explants of *I. zollingeriana* in agreement with Vaezi et al. (2015), who reported no regeneration on callus derived cotyledon explants of *Trigonella foenum-graecum* L cultured on MS medium supplemented with BAP and NAA. It was implied that the different types of explants with or without organ regeneration had been influenced by auxin and cytokinin ratio in the cultures medium (Skoog and Miller, 1957; Iwase et al., 2011; Vaezi et al., 2015).

Antibacterial Activity Study

Antibacterial activity against *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823 bacteria on methanol extracts of hypocotyl, cotyledon and leaf induced calli were variably positive.

***S. aureus* ATTCC25923**

The methanol extracts obtained from hypocotyl induced calli on combinations of BAP + NAA presented the highest antibacterial activity against *S. aureus* ATTCC25923 with inhibition zones in range of 0.9-2.3 cm (Table 2). The antibacterial activity or inhibition zones of the methanol extracts of calli increased when 0.5 or 1.0 mg/L BAP + 0.15 mg/L NAA (two combinations) was used. However, at increased concentrations of 1.5, 2.0 and 2.5 mg/L BAP + 0.15 mg/L NAA (three combinations), the inhibition zones were smaller compared to the inhibition zones noted on methanol

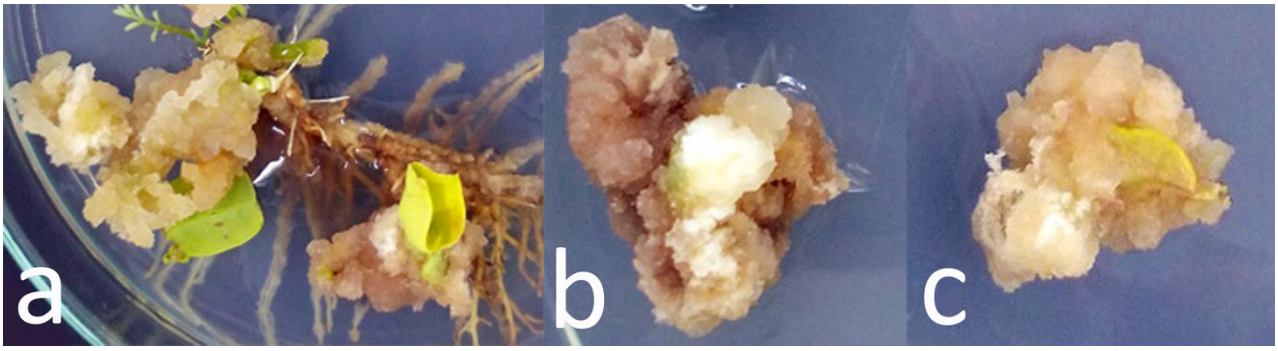

Figure 1. Callus induction on different explants of *Indigofera zollingeriana* (a) hypocotyl with roots on MS medium containing 1 mg/L BAP + 0.10 mg/L NAA (b) cotyledon node on MS medium containing 2.5 mg/L BAP + 0.10 mg/L NAA (c) leaves on MS medium containing 2.5 mg/L BAP + 0.15 mg/L NAA

extracts of 1.5, 2.0 and 2.5 mg/L BAP + 0.10 mg/L NAA (three combinations) induced methanol extracts of hypocotyl based calli. An inhibition zones (1.2 cm) of methanol extracts was obtained from hypocotyl from plants germinated on 0.1 mg/L GA_3 (control treatment No. 1).

The methanol extracts of cotyledon based calli induced on 0.5-1.0 mg/L BAP + 0.10-0.15 mg/L NAA and 1.5 mg/L BAP + 0.10 mg/L NAA did not show any antibacterial activity. However, the methanol extracts of the calli induced on 1.5 mg/L BAP + 0.15 mg/L NAA and 2.0-2.5 mg/L BAP + 0.10-0.15 mg/L NAA induced cotyledon based calli showed antibacterial activity with inhibition zones of 0.8-1.2 cm. The inhibition zones of methanol extracts obtained from cotyledon explants of plant germinated on 0.1 mg/L GA_3 remained 1.0 cm.

In case of leaf based calli, antibacterial activity ranged 1.2-1.5 cm. Antibacterial activities was noted on the methanol extracts noted on leaf based calli induced on 0.5-1.0 mg/L BAP + 0.1 mg/L NAA (two combinations), and 1.5 mg/L BAP + 0.15 mg/L NAA induced leaf based calli. The largest inhibition zone was noted on using methanol extracts obtained from 0.5 mg/L BAP + 0.1 mg/L NAA induced leaf based calli. The inhibition zones of the methanol extracts obtained from leaf explants of plants germinated on 0.1 mg/L GA_3 remained 1.0 cm.

Inhibition zones of 2.1, 1.5, 0.8 and 2.5 cm were noted when the antibiotics Erythromycin, Gentamycin, Penicillin and Chloramphenicol were used in the same order against *S. aureus* ATTCC25923 in control 7, 8, 9 and 10 treatments (Table 3).

***P. aeruginosa* ATCC25823**

The methanol extracts obtained from hypocotyl based calli induced on 0.5 mg/L BAP + 0.10 mg/L NAA presented the highest antibacterial activity against *P. aeruginosa* ATCC 25823 with inhibition zones in range of 2.4 cm (Table 2). It was followed by 1.5 cm long inhibition zones induced on methanol extracts obtained from hypocotyl based calli induced on 2.0 mg/L BAP + 0.10 mg/L NAA. The methanol extracts with 0.1 mg/L GA_3 pretreated plants used as control 4 remained 1.3 cm. The methanol extracts of rest of the treatments failed to induce any inhibition zone.

The methanol extracts obtained from cotyledon based calli induced on 0.5 mg/L BAP + 0.10 mg/L NAA presented the highest antibacterial activity against *P. aeruginosa* ATCC25823 with inhibition zones of 1.2 cm (Table 2). It was followed by 1.1 cm long inhibition zones induced on methanol extracts obtained from cotyledons germinated on 0.1 mg/L GA_3 pretreated plants used as control 5. Methanol extracts of the rest of the treatments failed to induce any inhibition zone.

No inihibition zone was noted on methanol extracts leaf based calli induced on any combination of 10 different BAP+NAA treatment or control treatment. Inhibition zones of 1.9, 1.7, 1.0 and 2.6 cm were noted, when the antibiotics Erythromycin, Gentamycin, Penicillin and Chloramphenicol (Control 7, 8, 9 and 10) were used in the same order against *P. aeruginosa* ATCC25823 (Table 3).

The antibacterial activities both Gram-positive and Gram-negative bacteria against extracts of of hypocotyl explants were comparatively higher compared to the control treatment or the extracts obtained from other explants. Similarly, Ravinder Singh (2011) reported higher inhibitory activities on leaf based callus extracts of *Premna serratifolia* compared to the extracts of leaves growing under natural conditions. Staba (1980) has also reported that rate of cell multiplication and cell division influenced increasing secondary metabolites production. There was no antibacterial activity againts both Gram-positive bacteria except two combination of PGR and Gram-negative bacteria on callus leaf extracts in agrement with Jisha and Benjamin (2009), who studied antibacterial activity on leaves and callus leaf extract of *I. tinctoria*. They reported that callus leaf extract showed no inhibiton zones on all bacterial treatments, while antibacterial activities were noted from leaves extracts.

The study conducted by Jahan et al. (2013) who reported that use of leaf exctract of *in vitro* raised plant on *Tylophora indica* had higher inhibition zone of *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823 than leaf callus extracts, as leaf extract of parent plant was low compared to previous mentioned extracts due to nutritional and hormonal manipulation added to culture medium.

It might be due to secondary metabolite accumulation of callus responsed against antibacterial or human pathogens influenced by plant growth regulator in agreement with Goyal et al. (2008). Jain et al. (2012), who noted that the medium with suitable concentration of individual or combination of auxin and cytokinin controls both callus growth and secondary metabolite production in *in vitro* cultures. The

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Table 2. Antibacterial activities of callus based methanol extracts obtained from hypocotyl, cotyledon, leaf explants of *I. zollingeriana* against *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823

Treatments to to induce calli on explants taken from plants germinated on 0.1 mg/L GA_3 *		Inhibition zone (cm) formed against *S. aureus* ATTCC25923 by callus extracts of			Inhibition zone (cm) formed against *P. aeruginosa* ATCC25823 by callus extracts of		
BAP (mg/L)	NAA (mg/L)	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf
0.5	0.10	1.6	0.0	1.5	2.4	1.2	0.0
0.5	0.15	2.0	0.0	0.0	0.0	0.0	0.0
1.0	0.10	1.8	0.0	1.3	0.0	0.0	0.0
1.0	0.15	2.2	0.0	0.0	0.0	0.0	0.0
1.5	0.10	2.1	0.0	0.0	0.0	0.0	0.0
1.5	0.15	1.3	1.0	1.2	0.0	0.0	0.0
2.0	0.10	2.3	0.8	0.0	1.5	0.0	0.0
2.0	0.15	2.0	1.2	0.0	0.0	0.0	0.0
2.5	0.10	1.5	1.0	0.0	0.0	0.0	0.0
2.5	0.15	0.9	0.9	0.0	0.0	0.0	0.0
Methanol extracts of pretreated Control treatments plants with 0.1 mg/L GA_3**		**1**	**2**	**3**	**4**	**5**	**6**
		1.2	1.0	1.0	1.3	1.1	0.1

**Liquid extracts obtained after pretreatment with liquid GA_3 followed by culture on MS medium for 30 days and post treatment with 10 combinations of BAP+NAA followed by bacterial culture on agar solidified Mueller-Hinton (MH) medium*

*** Liquid extracts obtained after pretreatment with liquid GA_3 followed by culture on MS medium for 30 days and no post treatment followed by bacterial culture on agar solidified Mueller-Hinton (MH) medium*

Table 3. Antibacterial activities of erythromycin, gentamycin, penicillin and chloramphenicol against *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823

Control treatment	Inhibition zone (cm) formed against S. aureus ATTCC25923	Inhibition zone (cm) formed against P. aeruginosa ATCC25823	Antimicrobial index (%)
7- Erythromycin	2.1	1.9	100
8- Gentamycin	1.5	1.7	100
9- Penicillin	0.8	1.0	100
10-Chloramphenicol	2.5	2.6	92-88

results of this study are fully supported by literature and it is confirmed that the plant extracts are effective against Gram-positive and Gram-negative bacteria. However, the extracts from different explants under the influence of variable PGRs' combinations have variable capability to kill the two type of bacteria (Mohajer et al. 2012). In addition, Soorni and Kahrizi (2015) reported higher amounts of secondary metabolites sometimes could be produced by callus cultures excised from plant tissues. These results also indicated the significance and superiority of *I. zollingeriana* hypocotyl plant extracts with induction of variable inhibition zones formed against *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823.

Different inhibition zones on various explants could vary depending on the source of explant and the characteristics of extraction solvents. Johnson and Babu (2010) has observed that some extraction solvents may act as potential inhibitors to both Gram-positive and Gram-negative bacteria. Praveen and Nair (2014) confirm that the increased concentration of extraction solvents act linearly with the increased antibacterial activity of *Myxopyrum smilacifolium* Blume. Other studies also show that the different antibacterial potentials of the callus and leaf methanol extracts on *Melaleuca alternifolia* could be due to changing composition of the bioactive compounds in part of plant used as source. This could improve and reduce ability of the solvent extracts to extract in their antibacterial activities (Jeyakani Santosh and Rajalaksmi, 2016). The results of the present study are in line with these studies reported earlier.

The results of this study were very encouraging and and showed the presence of biological active compounds in methanolic extracts of *I. zollengriana.* The antibacterial activity of the tested extracts of *I. zolingeriana* showed significant reduction in bacterial growth in terms of zone of inhibition in relation to the type of explant and treatments. All callus formation seemed to be dependent to explant and phytohormone concentrations with related antibacterial activity in agreement with Frank et al. (2000). Maximum antibacterial activities in terms of inhibition zones was noted using extracts from callus induced on hypocotyls. Varied but very similar inhibition zones were noted on the extracts obtained from calli of cotyledon and leaf origin. The antibacterial activities showed that callus extracts had higher variability inhibition zone than extracts obtained from the control treatments of

respective explants. This confirms that their occured chemical changes in the explants under the influence of hormones that influenced their antibacterial activities. Control treatments 1, 2, 3, 4, 5, 6 had minimum or poor inhibition zones. Inhibition zones due to Erythromycin, Gentamycin, Penicillin, and Chloramphenicol (control treatment 7, 8, 9, and 10) were comparable to the inhibition zones from the extracts of the 3 explants in agreement with (Borgatti,1998). According to the antimicrobial index, highest inhibition zones of hypocotyl extracts on both Gram-negative and Gram-positive bacteria (Table 2) showed 100% activity compared to Erythromycin, Gentamycin and Penicillin, when Chloramphenicol showed 88-92% activity, respectively (Table 3). It could be determined that the mentioned results had high antibacterial activity againts compared to Erythromycin, Gentamycin, Penicillin and Chloramphenicol.

There are many reports on the production of secondary metabolites and phenolic acids and antibacterial in many plant species including the plants belonging to the genus Indigofera; however, there are no reports about antibacterial activities of the extracts of *I. zollingeriana* cells or organ culture. Esimone et al. (1999), Ngoci et al. (2012) and Santos et al. (2015) have also reported antibacterial activities leaf, root extracts of *I. suffruticosa, I. lupatana, I. dendroides* against Gram-positive bacteria (*S. aureus*, *P. aeruginosa, Bacillus subtilis*) and Gram-negative bacteria (*K. pneumoniae, E. coli*).

Previous study by Christie et al. (1969) showing naturally occurring amino acid indospicine that is also present in aqueous extracts of *I. linnaei* (Hoffman and Gallaher 2007; Medeiros et al., 2011) as a natural toxin of the plant (Terras et al., 1995). Similar toxic peptide or protein fractions have been reported in aquous extracts of leaves of of *I. oblongifolia* by Dahot (1999) and change variably under the influence of PGRs. Contrarily, Leite et al. (2002) presume that biological activity of aqueous leaf extracts of *I. suffruticosa* could be due to the presence of lectins. The results indicated that *I. zollengriana* extracts in this study also have significant but variable antibacterial activity against both Gram-positive and Gram-negative bacteria depending on the type of explant used.

Conclusions

The results of this study signified that antibacterial activity had affected explants source (type of tissue or organ), extracts source and use of plant growth regulator. The methanol callus extracts of hypocotyl explant of *I. zollingeriana* have great potential as antibacterial compounds against *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823 and can be used in the treatment of infectious diseases due to these bacteria. The understanding that *I. zollingeriana* plant cells, tissues, and organs carry distinguished compounds of medicinal importance is very important. In conclusion, further and more specific research is needed to establish and determine secondary metabolites as well as accumulation of antibacterial compounds with phytochemical screening on these and other callus extracts of this plant.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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The relationships between some phenological and morphological properties of chickpea (*Cicer arietinum* L.) and the possibilities of using these properties in selection: The Western Mediterranean Region Model

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Abstract

In this study, the relationships between grain yield and yield characteristics were analyzed by running correlation and linear regression analyses. In the study, the herbal properties of 18 registered chickpea varieties and 3 local chickpea lines were used. The trials were continued for two years in two locations in the transitional zone of the Antalya-Korkuteli-Ulucak village and the coastal conditions of Antalya Aksu in the western Mediterranean region. According to the results of correlation and regression analysis, significant correlations were found between the yield and the number of the days to 50% emergence ($r = -0.6707$ and $p<0.01$), the number of days to 50% flowering ($r = -0.6446$ and $p<0.01$), number of days to maturity ($r = -0.7303$ and $p<0.01$), plant height ($r = 0.4304$ and $p<0.01$), first pod height ($r = 0.5990$ and $p<0.01$), number of pods per plant ($r = -0.1681$ and $p<0.05$) and the number of seeds per pod ($r = 0.2696$ and $p<0.01$). Although the data obtained as a result of the regression analysis did not exactly match the data determined, it was determined that close or average values could be reached, which may be beneficial for breeding activities.

Keywords: Correlation, Chickpea varieties, Linear regression analysis

Introduction

Due to the narrowness of adaptation boundaries (Şehirali and Özgen, 1988; Singh and Bejiga, 1990; Bozoğlu and Gülümser, 2000; Sayılğan and Kocatürk, 2019), it is more appropriate to carry out breeding activities in the chickpea plant as sub-programs and regionally. In genotype choices with high adaptability in regional breeding studies, the yield is generally emphasized as the final output, and in the existing ecological conditions, the varieties with the highest yield and high stability are preferred. The genetic structure of the plant, the effect of the environment and the effects of cultural processes separately or together have an impact on the yield. These effects make themselves felt in different parts of the plant during the plant development period and directly or indirectly influence the yield. Although this situation brings about a variety of disadvantages, it also provides future-oriented inferences based on predictable results that facilitate breeding. In the observation garden stage of breeding programs initiated using thousands of genotypes, the work schedule is shortened, the workload is reduced, and more efficient outputs can be achieved thanks to the fast, easy-to-diagnose and convenient separation criteria. The phenological and morphological features that are thought to be effective on especially the yield are emphasized in these studies.-

Correlation coefficients, direction and significance level between these plant characteristics were determined. The significance level provides us with some clues as to whether the relationship between variables can be taken into account. In the case of high probability (95-99%) relationships, modeling can be made with the regression analysis to make predictions about

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future situations based on the existing variable data. The effect of the properties, which were found to have high correlation, was determined by conducting the linear regression analysis. Evaluations were made through the model formulas obtained as a result of the regression analysis.

Materials and Methods

In this study, were used eighteen registered varieties and three local populations In the study, plot observation averages of plant properties belonging to two years, two different locations and eighteen registered varieties and three local populations were evaluated. The trials of the study from which the data were obtained were established in the coastal zone of Aksu district of Antalya province and in the transitional zone of Ulucak village of Korkuteli district of Antalya province. Trials were carried out for two years at two locations. In this study, evaluations were made for each property according to the technical instruction principles for chickpea issued by the "Seed Registration and Certification Center", for morphological properties according to plot averages determined over 10 plants, and for phenological values by using 336 observation data per each related to daily averages. Correlation analyses between the plants and regression analyses and relevant tables and graphs were created by using the appropriate statistical analysis package program JUMP.

Results and Discussion

Correlation analysis is a useful method used to determine the relationship, strength and direction between multiple variables. The measurement of the relationship between the two variables is the correlation coefficient (r).

Table 1. The table showing the relationship, strength and direction of plant properties

	NDE	NDF	NDM	PH	FPH	NBRPP	NPOPP	NSEPPO	100SW	HS
NDE										
NDF	0.4679**									
NDM	0.6369**	0.4853**								
PH	-0.4842**	-0.1889*	-0.2248**							
FPH	-0.6484**	-0.3552**	-0.3930**	0.8224**						
NBRPP	-0.0409ns	0.2009*	0.0023 ns	0.3545**	0.2186**					
NPOPP	0.2457**	0.3735**	0.0858 ns	0.0690 ns	-0.1555*	0.6136**				
NSEPPO	-0.4062**	-0.3923**	-0.2427**	0.2935**	0.3613**	-0.1219*	-0.3393**			
100SW	-0.0234 ns	-0.2479**	0.0823 ns	0.1526*	0.0220 ns	-0.0715 ns	0.0402 ns	0.1794*		
HS	0.0327ns	-0.0202 ns	0.0846 ns	-0.0519 ns	-0.0548 ns	-0.0456 ns	-0.1276*	0.1481*	0.0689 ns	
Y	-0.6707**	-0.6446**	-0.7303**	0.4304**	0.5990**	-0.0291	-0.1681*	0.2696**	0.0666	-0.1004 ns

* and ** indicates significance at $p<0.05$ and $p<0.01$, respectively, ns: indicates not significant. NDE: Number of day to 50% emergence (day), NDF: Number of day to 50% flovering (day), NDM: Number of days to maturity (day), PH: Plant height (cm), FPH: First pod height (cm), NBRPP: Number of branches per plant (average), NPOPP: Number of pods per plant (average), NSEPPO: The number of seeds per pod (average), 100SW: 100 seed weight (gr), HS: The humidity of seed at harvest (%), Y: Yield ($kg.ha^{-1}$).

The correlation coefficient is a value between -1 and +1, and the exact state of -1 (negative) and +1 (positive) are the indicators of perfect correlation. There can also be no relationship between the variables (r = 0). In general, in negative correlation, one of the variables increases while the other one decreases. In positive correlation, one of the variables increases while the other one also increases. Correlation analysis results of the properties discussed in this study are given in Table 1.

Although the number of similar studies has decreased in the last two decades, the relationships between yield and some phenological and morphological vegetative properties have been examined in the studies conducted since 1970s. Although there are some studies reporting that the relationship between yield and the number of days to 50% flowering is significant and positive (Ali, 1990) or insignificant (Singh, 1978), there also exist some study results which are similar to the significant and negative relationship we identified in this study (Salih, 1982; Wahid and Ahmed 1999; Yeşilgün, 2006). When the determination process is analyzed in a historical perspective, it can be seen that the results found in this research are similar to the recent research results.

In this research, the number of the studies that reported a significant and positive relationship between the yield and the number of branches per plant, which we determined as insignificant, (Sharma et al., 1970; Mishra, 1973; Gufta et al., 1974; Eser, 1975; Khorgade et al., 1985; Uddin et al., 1990; Özdemir 1996; Khorgade et al., 1988; Khedar & Maloo, 1999; Atta et al., 2008) is higher. Sing (1978) also reported the same

relationship as significant without specifying its direction.

Despite the significant and negative relationship we found between the yield and the number of pods per plant, the number of studies which determined this relationship as significant and positive (Sharma et al., 1970; Joshi, 1972; Mishra, 1973; Dabholkar, 1973; Eser, 1975; Chand et al., 1975; Uddin et al., 1990; Abdali, 1992; Özdemir, 1996; Anlarsal et al., 1999; Khorgade et al., 1988; Wahid and Ahmed 1999; Arshad et al., 2002; Karaköy, 2008) is quite high. Naidu et al. (1987) reported that the same relationship was insignificant.

The significant and positive relationship between yield and the number of seeds per pod we identified in this study is in compliance with many studies conducted in the past (Singh, 1978; Joshi, 1972; Gufta et al., 1974; Dabholkar, 1973; Eser, 1975; Abdali, 1992; Özdemir, 1996; Anlarsal et al., 1999). Khorgade et al. (1988) found that the relationship between yield and the number of seeds per pod is also significant and negative in their study. The relationship between yield and plant height which we determined in our study and in many other studies was significant and positive (Sharma et al., 1970; Work, 1975; Ali, 1990; Özdemir, 1996; Wahid and Ahmed, 1999). However, this relationship was reported to be significant but negative by Mishra (1973).

The significant and negative relationship between yield and maturity is similar to that reported by Berger et al. (2004). The same relationship was reported to be significant and positive in many previous studies (Uddin et al., 1990; Salih, 1982; Ali, 1990; Khorgade et al., 1988; Wahid and Ahmed, 1999; Atta et al., 2008); however, Singh (1978) reported this relationship as insignificant.

Similar to our findings, there are studies that determined the relationship between yield and 100 seeds as insignificant (Eser, 1975; Naidu et al., 1986; Anlarsal et al., 1999). The same relationship was mainly reported as significant and positive in various studies (Sharma et al., 1970; Joshi, 1972; Mishra, 1973; Khorgade et al., 1985; Uddin et al., 1990; Eser et al., 1991; Khorgade et al., 1988; Khedar and Maloo, 1999; Karaköy, 2008; Atta et al., 2008).

The linear regression analysis was performed in order to reveal the properties of the plant which, as a result of correlation analysis, were found to have high probability of relationship by 95-99% with yield (the number days to 50% emergence (days), the number of days to 50% flowering (days), maturity days (days), plant height (cm), first pod height (cm), the number of pods per plant (number), the number of seeds per pods (number), 100 seed weight (gr)) and strengthen the predictions about future conditions (Table 2).

Table 2. Regression control values for the relationship between yield and plant properties

Properties	Properties	R^2	$\underline{R^2}$	MSE	F	p
Y(kg.ha^{-1})	NDE (day)	0.449853	0.448206	78.1341	273.1101	**
Y(kg.ha^{-1})	NDF (day)	0.415487	0.413737	80.75077	237.4159	**
Y(kg.ha^{-1})	NDM (day)	0.533292	0.531894	72.15599	5.2619	**
Y(kg.ha^{-1})	PH (cm)	0.185225	0.182786	95.33852	75.9292	**
Y(kg.ha^{-1})	FPH (cm)	0.358771	0.356851	84.57776	186.8747	**
Y(kg.ha^{-1})	NPOPP (average)	0.028256	0.025346	104.118	9.7118	ns
Y(kg.ha^{-1})	NSEPPO (average)	0.072703	0.069927	101.7089	261.867	**

*and **: indicates significance at $p<0.05$ and $p<0.01$, respectively, ns: indicates not significant. MSE: Means square error

The number of parcel emergence days detected in the trials was completed between 9 and 21 days. There is a negative relationship between yield and the number of days to 50% emergence. It is seen that the efficiency is decreased significantly when emergence is completed in 14 days. This may have stemmed from many factors, from variety characteristics to soil temperature and humidity. However, in any case, it can be expected that extension of the number of days to 50% emergence in the Western Mediterranean will lead to decrease in yield as it will mean that it will take longer than other phases in the vegetation process.

When the expected values to be obtained as a result of the formula were analyzed, the expected yield value for genotypes that complete the number of days to 50% emergence in 9 days was predicted as 2980 kg.ha^{-1}, and for genotypes completing it in 21 days, it was predicted as 1190 kg.ha^{-1}.

Mainly two groups were formed as 9-10.5 days and 11.5-16 days. It is seen that this situation is in parallel with the early completion of the emergence at the coastal location and the later completion in the transitional zone and that as the number of days to %50 emergence increases, the intensity in Figure 1 decreases.

The number of days to 50% flowering ranged from 37 to 68 days (Figure 2). The relationship between yield and the number of days to 50% flowering is negative. As the number of days to 50% flowering increases, the yield decreases. Concentration was formed in three groups between 37-38, 40-52 and 55-64 days. The expected yield value for the number of 50% flowering days is expected to be 2940 kg.ha^{-1} for the genotypes which complete it in 37 days and 1420 kg.ha^{-1} for those which complete it in 55 days. There are considerable differences between the detected and expected values. The varieties giving the highest yield average (Çakır, 2390 kg.ha^{-1}; Çağatay, 2380 kg.ha^{-1}) completed their 50% flowering in 47.7 days and 46.4 days, respectively, whereas the local population with the lowest yield (Aksu B1, 1340 kg.ha^{-1}) completed it in 45 days.

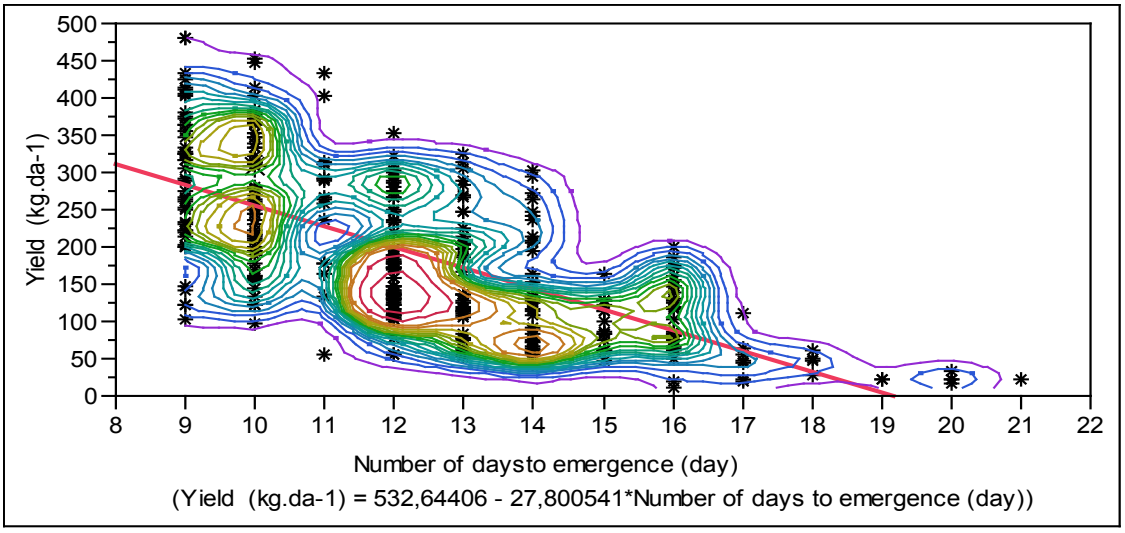

Figure 1. Linear change that occurs in yield based on the number of days to 50% emergence, its density and formula

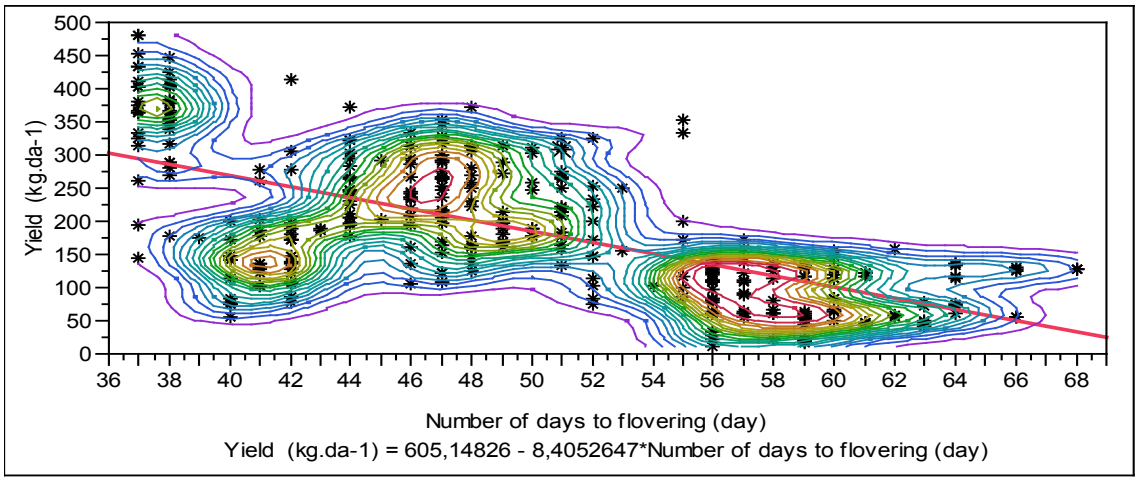

Figure 2. Linear change that occurs in yield based on the number of days to 50% flowering, its density and formula

Although the yield values are different, it can be said that the yield decreases as the number of days to 50% flowering is prolonged according to the data obtained with the prediction formula.

The number of days to maturity ranged from 77 to 119 days (Figure 3). It can be said that the relationship between yield and maturity is more linear. The maturity period affects the yield up to day 89, the efficiency decreases as it is prolonged, and the yield decreases sharply over 93-108 days. The expected yield value for genotypes reaching maturity in 77 days was 3150 kg.ha^{-1}, and it was estimated that there was a risk of getting no yield for the genotypes which matured in longer than 105 days (940 kg.ha^{-1}).

Plant heights ranged from 21.5 to 84.5 cm (Figure 4). The relationship between yield and plant height is positive. In other words, it can be said that as the plant height increases in the region, the yield increases. However, the main factor preventing the yield is the high temperature effects in the region which the plant is exposed to during the maturity process. Many genotypes dried up before completing the vegetation period. For all genotypes, the fact that they were at their highest level at this stage of development or completion of development can be considered as the reason for this situation. However, the main concentration occurred between 38 and 58 cm plant heights and around the yield between 100 and 3500 kg.ha^{-1}. The expected yield value for 21.5 cm. is 630 kg.ha^{-1} and for 84.5 cm it is 2760 kg.ha^{-1}.

The first pod height ranged from 10 to 52.3 cm (Figure 5). The relationship between yield and the first pod height is positive. A continuous increase in yield is observed up to the first pod height of 39 cm. All genotypes with the first pod height of 25 cm and above yielded 1000 kg.ha^{-1} and above. The main genotype density is 10 to 28 cm of the first pod height. The genotypes of high yield with the first pod height of 28 cm (2200 kg.ha^{-1}) and 39 cm (3100 kg.ha^{-1}) were observed to be dense. The expected estimated yield value for the first pod height of 10 cm is 750 kg.ha^{-1}, and for the first pod height of 52.3 cm, it is 4110 kg.ha^{-1}. Here, the relationship between the first pod height and yield may be valid for the genotypes that have completed their physiological development, because it was observed that the yields of the genotypes drying early, which could not complete the maturity period even though the height of the first pod was high, were quite low.

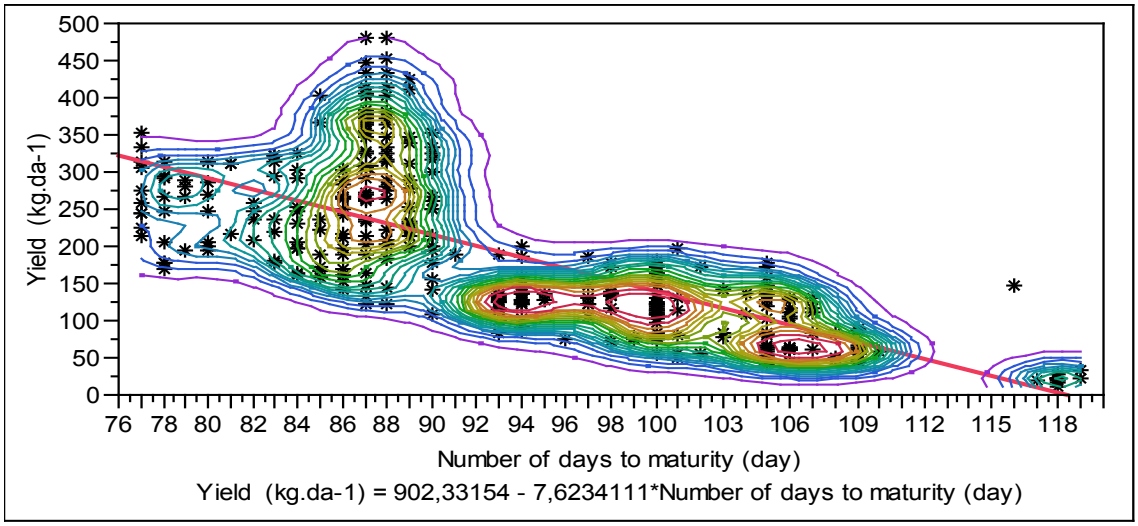

Figure 3. Linear change in the yield based on the number of days to maturity, its density and formula

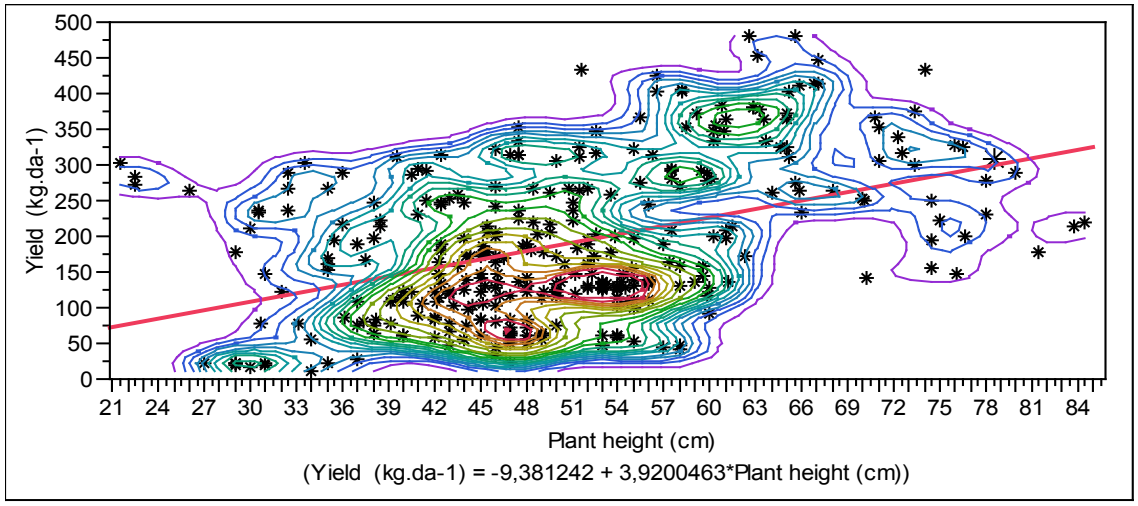

Figure 4. Linear change occurring in yield based on plant height, its density and formula

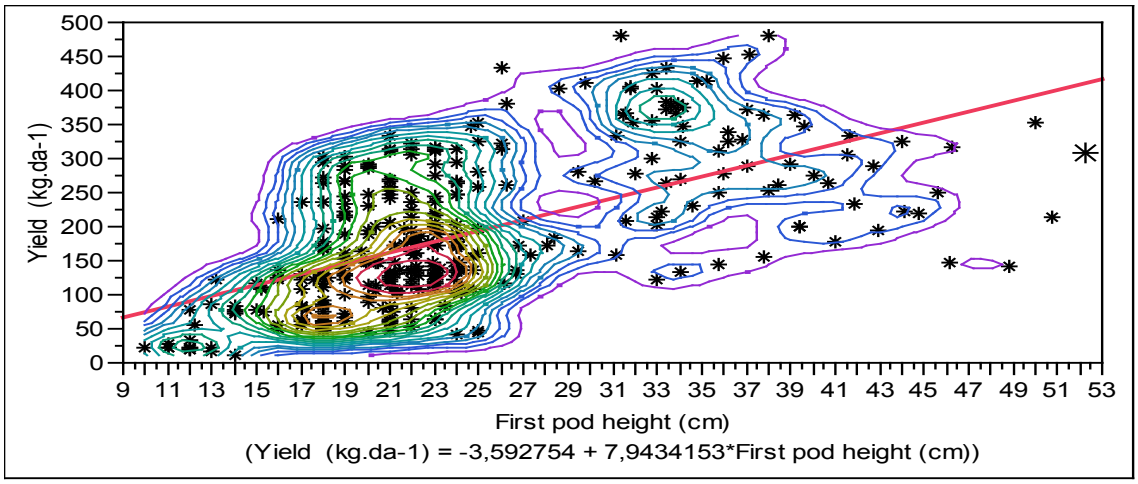

Figure 5. Linear change occurring in the yield depending on the first pod height, its density and formula

The correlation ($p<0.05$) with the number of pods per plant was significant and (-r) negative, but it was determined that it did not have a significant effect on yield as a result of the regression analysis (Figure 6).

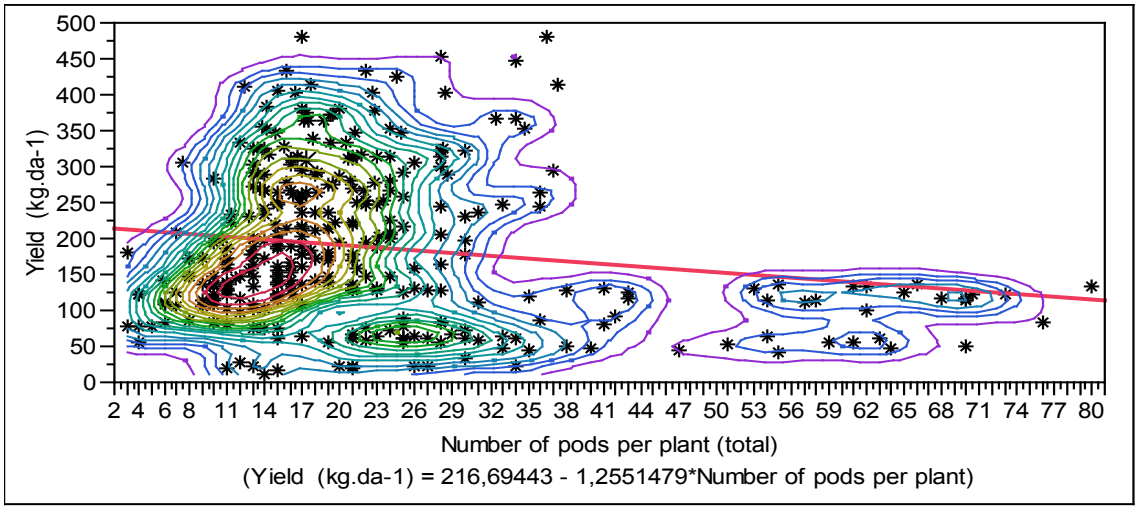

Figure 6. Linear change occurring in the yield depending on the number of pods per plant, its density and formula

The number of the filled pods which should be in the genotypes that normally survive the maturity period without any problem is positively related with the yield. However, regarding the number of pods per plant, which belonged to the trial material varieties, there was no significant difference between the high-yielding and low-yielding genotypes. It was observed to be more important to determine the rate of the filled pods rather than the genotypes with high number of pods. Although some of the genotypes had many pods in the plant, it was observed that the number of seeds per pod remained low due to early drying. This already manifested itself in the yield in which the number of seeds per pod was important and positive.

The number of seeds per pod ranged from 0.1 to 2.2 (Figure 7). The relationship between yield and the number seeds per pod is positive. The highest yields are observed in the genotypes with 1.1 and 1.3 seeds per pod. In general, it is seen that, as the rate of fullness increases, the yield also tends to increase. The expected yield value depending on the number seeds per pod for 0.1 seeds/pod is 1180 kg.ha^{-1}, and for 2 or more seeds per pod, it is 2590 - 2730 kg.ha^{-1}. In general, the majority of the genotypes yields as low as 1.0 seeds per pod.

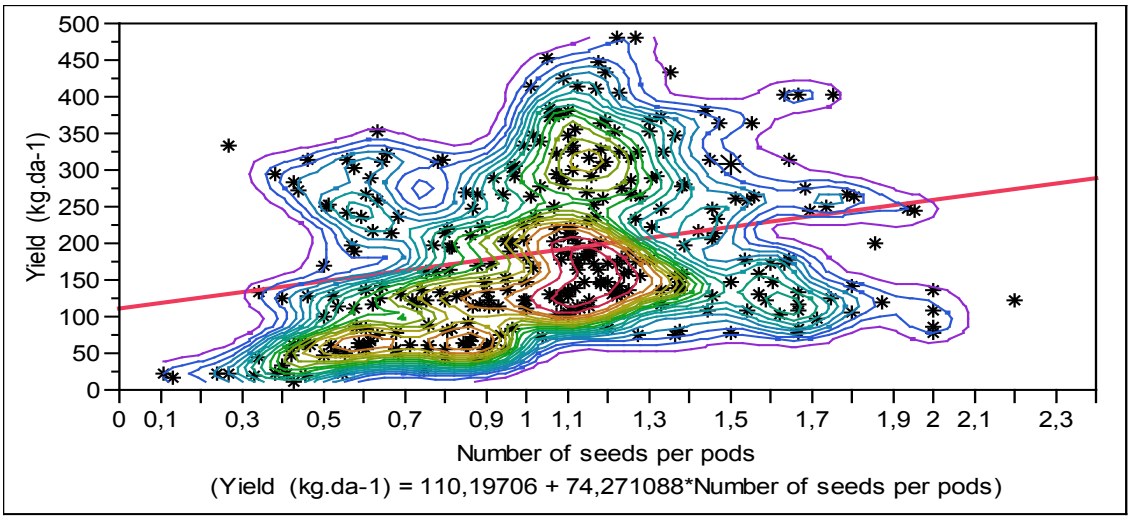

Figure 7. Linear change occurring in the yield depending on the number seeds per pod, its density and formula

Conclusion

It is understood that the number of days to 50% flowering , the number of days to maturity, first pod height, the number of pods per plant, the number of seeds per pod, plant height, the number of branches per plant and 100 seed weight for chickpea are the most researched features in relation to the yield. The results of the studies can be said to have been successful considering the increases in yields per unit area from past to present. It is observed that while the researches related to yield and facilitating selection studies were carried out intensely until 1999, they decreased from 2000 and onward. It is known that earliness in maturity has increased and maturity periods are reduced due to the effects of phenological deviations resulting from global climate change, which has accelerated recently (Penuelas and Filella 2001; Walther et al., 2002; Craufurd and Wheeler, 2009; Visser et al., 2010; Sayılğan, 2016; Yavaş and Ünay, 2018; Gülser et al., 2019). In order to develop varieties with high adaptation to these conditions, revealing the effects of phenological features and new developments in plant morphology on yield is important in terms of the success of the

breeding programs and prospective predictability.

Multidimensional evaluation of the data obtained and updating the situation by comparing it with the data obtained in the past are especially important for long-term breeding programs, because the temporal change in environmental and genotype characteristics causes the plants to acquire features that can adapt to new situations. Or those which cannot adapt disappear in this process. Today, the so-called "data mining" work saves a lot of time and labor when they are utilized well. In this study, a total of 3696 observation data of plant characteristics obtained from the present research and the results of similar studies conducted on chickpea plant starting from 1960s till today were used. The correlation coefficients of plant properties and their significance levels were determined.

As the facilitating separation features in the selection with a purpose of efficiency in breeding activities in the Western Mediterranean, it was determined that the genotypes which complete the number of days to 50% emergence early (9 - 12 days), reach the number of days to 50% flowering early (35-50 days), have physiological maturity days less than 100 days, complete the plant height early (30 - 80 cm) and passes to physiological maturity stage, has the first pod height between 20 and 45 cm, has higher number of filled pods rather than the number of pods, and has the number of seeds per pod between 0.7 and 1.9 pods may be more productive.

As a result of the linear regression analysis, the causal relationship between the yield and such plant properties as the number of days to 50% emergence (Yield = 532.64406 - 27.800541 * the number of days to 50% emergence), the number of days to 50% flowering (Yield = 605.14826 - 8.4052647 * the number of days to 50% flowering), the number of days to maturity (Yield = 902.33154 - 7.6234111 * the number of days to maturity), plant height (Yield = -9.381242 + 3.9200463 * Plant height), the first pod height (Yield = -3.592754 + 7.9434153 * the first pod height), the number of pods per plant (Yield = 216.69443 - 1.2551479 * the number of pods per plant) and the number of seeds per pod (Yield = 110.19706 + 74.271088 * the number of seeds per pod) was tried to be estimated. Although the results of the linear regression performed with full reality do not coincide with the data determined, it was determined that the results could be obtained at the level of average values, which can be very beneficial for breeding activities.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Research Article

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Analysis of Household Energy Expenditure in Umuahia North Local Government Area of Abia State, Nigeria

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Abstract

Understanding household energy expenditure is important to encourage policies that support the provision of cleaner, efficient and cost effective sources of energy to households. This project analyzed household energy expenditure in Umuahia North Local Government Area (LGA) of Abia State, Nigeria. The available domestic energy commonly used by the households' was kerosene, firewood, Liquefied Petroleum Gas (LPG), charcoal, and electricity. The most used domestic energy types was kerosene (1st), followed by Liquefied Petroleum Gas (2nd); then firewood (3rd) and charcoal (4th). Multiple regression result for the factors influencing household energy expenditure in the study area shows that five out of eight explanatory variables employed in the model significantly affected the respondent's households' energy expenditure. These variables were household size, sex, household income, education and frequency of cooking. Also, results from the ordered probit model shows that the significant variables influencing the choice of domestic energy expenditure was age and gender of the household head, their income level, educational level and occupation. Lack of financial resources, high cost of cleaner energy types and distance from the place of purchase significantly affected household energy expenditure in the study area. It is recommended that provision of cleaner, efficient and cost effective sources of energy be made available to households.

Keywords: Household Energy Expenditure, Fuel-wood, Liquefied Petroleum Gas (LPG), Ordered probit model, Umuahia North Abia State

Introduction

Energy is a basic necessity of life for meeting domestic, social and industrial needs (Momodu, 2013). Sufficient and steady energy supply for industrial and domestic uses are nuts and bolts for keeping socio-economic life moving. Energy is required at all times for various purposes, particularly at the household and industrial level. Life becomes difficult and meaningless without the availability of adequate and regular energy supply for domestic and industrial uses.

Household energy expenditure refers to the amount of energy resources that are spent by households for cooking, heating, lighting and powering gadgets and other electronic devices. According to the International Energy Agency - IEA, (2014), the various energy resources include: biofuel and waste, kerosene, electricity, gas, petroleum, diesel, and solar.

Household energy can be majorly categorized into expenditure proportions such as; cooking, lightening, heating and cooling, as well as transportation purposes. For satisfying the needs of cooking, the various sources available include; animal dung, plant residues, fuel-wood, kerosene, gas and electricity, (Julius, 2013). For lightening purposes, the various choices mainly include; electricity/solar, petroleum/diesel (used for fuelling generators), kerosene, candles and traditional lamps as well as firewood (Abubakar *et al*., 2015).

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Additionally, for the purpose of space heating and cooling (also drinks cooling), the various energy sources available consist of mainly electricity and petroleum/diesel power generator. Lastly, for transportation purposes the major choice available are; petroleum, kerosene and diesel for fuelling various transport vehicles, aircraft and motor cycles.

However, it is argued that more than 2.5 billion people worldwide depend majorly on traditional biomass fuel as their major source of energy for cooking, heating and lighting (Kowsari and Zerriffi, 2011). Such traditional biomass fuels are widely used particularly in developing countries (Yamamoto *et al*., 2009). A major example is the fuel-wood used in cooking and lighting. To overcome the negative effects of traditional biomass fuels on human health and the environment, and to improve living conditions in developing nations, there is a need for cleaner and efficient sources of energy that do not damage the environment and health of humans and animals. Understanding household energy expenditure is important to encourage policies that can support the provision of cleaner, efficient and cost effective sources of energy. In this regard, research which shows how different socioeconomic factors influence a household‘s energy expenditure is required. Therefore, this project analyzes household energy expenditure in Umuahia North LGA of Abia State, Nigeria.

Objectives of the Study

The broad objective of this study is to analyze the household energy expenditure in Umuahia North LGA of Abia State, Nigeria.

The specific objectives are to:

ι. examine the socio-economic characteristics of the respondents;

ιι. identify the domestic energy types and extent of use in the study area;

ιιι. determine the factors that influence household energy expenditure in the study area;

ιϖ. analyze the factors influencing the choice of household energy types in the study area;

ϖ. examine the constraints faced by household in the use of household energy.

Research Methodology Study Area

The study was carried out in Umuahia North Local Government Area (LGA) of Abia State, Nigeria. The LGA is one of the 17 LGAs of Abia state. It was created by the government of Ibrahim Babangida in August, 1991. Currently, its headquarters is in the city of Umuahia (Capital of Abia State). Umuahia North LGA is located within the tropical rain forest ecological zone of Nigeria. It occupies a land mass of 14,464 square kilometers and has geographical coordinates of $5^0 32^1$ North and $7^0 29^1$ East. The majority of the indigenes are farmers and others are civil servants, teachers, businessmen and craftsmen. The soil type of the area is predominantly sandy loan with some swamp areas especially along the river banks. This support the growing of staple food crops such as cassava, yam, maize, potatoes and vegetables.

Method of Data Collection and Sampling Technique

The study employed primary data. The data was collected through the aid of a well-structured questionnaire administered to the randomly selected households.

Multistage random sampling technique was used to select the respondents. In the first stage, three (3) autonomous communities were selected in Umuahia North LGA. The second stage involved the random selection of two (2) villages from each of selected communities. In the third stage, ten (10) households were selected from each of the villages and these resulted to 60 households' being selected for the study.

Analytical Techniques

Descriptive and inferential statistics was used to analyze the specific objectives of the study. Objective (i) and (ii) was analyzed with descriptive statistical tools such as frequency, mean, percentage and chart.

Objective (iii – factors influencing household energy expenditure) was analyzed with the application of multiple linear regression. The implicit form of the regression model is shown below:

$Y = f(x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8)$ -(1)

Y = Household energy expenditure (₦/ month)

X_1 = Age (Years)

X_2 = Household size (number of persons)

X_3 = Sex (male = 0, female = 1)

X_4 = Household income (₦ / month)

X_5= Education (number of years spent in acquiring formal education).

X_6= Marital status (Married = 1, otherwise = 0)

X_7= Occupation (Farming = 1, Non farming = 0)

X_8= Frequency of cooking (number of times of cooking foods per month).

Objective (iv): Factors influencing the choice of household energy types was analyzed with an Ordered Probit model. Following Campbell *et al*., (2003), the standard ordered probit model is widely used to analyze discrete data of this variety and is built around an ordinal regression of the following form:

$\tilde{y} = N'\beta + \mathcal{E}$ - - - (2)

Where N' and β are standard variables and parameter matrices, and Ɛ is a vector matrix of normally distributed error terms. Obviously, predicted grades ($\tilde{y}$) are as follows;

$\tilde{y}_{\text{ordered probit}}$

(3 = firewood, 2 = charcoal, 1 = gas cooker/stove)

N_1 = Age (Years),

N_2 = Household Size (Number of Persons)

N_3 = Gender (Female = 1, Male = 0)

N_4 = Household Income (₦/month)

N_5 = Education (Number of years spent in acquiring formal education)

N_6 = Marital Status (Married = 1; otherwise = 0)

N_7 = Occupation (Farming = 1; otherwise = 0)

N_8 = Frequency of cooking (Number of times of cooking food per month)

Ɛi = Error term distributed across observations and is normalized with the mean and variance of zero and one.

β›s = Coefficients

Objective (v) was analyzed with the use of Likert Scale.

In the use of the Likert scale, the researcher considered the mean score of 3.00 to be the accepted constraints; while any constraints below 3.00 were rejected. The score of ***3.00*** was calculated using the weightings attached to the response options of:

Strongly Agree (SA)	= 5
Agreed (A)	= 4
Dis – Agree (DA)	= 3
Strongly Disagree (SD)	= 2
Neutral (NU)	= 1

Hence, $\frac{5+4+3+2+1}{5} = \frac{15}{5} = 3.0$

Results and Discussion

Socio–Economic Characteristics of Respondents

This sub-section presents the findings of the research with reference to socio-economic characteristics that affect the households in the study area.

Table 1. Summary of Socio Economic Profile of Respondents

	Frequency		Percentage (%)
Gender			
	Male	29	48.33
	Female	31	51.67
Total		60	100
Age (Years)			
	25-35	9	15.00
	36-46	21	35.00
Minimum (25)	47-57	12	20.00
Maximum (76)	58-68	12	20.00
Mean (48.87)	69-79	6	10.00
Total		60	100
Marital Status			
	Single	13	21.67
	Married	41	68.33
	Divorced	4	6.67
	Widowed	2	3.33
Total		60	100
Educational Level			
	No Formal Education	12	20.00
	Primary Education	10	16.67
	Secondary Education	25	41.67
	Tertiary	13	21.66
Total		60	100
Household Size (Number of Persons)			
		17	28.33
Minimum (2)	2-4	29	48.33
Maximum (10)	5-7	14	23.34
Mean (6.01)	8-10		
Total		60	100

Source: Field Survey Data, 2018

Table 1 portrays the socio economic profile of the households. The socio-economic characteristics encompass the respondents' gender, marital status, educational level and household size. From Table 1, it is observed that a total of 48.33% of the respondents were males; while 51.67% were females. This result indicate that greater percentage of the household heads were females. This is contrary to the typical and natural household structure in the traditional African setting where most household heads are males. Onoja (2012) observed that females only become the household head in the event of death of their husband, separation or outright divorce. The result infers that females are more involved in domestic energy procurement, as well as cooking in their respective households.

Age is an important criterion in accessing the socio-economic effects of household energy expenditure; this is so because households' heads that are adult are more likely to engage in energy issues than dependent age group. Majority of the respondents were aged 36-46 years (35%). A total of 47-57 years and 58-68 years represented 20% (respectively) of the population sampled. Only 10% of the respondents were aged 69-79 years. The mean age of the respondents was 49 years.

The result implies that preponderance of the respondents were middle aged and productive. This indicates that the household head were adult and within the economically active age group. This result confirms that mainstream of the household head mostly partake in domestic energy utilization. The inference is that the choices over which fuel to use for cooking, lighting or heating in a household are taken by adults.

Expectedly, majority (68.33%) of the sampled households' were married; others were widows (3.33%), divorcees/ separated (6.67%) and 21.67% were single. This connotes a higher level of social responsibility in terms of household energy procurement and utilization among the households.

A total of 20% of the household head had no formal education. Consequently; 16.67%, 41.67% and 21.66% had primary school education, secondary education and tertiary education. This indicates that greater percentage of the respondents have moderate formal education and thus may have knowledge of the use of the various household cooking fuel appliances; and thus, would be able to procure and utilize the various domestic energy types.

The distribution of the household size shows that 28.33% had family size of 2-4 persons, 48.33% had household size of 5-7 persons, while 23.33% had family size of 8-10 persons. The average household size was estimated at 6 persons. This is an indication of a moderate family size. Ibidun and Afeikhena, (2006) posits that the number of persons in a household is expected to influence the amount that would be spent on energy products and food. Therefore, if a household's need of energy is much, alternative sources that are cheaper might be sourced. This suggests that the household size of the respondents determine the quantity of energy to be consumed. Larger households sizes are expected to cook several times; hence, a higher demand for domestic energy.

Available Energy Types and Extent of Use by the Households' in Umuahia North Local Government Area of Abia State

Table 2 shows the domestic energy types and usage by the households in the study area. The domestic energy availability represents the existing domestic energy utilized by the respondents. The rate of usage of the various domestic energy types is represented by their frequencies/percentages. Multiple responses were recorded; this implies that most of the respondents could use one or more domestic energy types concomitantly.

Table 2. Energy Types Availability and Usage by Households' in Umuahia North L.G.A., Abia State, Nigeria

Energy Types	Frequency*	Percentage (%)*
(i) Kerosene	30	50.00
(ii) Firewood	9	15.00
(iii) LPG	32	53.33
(iv) Charcoal	7	11.67
(vi) Electricity	5	8.33
Total Respondent*	60	100

* = Multiple Responses. Source: Computed from field survey data, 2018

Table 2 shows the energy types available and frequency of usage by the households in the study area. We begin the empirical analysis by presenting the distribution of households' choice for energy in the study area. This was done by employing simple descriptive statistic such as table, percentage and frequency count. The available household energy types commonly used by the respondents in Umuahia North Local Government Area were Kerosene, Firewood, Liquefied Petroleum Gas (LPG), Charcoal, and Electricity. The energy usage per month is represented by their frequencies or percentages. The higher the frequency or percentage, the greater the energy usage by the households.

About half of the sampled households (50%) uses Kerosene as their chief domestic energy. A total of 15.0%, 11.67% and 8.33% of the household employ Firewood, Charcoal and Electricity as their domestic energy source. Preponderance of the households (53.33%) in the study area utilizes Liquefied Petroleum Gas (LPG) as their principal domestic energy type.

The higher percentage of LPG (53.33%) usage among the respondents might indicate that the households' in the study area are high income earners. High income earners mostly use LPG which they prefer. Njong and Johannes (2011) observed that the high preference for LPG is due to its clean nature, speed and convenience.

Approximately 8.33% of the households in the study area use electricity for cooking. The results of the analysis indicated that the use of -electricity was very low in Umuahia North due to its irregular supply. Igbinovia and Orukpe (2007) noted that the situation in rural areas is worse, where there are countless uncompleted rural electrification projects. Togola (2005) reported that about 73% Nigerians lack access to electricity, thereby making economic development very difficult. Igbinovia and Orukpe (2007) also observed that utilization of adequate form of energy (such as electric energy for cooking, heating and powering gadgets) is a propellant for job creation and socioeconomic development. Inadequate access to electricity is a major limitation to development cottage industries in Nigeria. Synoptically, the high cost of elctricity tariff, irregularity and risk involved is possibly the reason it is among the least household energy utilized in the study area.

A total of 50% of the respondents in Umuahia North LGA use Kerosene as household energy. Umuahia North LGA

is considered an urban area due to the presence of higher infrastructural facilities and the location of Government house. Kerosene was mostly consumed by the households in the study area because of easy accessibility and its production of cleaner energy compared with the use of fuel wood. Most households in Nigeria use it for cooking through Kerosene stoves and for lighting via Kerosene lanterns. This result is in agreement with Onoja (2012) who observed high utilization rate of Kerosene in Kogi State capital (Nigeria).

The lower percentage of Charcoal utilization for cooking (11.67%) implies that the traditional energy sources have reduced in importance in the study area. Brew-Hammond and Kemausuor (2009) observed that in the absence of affordable modern fuels and electricity, 90% of the Sub-Saharan Africa population relies on traditional fuels for cooking, heating and lighting.

In the case of Firewood, 15% of the households in the study area used Firewood. Onoja, (2012) observed that many households remain subsistently dependent on fuel wood due to socio-economic (e.g. income and wealth), demographic (e.g. family size, household composition, lifestyle and culture) and location attributes (e.g. proximity to sources of modern and traditional fuels) in addition to fuelwood availability. Although the use of fuelwood as domestic source of energy is regarded as an indication of poverty, fuelwood may still be the most readily affordable source of domestic energy for the masses in Nigeria and other developing nations. Sambo (2009) argues that sourcing of household fuel for domestic and commercial uses is a major cause of desertification in the arid-zone states and erosion in the southern part of Nigeria. He further stated that the consumption of firewood is worsened by the inefficient combustion of the wood, producing smokes and sooths which are hazardous to human health, especially to women and children who mostly do the cooking in homes.

Table 3. Daily, Weekly and Monthly Average Energy Utilization by Households' in Umuahia North L.G.A., Abia State, Nigeria

Energy Types	Daily	Weekly	Monthly	Total	Usage
(i) Kerosene	3.0	21.0	90.0	114.0	1st
(iii) LPG	2.7	18.9	81.0	102.6	2nd
(ii) Firewood	1.3	9.1	39.0	49.4	3rd
(iv) Charcoal	0.6	4.2	18.0	22.8	4th

Source: Computed from field survey data, 2018

Statistic for households' choice for cooking fuel in Umuahia North L.G.A., Abia State, was presented in Table 3. The most used domestic energy type was Kerosene (1st), followed by Liquefied Petroleum Gas (2nd); then firewood (3rd) and charcoal (4th).

The most recurrent (daily, weekly and monthly) energy usage by the respondents is Kerosene. The implication of the results showed that the consumption of kerosene had dominated all other domestic energy sources in the study area because of its convenience and diversified use as a source of lighting in Kerosene lanterns and cooking with kerosene stoves. This result is in accordance with Ouedraogo (2006), who stated that Kerosene is the most popular domestic energy in urban Nigeria. Though, fire wood is still a veritable source of domestic energy in the rural areas; kerosene usage is currently more popular due to the problem of fire wood scarcity and the health imperatives of the use of fire wood. This result is in agreement with Chukwuezi (2009), who stated that the utilization of fuel wood/firewood has serious health impact, because open fires in the home produce unventilated smoke. Chukwuezi (2009) also noted that the Nigerian government has put in place distribution mechanisms that ensured availability of Kerosene. However, there had been some perennial scarcity and product adulterations.

The result from Table 3 shows that LPG is the second most used household energy by the respondents in the study area. This could be as a result of the cleaner energy derived from Liquefied Petroleum Gas. Liquid Petroleum Gas if compared to Kerosene or fuel wood, has clear heath, environmental and productivity benefit.

Firewood or fuel wood was the third most used household energy type as against other alternative sources of energy for cooking among the sampled households in Umuahia North LGA of Abia State. This may be influence not only by the availability of forest in the region, rather, prevalence of incidence of poverty in Nigeria. The International Energy Agency (IEA, 2006) stated that about 70% of rural households in sub-Saharan Africa rely on fuel wood as cooking energy. Such high usage of fuel-wood is totally not environmentally friendly. It has negative impact on atmosphere and peoples' lives according to Nlom and Karimove (2014). Apart from deforestation, desertification and soil erosion; the use of fire wood has a very low thermal efficiency and the smoke is also hazardous to human health, especially to women and children who mostly do the cooking in households. In like manner, Chukwuezi (2009) stated that the populace are not aware of the implication of consumption of firewood except the smoke and blackened pots and walls. The associated environmental and health hazard of consumption of fire wood are sore and redness of eye, burning and irritation of the body due to burning of

the biomass; it also lead to exhaustion, tiredness and illness as a lot of enormous physical energy implored in lighting and fanning the wood; as well as discomfort due to heat trapped in the kitchen and smelling of clothes due to settled smoke on it among others.

The use of Charcoal as household energy represents the fourth most preferred domestic energy utilized in the study area. This result implies that Charcoals and fire wood recorded high usage among the sampled households. Similar result had been found by Nnaji *et al*., (2012) who stated that fire wood constitutes about 80% of domestic energy utilization in developing nations. Muller and Huijie (2016) posited that the cause for severe environmental and health problems is the use of firewood and Charcoal. For example, the incomplete burning of these fuels is responsible for indoor air pollution, mostly associated with carbon monoxide, particulate matter, sulphur dioxide and nitrogen dioxide. These pollutants play a major role in generating respiratory diseases and cardiovascular mortality. The consumption of these fuels also spurs climate change by releasing carbon dioxide into the atmosphere. In turn, climate change damages agricultural production and subsequently threatens the nutritional health of human.

Factors Influencing Household Energy Expenditure in Umuahia North L.G.A., Abia State, Nigeria

Factors Influencing Household Energy Expenditure in the study area was analyzed with Multiple Linear Regression Model of the Ordinary Least Squares (OLS). The result is presented in Table 4.

Table 4. Multiple Regression Result for Factors Influencing Household Energy Expenditure in Umuahia North L.G.A., Abia State, Nigeria

Variables	Parameters	Coefficient	Standard error	t – value
(β_0) Constant	β_0	-3433.661	3434.070	-1.000
(X_1) Age	β_1	24.858	44.374	0.560
(X_2) Household Size	β_2	398.726	206.036	1.935*
(X_3) Sex	β_3	-2507.065	1119.327	-2.240**
(X_4) Household Income	β_4	-0.014	0.007	-1.954*
(X_5) Educational Level	β_5	105.813	46.531	2.274**
(X_6) Marital Status	β_6	994.120	1417.872	0.701
(X_7) Occupation	β_7	91.136	1059.806	0.086
(X_8) Frequency of Cooking	β_8	87.141	36.356	2.397**
R		0. 571		
R^2		0.326	3695.760	
F – Statistics		3.08***		

** and * denotes significance of coefficient at 5%, and 10% levels respectively.
Source: Field Survey Data, 2018

Table 4 shows the multiple regression result for factors influencing household energy expenditure in Umuahia North L.G.A., Abia State, Nigeria. The regression analysis was carried out to identify the variables that significantly affected energy use among the households.

The result shows that five out of the eight explanatory variables used in the model significantly affected the household energy expenditure. These variables were household size (X_2), sex (X_3), household income (X_4), education level (X_5) and frequency of cooking (X_8). The multiple regression for the household energy expenditure in the study area has a multiple determination (R^2) value of 0.326, implying that 32.6 percent of the variation in the exogenous variables (X_1-X_8) was explained by the dependent variable (household energy expenditure). The F-ratio was 3.08 and statistically significant at 99% level of confidence; which implies that the model had a good fit. The constant term (β_0) was not significant, but has a coefficient of -3433.661. This implies that household energy expenditure will decrease by 3433.66 assuming other explanatory variables were held constant.

The household size of the respondents was positive and statistically significant at 10% level; with a coefficient of 398.726. This implies that a unit increase in family size of the respondents will result in 398.72 increase in monthly expenditure of domestic energy usage. The result denotes that the higher the household size, the more likelihood of increased expenditure on domestic energy. The sign of the variable conforms to *a priori* expectations. Generally, the more people in a household, the more mouth to feed and this conventionally would require more energy to cook the food hence increase in cooking energy expenditure. This result is synonymous with that of Mekonnen and Kohlin (2008) who opined that the rate of food consumption is a function of number of people.

Gender of the households was negative and statistically significant at 5% level with coefficient of -2507.065. This infers that male headed households had lower probability of using household energy. This is expected because females are traditionally responsible for fetching fuel wood in many

Nigerian States. On the other hand, household income of the respondents negatively influences monthly domestic energy expenditure in the study area with a coefficient of -0.014. Abdullahi *et al*., (2017) observed that low income households uses traditional stoves and cooking fuels such as animal dung, charcoal and wood, while those households with higher income used modern cooking technology and fuels. As income increases, households transit from traditional fuels and cooking stoves to modern fuels and cooking technology that may be cost-effective economically. Also, other already-processed food needing no cooking may be purchased more often as income increases.

The educational status of the households was positive and statistically significant at 5% level; with a coefficient of 105.813. This implies that a unit increase in educational status of the respondents will result in ₦105.81 increase in monthly expenditure of domestic energy. A possible reason for this finding is that education enhances individuals' awareness of the detrimental consequences of using inconducive energy types (firewood and charcoal) on people's health and the environment. Hence, the higher monthly expenditure on cleaner energy sources such as LPG or Kerosene. Lastly, the frequency of cooking was positive and statistically significant at 5% level; with a coefficient of 87.141. This implies that a unit increase in frequency of cooking will result in ₦87.14 increase in monthly expenditure on domestic energy. The more food a household cooks, the more the energy expended.

Factors Impelling the Choice of Household Energy Types in Umuahia North L.G.A., Abia State, Nigeria

The result of the analysis on the factors influencing the choice of household energy types in Umuahia North LGA., Abia State is presented in Table 5

Table 5. Results of Ordered Probit Regression for the Factors Influencing Choice of Household Energy Types in Umuahia North L.G.A., Abia State, Nigeria

Explanatory Variables	Parameters	Coefficient	Standard error	Z – value
(N_1) Age	β_1	0.0278	0.0179	1.55*
(N_2) Household Size	β_2	-0.0112	0.1090	-0.10
(N_3) Gender	β_3	0.5901	0.3502	1.68*
(N_4) Household Income	β_4	8.01E-06	2.78E-06	2.88***
(N_5) Educational Level	β_5	3.6893	1.3297	2.77**
(N_6) Marital Status	β_6	0.2082	0.4351	0.48
(N_7) Occupation	β_7	0.0136	0.0091	1.47*
(N_8) Frequency of Cooking	β_8	-0.0019	0.0114	-0.17
Log likelihood		-62. 6517		
Chi Square		23.10***		
Pseudo R – Square		0.1556		

***, **, and * denotes significance of coefficient at 1%, 5%, and 10% level respectively
Source: Field Survey Data, 2018

The results of ordered probit for factors influencing choice of household energy types is shown in Table 5. The non-zero censoring coefficients were of positive signs (cut 1, cut 2 and cut 3), with the lower censoring threshold at 2.281 and the upper threshold at 0.5476; each statistically significantly different from zero. The goodness of fit measured by the high Chi-square value of 23.1 which is significant at 99% level of confidence showed that the choice of explanatory variables included in the ordered probit model explained the variation in the choice of household energy types. The value of the pseudo R^2 is 0.1556; which explains 15.56% of factors influencing the choice of household energy types in the study area.

The household income of the respondents was a significant factor influencing the choice of household energy expenditure in the study area. The result of the household income of the ordered probit model was significant at 1% level with a positive coefficient of 8.01. The sign of the variable is in consonance with *a prori* expectations. The result of this study collaborates with the findings of Wange and Bessler (2006) in which they stated that the incomes of the consumer were significantly related to the choice of domestic energy consumed by the people of southern Nigeria. The result infers that as one's economic status increases he/she is less likely to partake in discriminate destruction of natural vegetation for energy consumption. This denotes that fuel wood is mostly patronized by those who fall below the socio-economic status threshold (*i.e.* those who are poor).

The educational level of the ordered probit model was significant at 5% level with a positive coefficient of 3.68. The result infers that the higher the level of education attained by household head the greater chances for his/her willingness to consume cleaner and efficient types of energy for domestic purposes. This suggests that educated household heads are less likely to engage in consuming fuel wood or charcoal, hence, reduces the tendencies of environmental degradation through deforestation and climate change.

Household heads that were not formally educated reported higher likelihoods of using charcoal and fuel wood. Adepoju

et al., (2012) found that irrespective of the educational status of the household heads, economic status was important in determining the choice of energy utilized. Conventionally, illiterate household heads are expected to have limited understanding of some environmental and health hazards that are associated with charcoals and fuel wood usage. They are also likely to have lower income. Gupta and Köhlin (2006) and Baiyegunhi and Hassan (2014) observed in India and Nigeria that a higher educational level induces households to move away from firewood dependence towards the use of Kerosene and LPG. In like manner, Gebreegziabher *et al*., (2012) found in Ethiopia that, the higher the education level, the less likely the households will choose wood, while the more likely the households will choose electricity and LPG.

The result of the ordered probit regression shows that the age of the household head was significant at 10% level with a coefficient of +1.55. This result suggest that as the age of household head reached certain level he/she will be more likely to use alternative sources of energy (LPG, Kerosene or Electricity) than the fuel wood or charcoal for cooking/ lighting. This infers that there is a particular age bracket that when reached household heads are more conscious about the disastrous effects associated with incessant consumption of fuel wood/charcoal. This is true if their education level is high. Also, the aged households may use his/her life time savings (or retirement benefits) for consumption of refined and cleaner energy types. Contrarily, Baiyegunhi and Hassan (2014) observed that an increase in the age of household head induces Nigerian rural households to shift away from natural gas towards fuelwood or charcoal. On the other hand, Rahut *et al*., (2014) shows that households with older heads prefer fuelwood to electricity in Bhutan, Indian.

The gender of the household head was significant at 10% level with a positive coefficient of 1.68. This result is in agreement with that of Rahut *et al.*, (2014), who observed that female-headed households prefer modern domestic fuels (LPG, Kerosene and Electricity) to traditional fuels (Firewood and Charcoals). This may be attributed to the fact that women are often responsible for household cooking. On the other hand, the result is in disagreement with the findings of Adepoju *et al*., (2012); who observed that female headed households may be poorer than their male-headed counterparts due to low access to production resources as a result of traditional gender issues in resource allocation. This can also be linked to the fact that female members of households are some time ago directly responsible for fuel wood gathering.

Constraints in the Use of Domestic Energy in the Study Area

The constraints associated with the use of household energy in the study area were analyzed with 5 point Likert scale. The following scales were Strongly Agree (SA), Agreed (A), Dis – Agree (DA), Strongly Disagree (SD) and Neutral (NU).

Table 6: Constraints Faced by Household in the Use of Domestic Energy

Responses	SA	A	DA	SD	NU	Mean	Decision
Lack of financial resources	15	35	10	0	0	4.08	Accepted
Scarcity of household energy	12	17	30	1	0	3.67	Accepted
High cost of household energy	12	26	15	5	2	3.68	Accepted
Distance from the place of purchase	8	11	26	15	0	3.20	Accepted
Adulteration	22	4	7	14	13	3.13	Accepted

Source: Field Survey Data, 2018

Table 6 shows the constraints faced by household heads in the use of domestic energy in Umuahia North Local Government Area of Abia State, Nigeria. From Table 4.6, it is observed that all the five constraints (lack of financial resources, scarcity of household energy, high cost of household energy, distance from the place of purchase and adulteration of the energy) significantly affected the household energy expenditure in the study.

In regards to scarcity of household energy, Momodu (2013) observed that energy for domestic purposes is determined by two major factors: availability and affordability. This implies that energy must be readily available and the price must be within the reach of the people especially the poor.

In terms of distance from the place of acquisition of to the place of utilization domestic energy, Abdullahi et al., (2017) noted that the prevailing poor road network of the country inhibits many people from having access to energy goods. Lack of good roads contributed to increase in the prices of goods in the country. This makes prices of kerosene and other energy goods to rise beyond the reach of the poor. The only alternative left for the poor is to adopt fuelwood for their energy needs. Momodu (2013) observed that women and children are involved in the collection and transportation of fuelwood from the bush to their homes. On many occasions depending on the situation, they have to travel far and wasted their time in the process. At times, women have to carry heavy loads to reduce the number of trips required to provide fuelwood for their households. They may head-load fuelwood as heavy as 35 kilogram or more over a long distance of up to 10 km in often difficult terrain. Carrying such heavy loads over long distance has adverse health implications on the women especially those within the child-bearing age. This may damage spice and cause difficulties during pregnancies and childbirth because substantial amount of energy is involved coupled with poor

access to good medical facilities in most of the rural areas in Nigeria.

Relating the issue of high cost of domestic energy; Chukwuezi (2009) noted that the inadequate and poor condition of infrastructure, especially, the energy infrastructure prevents people from getting regular supply of energy in Nigeria. For example, the four public refineries and private ones in the country cannot guarantee adequate production of petroleum products for local consumption.

Conclusion and Recommendation

Preponderance of the respondents was middle aged and productive. The inference is that the choices over which fuel to use for cooking, lighting or heating are taken by the adults.

Majority of the sampled households' were married. This connotes a higher level of social responsibility in terms of household energy procurement and utilization among the households.

Greater percentage of the households' have moderate education and thus may have knowledge of the use of the various household cooking fuel appliances; and thus, would be able to procure and utilize the various domestic energy types.

Results of the ordered probit regression for the factors influencing choice of household energy expenditure shows that gender, age, household income, educational level and occupation were the significant variables influencing the choice of domestic energy expenditure in the study area.

Lack of financial resources, scarcity of household energy, high cost of household energy, distance from the place of purchase and adulteration of the energy significantly affected the household energy expenditure.

Finally, there should be availability and utilization of cleaner energy types such as kerosene and LPG. The cost of kerosene should be further subsidized by the government to make the product affordable since is the most used domestic energy type in the study area. Also, there should be legislation to ensure that the commodity is readily available to households at all times.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

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

Ethical approval

Experimental procedures were approved by Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Research Article

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The use of *Marrubium vulgare* L. plant extracts in the control of fungal plant pathogens

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Abstract

In this study, we investigated anti-fungal properties of *Marrubium vulgare* L. plant extracts dissolved in four different solvents against five different plant pathogenic fungi, namely, *Fusarium oxysporum* f. sp. *lycopersici, Macrophamina phaseolina, Phytopthora infestans, Sclerotinia sclerotiorum* and *Rhizoctania solani*. We used 1.0%, 2.5% and 5.0% plant extract concentrations against these fungi species which cause a significant decrease in the production yield of certain plants. In all pathogenic fungi species analyzed, we observed an increase in the anti-fungal effect of *Marrubium vulgare* L. plant extract preparations in a dose-dependent manner. *Marrubium vulgare* L. extracts dissolved in chloroform and dichloromethane at 2.5% concentration showed higher inhibition against *Fusarium oxysporum* compared to other solvents at this concentration. At the same concentration, extracts dissolved in methanol resulted in higher anti-fungal activity against *Phytopthora infestans* and intermediate activity against *Macrophamina phaseolina* and *Sclerotinia sclerotiorum*. As a result, it can be stated that *Marrubium vulgare* L. plant extracts dissolved in different solvents display anti-fungal properties against certain plant pathogens.

Keywords: *Marrubium Vulgare* L., Anti-fungal activity, Plant extracts, Plant pathogens

Introduction

Marrubium vulgare L. which is a perennial plant from *Labiatae* family, is a very common plant in North Africa, Central and West Asia, and Southern Europe (Weel et al., 1999). *Marrubium vulgare* L. was reported to be used in the treatment of certain diseases including bronchitis, asthma, tuberculosis, respiratory system infections and diarrhea (Gruenwald et al., 1998). Many studies showed that *Marrubium vulgare* L. has various biological properties such as cytotoxicity (Argyropoulou et al., 2012), anti-cancer (Yamaguchi et al., 2006), anti-hypertensive (Bardai et al., 2001), analgesic (Meyre-Silva et al., 2005), antibacterial (Gonza'lez and Marioli, 2010; Bouterfas et al., 2018), antioxidant (Amri et al., 2017), antidiabetic (Boudjelal et al., 2012) anti-inflammatory (Schlemper et al., 1996), hypoglycemic (Roman Ramos et al., 1992) effects and wound healing (Bokaeian et al., 2014). Considering the biological importance of this plant, scientists have shown an increasing interest in the research of *Marrubium vulgare* L.

Plant fungal diseases cause serious problems in agricultural production and decrease annual yield significantly. Plant pathogenic fungus *Fusarium oxysporum* f. Sp. *Lycopersici* causes wilting in tomato (Rai et al., 2011), *Phytophthora infestans* induces late blight in tomato and potato (Marcin et al., 2012), *Rhizoctonia solani* and *Macrophamina phaseolina* give rise to root rot (Gautam et al., 2003) and *Sclerotinia sclerotiorum* infection results in white mold in plants (Visser, 2007). Several synthetic drugs are widely used for the control

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of diseases caused by these fungal pathogens; however, these chemicals lead to toxic residues in food and to environmental pollution. Recently, many researchers have focused on the use of environmentally-friendly biological methods for the protection of plants from damages and the loss of yield associated with plant pathogenic fungi (Koul and Dhaliwal, 2001; Copping and Menn, 2000). Plant extracts used in these methods are quite beneficial since they easily decompose, have no harm on the environment and have low phytotoxicity (Benner 1996). A review on the use of plants against plant pathogenic fungi species can be found elsewhere (Shuping and Eloff, 2017).

In the current study, we determined anti-fungal properties of *Marrubium vulgare* L. plant extracts dissolved in methanol, ethyl acetate, chloroform and dichloromethane against five plant pathogenic fungi species (*Fusarium oxysporum* f. sp. *lycopersici*, *Macrophamina phaseolina, Phytopthora infestans*, *Rhizoctania solani* and *Sclerotinia sclerotiorum*).

Materials and Methods

Preparation of plant extracts

100 g dried *Marrubium vulgare* L. plant was mixed with 250 mL methanol, chloroform, dichloromethane and ethyl acetate solutions separately after grinding, and stirred for 48h with a magnetic stirrer. Then, plant particles in solvents were filtered, solvents were evaporated and extracts were obtained in a viscous form. These extracts were diluted with 10 % aceton and kept at 4 ºC as 50% stocks for further use.

Microorganisms used in the study

Microorganisms used in this study (*Fusarium oxysporum* f. sp. *lycopersici*, *Macrophamina phaseolina*, *Phytopthora infestans*, *Rhizoctania solani* and *Sclerotinia sclerotiorum*) were obtained from stock cultures in Phytopathology Laboratory at Faculty of Agriculture, Tokat Gaziosmanpasa University.

Anti-fungal studies

Marrubium vulgare L. extracts prepared with different solvents were used in *in vitro* anti-fungal tests. *Marrubium vulgare* L. extracts dissolved in methanol, dichloromethane, ethyl acetate and chloroform were added to autoclaved Potato Dextrose Agar (PDA) as final concentrations of 1.0%, 2.5% and 5.0%. 5 mm mycelium disks for *Fusarium oxysporum* f. sp. *lycopersici*, *Macrophamina phaseolina, Phytopthora infestans, Rhizoctania solani* and *Sclerotinia sclerotiorum* pathogens were inoculated on solidified PDA growth media and placed in a 25 ºC incubator. Experiments were performed in triplicates. After 7-day incubation period, fungi mycelium radius measurements were carried out. PDA growth media without any plant extract was used as a control.

Evaluation of antifungal properties

In these anti-fungal tests, percent inhibition values of plant extracts against pathogenic fungi species were determined by comparing mycelium radial growth measurements of pathogens with that of controls. Inhibition rates were determined using the following formula by Deans and Soboda (1990):

MGI (%) = [(dc – dt) / dc] × 100

MGI = Inhibition (%)

dc = Radial growth in control petri dish (mm)

dt = Radial growth in petri dish with plant extract (mm)

Data visualization

Graphs were drawn in R statistical programming environment (R Core Team, 2018). Following R packages were used in data analysis and visualization: tidyverse (a collection of R packages including ggplot2 designed for common data science tasks such as data cleaning and visualization) (Wickham et al., 2019), readxl (data import into R from excel) (Wickham and Bryan, 2019). The following convention for star symbols indicating statistical significance was used in the plots: ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001 (ggpubr package, Kassambara, 2020).

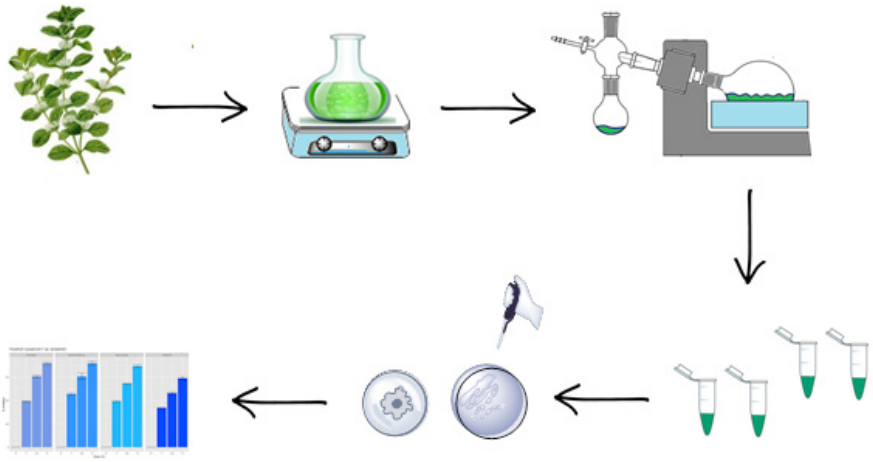

Figure 1. Stages of plant extract and fungus study

Results

The effect of *Marrubium vulgare* L. extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol on *Fusarium oxysporum* f. sp. *Lycopersici* mycelium growth was shown in Figure 2. Extracts dissolved in all solvents showed an increased anti-fungal activity in a concentration-dependent manner. The inhibitory roles of extracts on mycelium growth in terms of solvents were mostly parallel; however, extracts dissolved in methanol had lower inhibitory effect on mycelium growth compared to other solvents. This difference on inhibition of fungal growth might be due to the fact that different solvents can dissolve different organic metabolites in varying amounts. The highest anti-fungal activity on *Fusarium oxysporum* f. sp. *Lycopersici* relative to other solvents at the

same concentrations was observed with 2.5% plant extract in dichloromethane and 5.0% plant extract in chloroform, resulting in growth inhibition of 76% and 90%, respectively. 5.0% plant extract dissolved in methanol resulted in the lowest inhibitory effect (74%) compared to other solvents at this concentration.

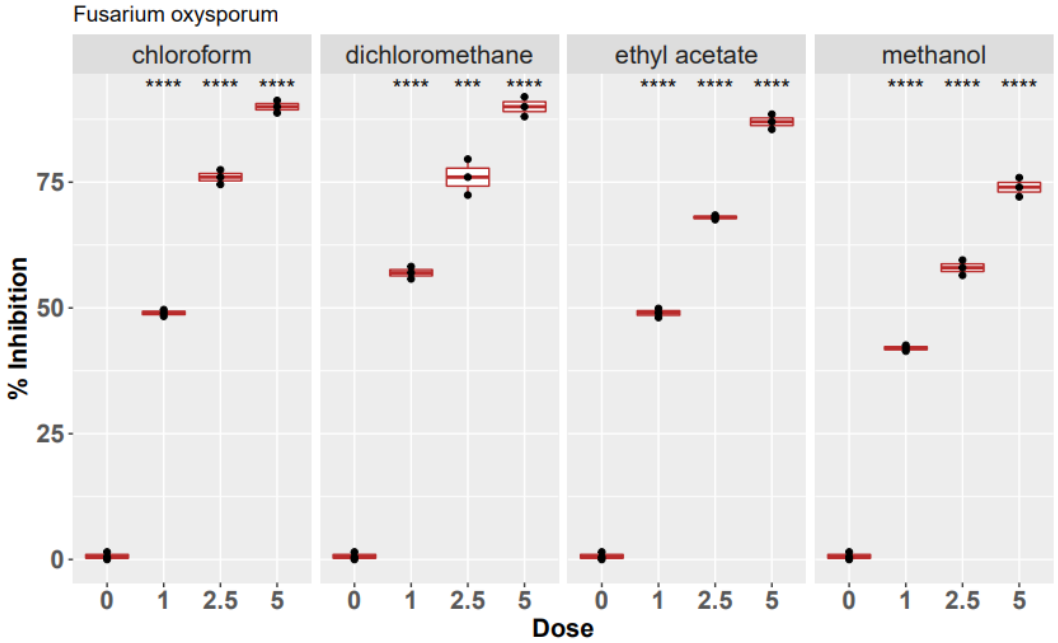

Figure 2. Percent inhibition of *Fusarium oxysporum* f. Sp. *Lycopersici* mycelium growth with increasing doses of *Marrubium vulgare L.* extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

The percent inhibition values for *Marrubium vulgare* L. extracts on *Macrophamina phaseolina* mycelium growth were given in Figure 3. The inhibitory effects of these extracts on *Macrophamina phaseolina* were seen to be parallel with that on *Fusarium oxysporum* f. sp. *Lycopersici,* similiar dose-dependent increase in inhibition was observed. For this fungi species, at the same concentration of *Marrubium vulgare* L. plant extract, we did not observe any significant difference between different solvents. At the highest dose (5.0%), inhibition rates were calculated to be between 88 – 90%.

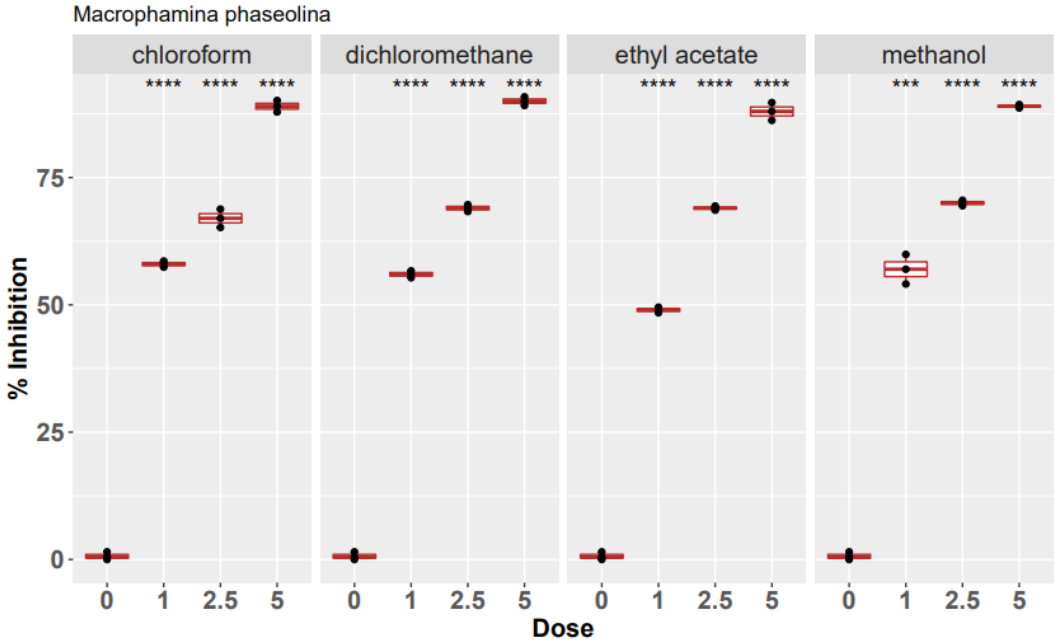

Figure 3. Percent inhibition of *Macrophamina phaseolina* mycelium growth with increasing doses of *Marrubium vulgare L.* extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

The highest inhibitory effect on *Phytopthora infestans* mycelium growth was seen when methanol was used as a solvent at all concentrations of plant extract tested (58% inhibition at 1.0% concentration, 76% at 2.5%, 89% at 5.0%). Figure 4 shows dose-dependent increase in the inhibiton of *Phytopthora infestans* with *Marrubium vulgare* L. extracts dissolved in four solvents. At the highest concentration used, plant extract in dichloromethane resulted in the second highest inhibition against *Phytopthora infestans.*

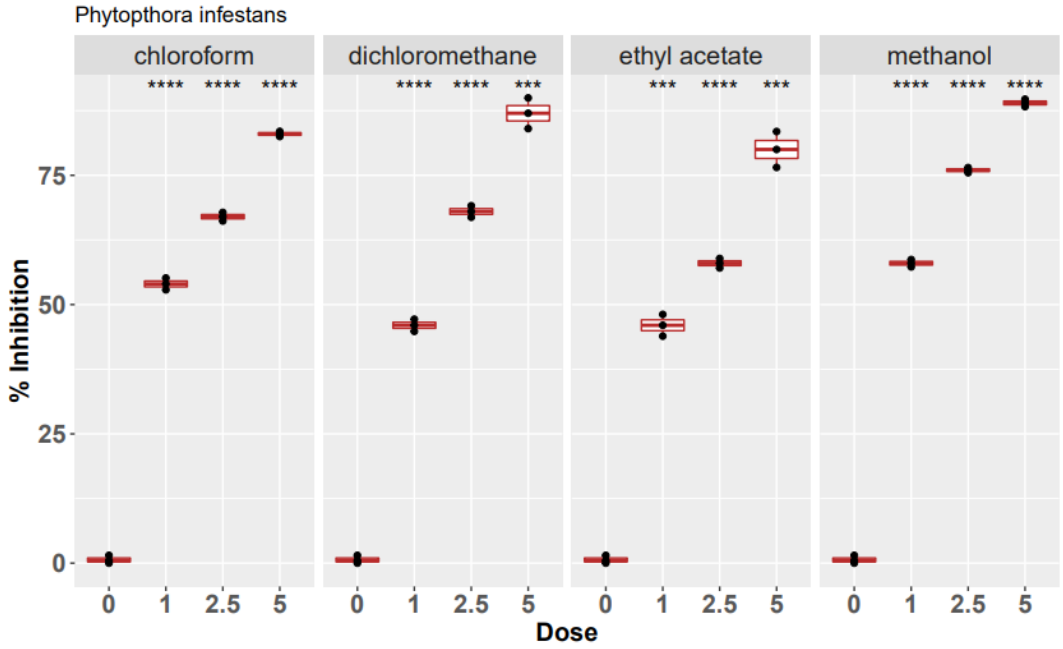

Figure 4. Percent inhibition of *Phytopthora infestans* mycelium growth with increasing doses of *Marrubium vulgare L.* extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

The anti-fungal effect of *Marrubium vulgare* L. extract against *Sclerotinia sclerotiorum* mycelium growth was the highest with plant extract dissolved in dichloromethane at 1.0% dose; mean inhibition for all solvents was 67.25% at 2.5% dose. However, at 5.0% dose, extract in dichloromethane showed the highest inhibitory effect against *Sclerotinia sclerotiorum* relative to other solvents at this concentration (91% growth inhibition). For other solvents, mean growth inhibition was 84% at the highest dose used. Results were summarized in Figure 5.

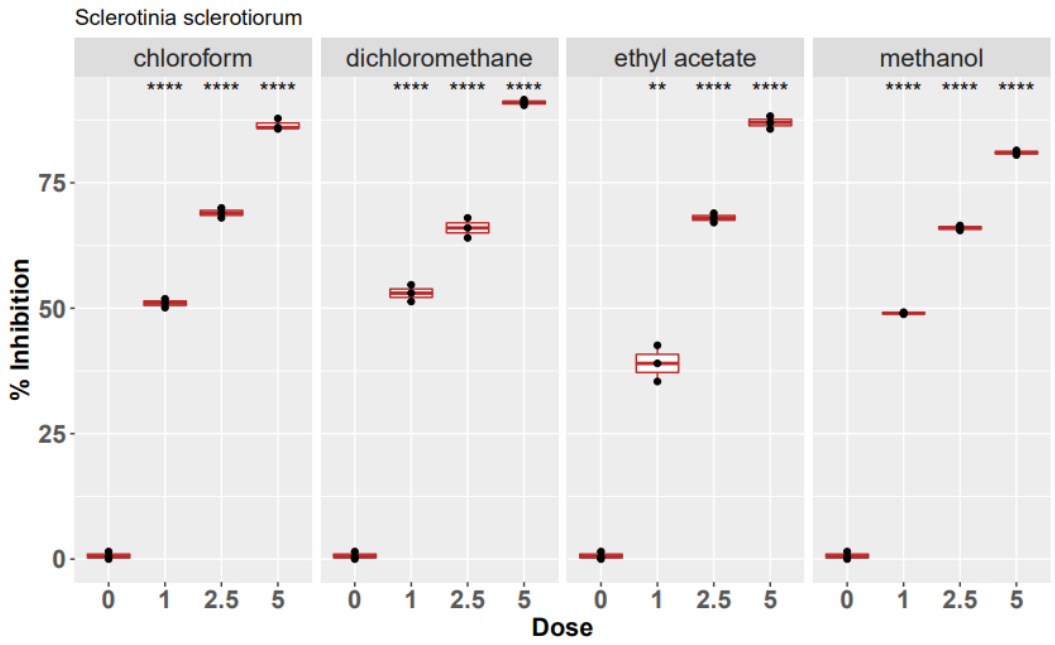

Figure 5. Percent inhibition of *Sclerotinia sclerotiorum* mycelium growth with increasing doses of *Marrubium vulgare L.* extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

Rhizoctania solani was shown to be least effected fungi species by the treatment with *Marrubium vulgare* L. extract in all solvents used in this study. Data on this fungus species were given in Figure 6. Highest growth inhibition (53%) against this pathogen was obtained with 5.0% *Marrubium vulgare* L. extract dissolved in methanol. Plant extract in dichloromethane followed methanol with 43% growth inhibition rate. At lower concentrations of plant extracts in solvents, significant inhibition percentages were not observed against *Rhizoctania solani.*

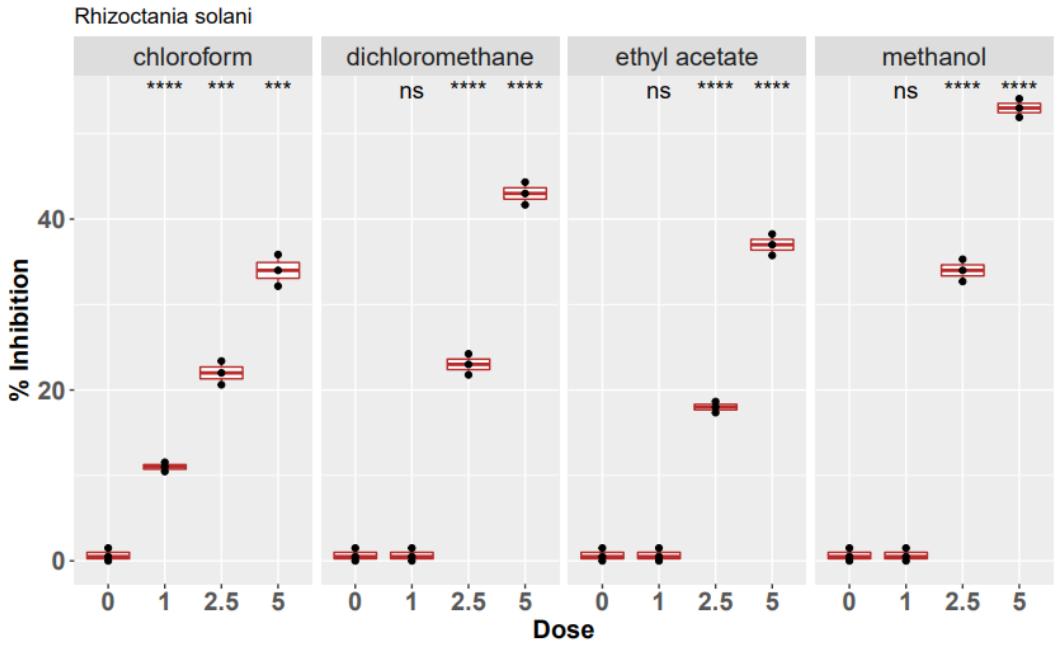

Figure 6. Percent inhibition of *Rhizoctani solani* mycelium growth with increasing doses of *Marrubium vulgare L.* extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

Conclusion

In the current study, anti-fungal properties of *Marrubium vulgare* L. extracts in different solvents against certain plant pathogenic fungi were determined. Today, diverse set of organic and inorganic molecules were synthesized in the laboratory conditions using different methodologies, and their multiple biological functions including anti-fungal properties were studied (Özbek et al., 2017; Gürdere et al., 2020; Özbek and Gürdere, 2020). However, in the present study, natural plant-based extracts were analyzed in terms of their anti-fungal activities. In all the analysis, we showed that increased dose resulted in higher inhibition on fungal mycelium growth. At 2.5% dose, higher anti-fungal activity against *Fusarium oxysporum* was observed when *Marrubium vulgare* L. extracts dissolved in chloroform and dichloromethane. At the same concentration, plant extract dissolved in methanol showed increased inhibitory activity on *Phytopthora infestans* growth; however, intermediate effects were observed against *Macrophamina phaseolina and Sclerotinia sclerotiorum* pathogens. Significant inhibitory effect against *Rhizoctani solani* was observed only with plant extract dissolved in methanol, though this anti-fungal effect is intermediate. Other doses and solvents did not show any considerable inhibition on mycelium growth of *Rhizoctani solani.* Finally, it can be stated that *Marrubium vulgare* L. extracts dissolved in methanol, dichloromethane, ethyl acetate or chloroform have anti-fungal properties against certain plant pathogens and can be used as biofungicides in the future to prevent agricultural losses associated with fungal infections.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

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

Ethical approval

Not applicable.

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Research Article

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Economics of production and marketing of apple (*Malus domestica*) in Mustang, Nepal

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Abstract

A study was carried out to assess the production and marketing status of apple in the Mustang district of Nepal in 2019. A total of 100 households were sampled by using simple random sampling technique and interviewed with a pre-tested semi-structured questionnaire. Descriptive statistics and independent-sample t-test was used for data analysis using SPSS and MS-Excel. Farmers were categorized into small farmers (n=68) and large farmers (n=32) based on the number of apple trees grown by farmers. The average area under apple cultivation was 6.64 ropani (0.3378 hactares). The overall average apple production was 2848 kg (2.84 Mt) and the large farmers had more apple production (7035 kg) as compared to small farmers (877 kg). The average annual household income from apple was NRs. 29,868 (248.90 USD). Apple farming was found to be a profitable farm enterprise with a benefit-cost ratio of 1.84. Two marketing channel was identified in the study area and Channel II was found more profitable. Education status of household head, ethnicity, number of economically active family members, experience on apple farming and visit of extension worker to apple farms were the significant factors that positively affected the production and marketing of apple. The satisfaction level of farmers from production and marketing of apple was found very poor (79%). Unavailability of inputs, lack of storage facilities, insect pest damage, poor technical knowledge and infrastructure were major production problems. Similarly, price variation, poor marketing infrastructure and linkage, high postharvest loss, poor bargaining power and low volume of production were the major marketing problems.

Keywords: BC ratio, Livestock standard unit, Marketing channel, Scaling technique

Introduction

Nepalese economy is predominantly an agricultural-based economy. Agriculture sector contributes 27% of GDP in the fiscal year 2018/19 (MoF, 2019) and employs 65.6% of the total Nepalese population (MoALD, 2017). The total area under fruit cultivation in Nepal was 150,387 ha while the productive area was 110,802 ha. The total fruit production within the country was 991,978 Mt with the productivity of 8.95 Mt/ha. Apple is a major temperate fruit of the country. The total area under apple production was 12,015 ha with the productive area of 3,707 ha (MoALD, 2017). Likewise, the global area under production was 4904305 ha with the production of 86142197 tonnes. Thus the productivity was 17.56 Mt/ha in the year 2018 (FAOSTAT, 2020). The area under apple production in Mustang was 1,115 ha but the productive area was only 360 ha. The production is about 4.500 Mt with the productivity of the 12.50 Mt/ha

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(MoALD, 2017). Globally, Apple accounts for about 50% of world's deciduous fruit tree production. China was the leading country in apple production accounting for about 41% of the total global production followed by the USA, India and Turkey (USDA, 2013).

Apple (*Malus domestica)*, belongs to the family Rosaceae and is perishable in nature. It is believed that the edible apple originated in Central Asia. It is small to medium tree with dark green serrated leaves and fleshy edible fruits (Collett, 2011).

Mustang district lies in the Gandaki Province of northern Nepal. The district headquarter located at Jomsom and covers an area of 3,573 km^2 and has a population (2011) of 13,452. The district straddles the Himalayas and extends northward onto the Tibetan plateau. Mustang is one of the remote areas in Nepal and is sparsely populated.

Mustang is a high hilly region located at an altitude of 2550 masl with an annual temperature of 12°C. 50% of the production form this region goes in the market and for processing.

Most of the farmers in the study area are poor and uneducated. According to the current census, the literacy rate of the district is only 66.2% (CBS, 2011). The marketing channels are not efficient in boosting the marketing of apple. In the year 2015/16, out of total 490 tons apples produced within the district, only 100 tons were marketed and remaining 390 tons of harvested apples were being used for domestic consumption (DADO, 2016). Intervention in the marketing of apple is required to increase the profitability of farmers and the promotion of agribusiness.

Apple production in mustang district is mostly subsistent in nature with low productivity. This may lead to the high inefficiencies in production and marketing of apple thereby hindering the commercialization of apple farming. To increase production, the smallholder farmers should efficiently utilize the limited resources for improving food security and generating farm income (Amos, 2007).

The climatic condition of high hills of mustang district is best suitable for apple cultivation. The sloppy hills of this region have huge potential for apple production and apple-based agribusiness enterprises. However, the comparative advantage of apple for this region is not yet seriously explored and exploited. No any study about the problems and prospects of apple sub-sector in mustang district are carried out yet. A coordinated study is required for suggesting measures to be taken for expansion of area under apple cultivation and maximizing apple production and profitability of farmers. PMAMP has prioritized apple in mustang district and established apple zone in Jomsom (PMAMP, 2017). However, researches on apple are primarily focused in Jumla district and its periphery. The efforts for commercialization of apple in mustang are still lacking and researches are very limited.

About 40% of people of Mustang are under the poverty line which is very high in comparison to the other parts of the country, the national average being 25.16% (UNDP, 2014). National Planning Commission aims to narrow down population under poverty line from 25.16% as of now to 17% by the end of 14th National Plan. It aims to achieve 4.7% annual growth in the agriculture sector. It expects to increase fruit production from 1,200,000 Mt in 2015/16 to 1,230,000 Mt by 2017/18. It also expects increased farm mechanization, technology development and adoption, increased raw material production for agro-based industries, improved marketing infrastructures and storage facilities (NPC, 2016). According to CBS (2011), the export of fruits was worth NRs. 486 million in 2010 while total imports of the same year were worth NRs. 4,715 million. Thus, Nepal suffered a trade deficit of NRs. 4,228 million in the year 2010.

The productivity is lower than that global average but higher than of national average. So, the research on production and marketing help to identify the pros and cones associated with it. Therefore the research on production and marketing of the apple in the Mustang was carried out.

Materials and Methods

Selection of the study area

This study was conducted in the Mustang district of Nepal. Gharapjhong Rural Municipality was purposively selected because it was the major area of apple production. Similarly, it was also the command area of Prime Minister Agriculture Modernization Project (PMAMP), Project Implementation Unit, Apple Zone, Mustang.

Sample size and sampling procedure

The list of small farmers, large farmers and traders of from PMAMP were used as a sampling frame which consists of 1000 households in the study area. From the sampling frame, 10% of households (100 households) were selected for the study. Simple random sampling technique was used for the selection of samples.

Data collection methods

Both primary and secondary data were collected for the study. The primary data was collected by conducting field survey using pre-tested household interview schedule, focus group discussions, key informant interview, rapid market appraisal and the case study of apple producers and traders.

Secondary data were collected from documents and publications of MOAD, FDD, AICC, NPC, NARC, PMAMP, AKC, Mustang and other government agencies. Similarly, reports and publications of various concerned NGO's and INGO's like FAO, UNDP along with journal articles were the sources of secondary data for this study.

Data collection procedure

Reconnaissance survey

Several preliminary field visits were carried out to be familiar with notable features of the study area like topography, land use, agricultural infrastructures, government/non-government service providers and community-based organizations. The information obtained from these observations acted as a vantage point for the preparation of the interview schedule and checklists for systematic data collection.

Interview schedule design

A semi-structured interview schedule was prepared to collect information from apple farmers. The questionnaire was designed to meet the objectives of the study. Similarly, checklists were prepared for focus group discussion and key informant interview.

Pre-testing of the interview schedule

The interview schedule was pre-tested with 10 households (10% of total sample size) for its validity and efficacy. The final interview schedule, after necessary modifications, was used to interview the sampled households.

Field Survey

The field survey was carried out in April 2019 to May 2019. Farmers were personally visited and interviewed by face-to-face interview technique. The objective behind the survey was clearly stated and their permission was sought. A good rapport was built before starting the interview to ensure that the information given by them is reliable and unbiased.

Data collection techniques

Household Interview

All randomly selected households were visited and interviewed with the help of the interview schedule. All the necessary data was collected on socio-economic and demographic characteristics, cost and return from the cultivation, major production and marketing problems associated with the apple.

Focus group discussion

A total of five focus group discussions were conducted using FGD checklists with the progressive farmer, ward representatives, presidents of farmer groups and members of the zone management committee. The information from these discussions was used to verify the responses from the household interview.

Key informant interview

A total of 10 interviews were conducted with key informants from Gharapjhong rural municipality, other non-government organizations, members of the zone management committee, progressive and lead farmers.

Data analysis technique

Data coding, entry and cleaning

The collected data were coded and entered in Statistical Package for Social Sciences (SPSS). The data was further cleaned by removing errors, inconsistencies and overlapped responses. The data was further analyzed using SPSS, Microsoft Excel and STATA.

Qualitative data analysis

The qualitative data were either analyzed qualitatively or further quantified to carry out the quantitative analysis.

Quantitative data analysis

The collected quantitative data were analyzed using both descriptive and analytical statistics.

Descriptive analysis

The average number of apple tree was found to be 126. Based on average apple trees, the farmers were categorized into small farmers (<126 trees) and large farmers (>126 trees). The further analysis was done by comparing between these two categories of farmers. The socio-demographic and economic characteristics were described using descriptive statistics.

Cost of production

The total cost of production per ropani was calculated by summation of variable and fixed cost. Variable cost includes input cost like fertilizer, irrigation, pesticide, planting, labour cost etc. Fixed cost includes the rental value of land and depreciation cost. The variable costs were separately calculated for the first and second year. From 3 to 15 years, the variable cost was increased each year by 10 percent. From 16 to 25 years, the cost was considered the same as that of 15 years but the production was assumed to be decreased by 20 percent than that of 15th year.

The variable cost was estimated by using the following formula:

$$\text{Variable cost} = C_{planting} + C_{labour} + C_{fym} + C_{irrigation} + C_{pesticide} + C_{management} + C_{other}$$

Where,

$C_{planting}$: Cost of planting (NRs.)

C_{labour}: Cost of human and animal labour used (NRs.)

C_{fym}: Cost of farmyard manure (NRs.)

$C_{irrigation}$: Cost of water/snow pond establishment (NRs.)

$C_{management}$: Cost of orchard management (NRs.)

C_{other}: Other miscellaneous costs (NRs.)

Similarly, the fixed cost was estimated by using the following formula:

Fixed cost = $C_{land\ tax} + C_{depreciation}$

Where,

$C_{land\ tax}$ = Cost of land tax (NRs.)

$C_{depreciation}$ = Depreciation cost of farm equipment (NRs.)

Gross return

The total gross return was calculated by multiplying the quantity of apple produced (kg) with the average price of apple (NRs.).

Profit/loss analysis

Profit or loss is the difference between gross return and total cost of production. If the difference is positive, it indicates that apple production is profitable while a negative value represents the loss in apple production in the study area.

Benefit-cost analysis

Benefit-cost ratio (BCR) is a quick and easy measure of the economic performance of any firm. BCR was calculated by using the following formula:

$$\text{Benefit-cost ratio (BCR)} = \frac{Gross\ return\ (NRs.)}{Total\ cost\ of\ production\ (NRs.)}$$

Scaling technique

Five-point scaling technique was used to measure the relative severity of production and marketing problems. The most severe, highly severe, moderately severe, less severe and least severe were given the scale values 1, 0.8, 0.6, 0.4 and 0.2 respectively. The index was calculated using the following formula:

I =

Where,

I: Index (0 < I <1)

S_i: Scale value at i^{th} severity

f_i: Frequency of the i^{th} severity

n: Total number of respondents

Results

Socio-economic and demographic characteristics of the respondents

Population distribution of age group

The population below 15 years in the study area was found to be less than a fifth (15.87 %), between 15 to 59 years (economically active group) was about seven in ten (71.64%) and above 60 years was a minority (12.47%)(Table 1).

Table 1. Population distribution by age group in the study area (2019).

Age group	Frequency
<15	84 (15.87%)
15-59	379 (71.64%)
>60	66 (12.47%)
Total	529

Ethnicity of apple farmers

It was found that the majority of the apple farmers in the study area were Janajati (88%), one in ten (10%) of farmers were Dalit and a very small farmers (2%) were Chettri. Among Janjati, small farmers hold 56 households and the large farmers hold 32 households (Table 2).

Table 2. Ethnicity of apple farmers in the study area (2019).

Ethnicity	Small farmers (n=68)	Large farmers (n=32)	Overall (N=100)
Chettri	2 (2.9%)	0	2 (2%)
Janajati	56 (82.4%)	32 (17.6%)	88 (88%)
Dalit	10 (14.7%)	0	10 (10%)

Education status of the household head

In total, more than two fifths (44%) of studied household head was found to be illiterate. The population of illiterate farmers was higher in small farmers (45.6) than that of large farmers (40.6) and rest 56% of farmers were literate. Among the literate household head, 17.64%, 33.82%, and 2.94% of the small farmers had received a primary, secondary and higher level of education respectively. Similarly, In the case of large farmers, 12.5%, 43.75% and 3.12% had received a primary, secondary and higher level of education respectively (Table 3).

Table 3. Education status of household head of apple farmers in the study area (2019).

Education	Small farmers (n=68)	Large farmers (n=32)	Overall (N=100)
Illiterate	31 (45.6%)	13 (40.6%)	44 (44%)
Primary	12 (17.64%)	4 (12.5%)	16 (16%)
Secondary	23 (33.82%)	14 (43.75%)	37 (37%)
Higher Level	2 (2.94%)	1 (3.12%)	3 (3%)

Religion followed of households

Majority of the households in the studied area follow Buddhism. On average, a very large majority (86%) of the household follow Buddhism while only minorities (14%) of the households follow Hinduism (Table 4).

Table 4. Religion followed by apple farmers in the study area (2019).

Religion followed	Small farmers (n=68)	Large farmers (n=32)	Overall (N=100)
Hinduism	14 (20.6%)	0	14 (14%)
Buddhism	54 (78.40%)	32 (100%)	86 (86%)

Landholding of households

The average landholding in the studied area was estimated to be 8.18 ropani (0.416 ha). The average landholding of the large farmers (14.37 ropani = 0.731 ha) was found significantly higher than that of small farmers (5.27 ropani = 0.268 ha) at 1% level of significance. The average khet land owned by farmers of the study area was found to be 6.54 ropani (0.3327 ha) of which 6.13 ropani (0.3119 ha) was irrigated land. The average cultivated land owned by farmers of the study area was found 7.02 ropani (0.357 ha). Large farmers (13.28 ropani = 0.675 ha) were found significantly higher than that of small farmers (4.07 ropani = 0.207 ha) at 1 % level of significance (Table 5).

Table 5. Distribution of landholding of respondents by apple farm size category in the study area (2019).

Type of land	[1]Small farmers (n=68)	[2]Large farmers (n=32)	Overall (N=100)	[1-2]Mean Difference	t value
Total owned land (ropani)	5.27 (0.268)	14.37 (0.731)	8.18 (0.416)	-9.1***	-4.56
Bari (ropani)	0.09 (0.00457)	0.69 (0.035)	0.28 (0.014)	-0.599*	-2.07
Khet (ropani)	4.07 (0.207)	11.78 (0.599)	6.54 (0.332)	-7.7***	-5.02
Kharbari (ropani)	0.53 (0.02696)	0.41 (0.02)	0.49 (0.024)	-0.12	0.35
Irrigated land (ropani)	3.56 (0.1811)	11.59 (0.589)	6.13 (0.311)	-8.03***	-4.82
Cultivated land (ropani)	4.07 (0.207)	13.28 (0.6756)	7.02 (0.357)	-9.2***	-5.6
Leased in (ropani)	0.32 (0.0162)	1.22 (0.062)	0.61 (0.031)	-0.89	-1.7

Notes: *, *** indicates the level of significance at 10% and 1% respectively, 1 hectare = 19.65 ropani, figures in parenthesis represents land units in hectares

Livestock standard unit of the household

Livestock rearing is an integral component of Nepalese agriculture. Livestock holding of each household was studied by calculating the Livestock Standard Unit (LSU). All the livestock species were converted into livestock standard unit by using the formula,

LSU = 1 cow + 1.5 buffalo + 0.6 pig + 0.4 goat/sheep + 0.02 poultry

The livestock holding unit was calculated to be 8.36 for small farmers whereas 32.36 for large farmers (Table 6).

Table 6. Livestock standard unit of the respondent by apple farm size in the study area (2019).

Livestock	Small farmers (n=68)	Large farmers (n=32)	Mean difference	t value
LSU	8.36	32.36	-24	2.93

Area, production, productivity and experience of apple farming

The average area of apple farm was estimated to be 6.64 ropani (0.337 ha). The average area under apple cultivation of large farmers (13.18 ropani = 0.670 ha) was found significantly higher than that of small farmers (3.35 ropani = 0.180 ha) at 1% level of significance.

The overall average productivity of apple in the study area was calculated as 8.57 Mt/ha. The average productivity of apple in the studied area was found significantly higher for large farmers (10.62 Mt/ha) than that of small farmers (4.94 Mt/ha).

The overall average production of apple among the studied area was found to be 2848 kg (2.84 Mt). The quantity of apple produce by large farmers (7035 kg) was found significantly higher than that of small farmers (877 kg) at 1 % level of significance.

Apple farmers in the studied area were found to have 15 years of experience in apple cultivation. Farmers having larger apple farms were found to have more experience (20 years) than that of small farmers (13 years) which was found statistically significant at 1% (Table 7).

Table 7. Area, production, productivity and apple cultivation experience by farmers category in the study area (2019).

Variables	[1]Small farmers (n=68)	[2]Large farmers (n=32)	[1-2]Overall (N=100)	Mean difference	t- value
Total area under apple cultivation (ropani)	3.55 (0.180)	13.18 (0.670)	6.64 (0.337)	-9.62***	-5.96
Total production (kg)	877	7035	2848	-6157***	-4.00
Productivity (Mt/ha)	4.94	10.62	8.57	-240	-2.78
Years of experience	13	20	15	-7.66***	-4.63

Notes: *** indicates the level of significance at 1%, 1 hectare = 19.65 ropani, kg = kilogram, Mt/ha = Metric tons/hectare, figures in parenthesis represents land units in hectares

Cost and return analysis

The total cost of apple production in one ropani on a year considering farmer's practices was estimated as NRs. 1,628,385 (13569.875 USD). The variable cost and fixed cost was accounted for to be NRs. 999,261 (8367.175 USD) and NRs. 629,124 (5242.7 USD) per ropani respectively. The total gross return from apples was estimated to be NRs. 3,001,568. The benefit-cost ratio was calculated as 1.84 representing profitable farm enterprise (Table 8).

Table 8. Cost and return estimation of apple production in a ropani of land in the study area (2019).

Particulars	Value
Variable cost (NRs./Ropani)	9,99,261 (8367.175)
Fixed cost (NRs./Ropani)	6,29,124 (5242.7)
Total Cost (NRs./Ropani)	16,28,385 (13569.875)
Gross return (NRs./Ropani)	30,01,568 (25013.06)
Profit	13,73,183 (11443.191)
BC ratio	1.84

NRs. = Nepalese Rupees, BC = Benefit cost,
1 United States Dollar = approx 120 NRs.,
figures in parenthesis represents currency in USD

Benefit-cost ratio

The benefit-cost ratio gives an idea about the recovery of expenditure incurred during the production by the return from the product. BC ratio in the studied area indicates that the large farmer (2.18) was profitable than that of small farmers (1.64) (Table 9).

Table 9. Benefit-cost ratio of the apple farmers in the study area (2019).

Variables	Small farmers (n=68)	Large farmers (n=32)	Mean difference	t value
BC ratio	1.64	2.18	-0.54	-1.76

Note: BC = Benefit cost

Marketing channel

Channel 1: Producer-Consumer

Channel 2: Producer – Trader – Consumer

Two marketing channels were found in the study area in which channel I was found more profitable than channel II. Considering Channel I, the marketing margin and producer's shares are 0 and 100 percent respectively. Similarly for the channel II (producer – traders – consumer) the marketing margin and producer's share was found 114.95 and 42.10 percent respectively (Table 10).

Table 10. Market channel, market margin and producer's share of apple farmers in the study area (2019).

Marketing Channels	Farm-gate price (NRs.)	Retail price (NRs.)	Market margin	Producer share
Channel I	117.5 (0.979)	117.5 (0.976)	0	100%
Channel II	83.55 (0.69625)	198.5 (1.65)	114.95	42.10%

NRs.: Nepalese Rupees, figures in parenthesis represents currency in USD, USD: United States Dollar

Production problems

Unavailability of inputs was identified to be the major problem in apple production with an index value of 0.81. It was due to the lack of market facilities in the study area for agriculture inputs like fertilizer, pesticide, phytohormones, lime etc.

Lack of storage facilities was ranked to be the second most serious problem for apple production with an index value of 0.74. It is due to the lack of storage facilities restricting to produce few products. Large quantities of apple were also damaged and farmers were unable to get good price due to lack of storage facilities.

Insect pests possess a severe threat to apple production. Damage by insect pests was ranked third serious problem in apple production with an index value of 0.73. The serious menacing insects were tent caterpillar, sanjose scale, apple woolly aphid and shoot borer while major diseases were powdery mildew, papery bark and foot and root rot disease.

The fourth major problem in apple production was identified to be poor technical knowledge about apple farming (0.406). Apple farming was an entirely different enterprise as compared to subsistence farming of food crops, farmers lacked information and skills about apple cultivation and improved orchard management practices. Farmers were found to be adopting faulty farming practices due to their ignorance.

The fifth major problem in apple production was identified to be lack of infrastructure with an index value of 0.312. Infrastructure includes irrigation, pruning and harvesting equipment which was essential for apple production (Table 11).

Table 11. Ranking of problems associated with apple production in the study area (2019)

Production problem	Index value	Rank (1= severe)
Unavailability of inputs	0.81	1
Insect pest damage	0.73	3
Poor technical knowledge	0.406	4
Infrastructure	0.312	5
Lack of storage	0.744	2

Marketing problems

The data revealed that the price variation (0.86) was the most serious marketing problem followed by poor marketing infrastructure (0.656), technical knowledge about the minimization of postharvest loss (0.546), poor bargaining power (0.496) and low volume of production (0.442).

There were no proper marketing infrastructures for the marketing of apple in the study area. There was difficult in the transportation to produce apple with very few processing industries of apple. The postharvest loss was also found to be very high. Poor storage facilities and poor handling of apple fruits during harvesting, distant market and poor transportation

facilities were found to be the major cause of high postharvest loss. The bargaining power of farmers was also found to be low as there were very few options available to farmers for marketing (Table 12).

Table 12. Ranking of problems associated with apple marketing in the study area (2019).

Marketing problem	Index	Rank (1= severe)
Low volume of production	0.442	5
Price variation	0.86	1
Poor marketing infrastructure	0.656	2
Technical knowledge	0.546	3
Poor bargaining power	0.496	4

Discussions

The primary centre of origin of apple is the region of Asia Minor, the Caucasus, Central Asia, Himalayan India, Pakistan and western China where at least 25 native species of *Malus* are found (Juniper et. al., 1998).

The relationship between farm size and productivity of the farm has been heavily debated. Farm size and yield can have a different type of relationships. There exists an inverse relationship in farm size and yield which means small farms are more productive than large farms. This is because small farms utilize family labour while large farms have to spend more on hired labour (Jha, 2000; Mazumdar, 1965; Dyer, 1997). Pender et al. (2004) found that age of household head, the main source of income, livestock holding, agro-climatic zones, landholding and participation in extension activities positively affected yield. These results are in line with our result as our findings demonstrated the education status, landholdings and livestock holdings were found higher in the large farmers than small farmers.

A positive relationship between farm size and its productivity was reported by Rao and Chotigeat (1981) which was due to the intensive application of nutrients by large farms than that of small ones. A thorough and careful review of the relationship suggested that the positive relation between these two widely debated variables can exist due to managerial factors which may be more efficient in large farms **(**Rao and Chotigeat, 1981**).** This results supports the findings of this study as higher productivity was demonstrated in larger farms than that of small farms.

The average production cost of apple crop is found to decrease with an increase in the number of plants. A study revealed that farmers having more than 500 plants in their orchards had to bear significantly lower variable cost per plant (Mehta et al., 2013). The 126 apple trees were used to distinguish between large and small farmers so this result couldn't demonstrate such relationships.

The benefit-cost ratio of apple is found to be higher than the food crops due to its high value and higher production and productivity. A study in Iran estimated benefit-cost ratio to be 1.77 (Fadavi et. al., 2011)**.** The BC ratio for small famers was comparable to this result but there was higher BC ratio for the large holder farmers in the study area. This results slightly supports the findings of this study.

Large farms were found to have relatively higher economic productivity attributed due to relatively better management and financial ability and hence were relatively more successful as compared to small farms (Fadavi et al., 2011). Similar result was demonstrated in this study.

It was reported that the marketing channel that involved transaction of apples from producer to primary wholesaler, secondary wholesaler, retailer and consumer is most efficient (Chand et al., 2017). But, our studied identified only two marketing channels in the study area.

The major marketing problems were identified to be lack of transportation facility, lack of market information, perishability of product, lack of packaging materials, lack of processing facility, price instability and lack of storage facility (Amgai et al., 2015). The marketing system of apple crop is quite simple. Most of the farmers, considered in a study, are found to prefer commission agent for channelizing their products in the market. Problems related to lack of marketing intelligence information are the major problems in the marketing of apple fruits (Mehta et al., 2013).

Several constraints may hinder apple marketing. The major marketing problems of apple in Mustang district, Nepal were identified to be lack of transportation facility, lack of market information, perishability of product, lack of packaging materials, lack of processing facility, price instability and lack of storage facility (Amgai et al., 2015). Problems related to lack of marketing intelligence information are the major

problems in the marketing of apple fruits in India (Mehta et al., 2013). A study from the Mustang district establishes a fact that apple farmers make a lucrative profit when the region is connected by road transportation (Sachs, 2017). This study identified price variation as the major marketing problem in the study area.

The average national retail price of apple in the fiscal year 2015/16 was NRs. 169 per kg. The seasonality of apple production results from a fluctuating price in different months: highest in summer and lowest during winter (MoALD, 2017).

Kashyap and Guleria (2015) found 4 marketing channels with 4 intermediaries which contradict with our result. Our study revealed 2 marketing channel with 1 intermediary.

There were several constraints to the promotion of apple farming in Nepal. The major farm-level problems prevailing in apple production in Nepal are small landholding and farmers' obligation to grow staple food crops, relatively longer gestation period of fruits, lack of technical know-how, unfavourable climatic conditions like hailstone and erratic rainfall, higher incidence of pests, lack of quality saplings and other inputs and damage by wild animals (Shahi, 2005). This study revealed the unavailability of the inputs as the major problem.

Conclusion

Apple farming in Mustang district is dominated by Janajati and the majority of the community followed Buddhism. Apple production in larger farms was found more profitable which was supported by higher BC ratio. Among the two marketing channel, Channel I was found to be more profitable. Unavailability of inputs was found as the major production problem whereas price variation was found as the major marketing problem.

Compliance with Ethical Standards

Conflict of interest

There is no conflict of interest associated to the research, authorship and publication of this article.

Ethical approval

Ethical approval for the study was obtained from Prime Minister Agriculture Modernization Project, Project Implementation Unit, Apple Zone, Mustang.

Consent to participate

Survey participants were not particularly vulnerable and data was processed in anonymized form. The survey participants had the possibility to escape the questions and terminate the enumeration process.

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Authors' contributions

Bikash Gayak: He was involved in the overall process of the research beginning from the preparation of the questionnaire, data collection, data analysis, data interpretation, preparation of manuscript, correction of the manuscript to final publication.

Subodh Raj Pandey: He was involved in the overall process of the research beginning from the preparation of questionnaire, data collection, data analysis, interpretation, preparation of manuscript, correction of the manuscript to final publication.

Sandesh Bhatta: Major supervisor, provided valuable feedback during the entire research period.

Data availability

The data generated and analyzed during the current research are available from the authors on reasonable request.

Consent for publication

The publisher had the author's permission to publish this contribution.

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Research Article

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Mitigating the constraints of high temperature and low humidity conditions of climate change on grapevine physiology and grape quality with iron and calcite pulverizations

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Abstract

Analysis of physiological adaptive mechanisms developed by grapevines to deal with environmental adversities is of prime strategy to maintain more efficient viticulture. In this context, certain exogenous treatments have been tested for effectiveness on enhancement of the grapevine growth against to constraints such as climatic extremes among which drought and high temperature predominate. Iron and micronized calcite pulverizations were performed three times during the vegetation period to soilless grown five years old grapevines of 'Italia' cultivar in controlled glasshouse in order to assess their possible effects on certain physiological and agronomic features of the vines imposed to mild stress condition of elevated air temperature (with midday means around 37.5±5.6 5 °C), decreased humidity in both air and growth substrate. Fe treatment increased the stomatal conductance in the hottest period of the experiment. The treatments did not affect the leaf temperature, while the chlorophyll and relative water contents of the leaves were improved by all the applications. The leaf mass and pruning residue measurements revealed that the individual application of Fe or calcite induced the vegetative development of the vines. Fe pulverization, with calcite in particular, remarkably increased the cluster mass and the size, although the biochemical features of the must were not affected by the treatments. Therefore, the use of Fe chelates supplemented with micronized calcite would be recommended to enhance grapevine development and grape quality on the face of ever-increasing global warming incidence.

Keywords: Table grapes, Climatic extremes, Vine physiology, Grape quality

Introduction

Water shortage and depletion of soil fertility are among the most essential constrains restricting the agricultural productivity worldwide. As the agricultural productivity has already impacted by climate change characterized by elevated temperature and decreased humidity (Webb et al., 2007), adapting agricultural practices to changing environment is essential as its trends will continue. Adaptation to changing environment is prime consideration in coping with the climate change besides to alleviating strategies across the world (Hinkel, 2011). Since the adaptation to climate change incidences is the prime development concern in developing countries, it is a key to have really affordable and feasible methods in the area. In this context, many crop management strategies and applications based on land and water use that fit with necessities of climate resilient development in agricultural are being examined and promoted across the world (Hu et al., 2018; Sabir et al., 2020).

Conventionally developed methods of producers have to be considered as the initial point in enhancing new approaches for adaptation to have feasible and applicable techniques in the agricultural area. However, the effects of global climate change

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on agricultural lands will be locally unique and hard to measure because of the environmental variations and complications in production techniques. Nonetheless, assessment of climate change effects at land level can be improved by scrutinizing the local experiences and applications (Tiyo et al., 2015; Debela et al., 2015). It is not intensively evaluated at conventional level how adaptation is applied, because the plants have been showing potentially different responses when the climate is getting warmer. However, in order to alleviate the harmful influences of climate change, the selection of adapted genotypes is a better strategy besides many other cultural practices employed simultaneously to obtain better results.

Grapes are commonly cultivated in regions having a warm temperature and dry climate across the world. Although such conditions contribute to good productivity in general, they also cause the decrease in grape quality when, in particular, climate extremes occur. Therefore, to ensure profitable yield and quality, it is often necessary to apply certain exogenous treatments. Grape growers are frequently familiar with the application of Fe chelates to cure or safeguard the grapevines against the stress condition (Ozdemir and Tangolar, 2007). Physiologically, Fe mediates the development stages of plants based on the fact that chlorophyll synthesis and the photosynthetic chain reaction are closely related to Fe status of the plant (Katyal and Sharma, 1980). For this reason, under the stressful environmental condition, Fe chelate pulverization may mitigate the adverse effects of stress factors on plants. Therefore, this study was carried out to investigate the possible effects of micronized calcite and Fe chelate on the physiology, vegetative development and grape quality of table grape cultivar 'Italia' imposed to mild stress conditions of high temperature and low humidity established in controlled glasshouse.

Materials and Methods

Experimental Design

The present experiment was performed in the research glasshouse of Selcuk University, Konya Province, using a soilless culture established with plastic pots (about 70 L in solid volume) containing a growth substance of peat and perlite mixture. The five years old healthy vines of 'Italia' cultivar grafted on 99 R Rootstock (*Berlandieri* x *Rupestris*) were drip irrigated using irrigation lines equipped with individual dropper of 4 L h^{-1} for every grapevine. The vines were designed with east-west orientation with a rectangular configuration of 0.5x1.0 m intervals. In the winter season prior to shoot growth, the grapevines were spur pruned to leave spurs having two buds each according to fruitfulness feature of the cultivar. In early spring, five or six shoots per vine were ensured to elongate to maintain homogenous vine development for optimum assessment of application influences. Nine healthy grapevines for each application were chosen based on the homogeneity in plant growth. Long term mild water stress condition was established with using tensiometers for long term tracking of substrate matrix potential as defined by Satisha et al. (2006) and Sabir and Sari (2019). The climate change events were simulated by simultaneous occurrence of elevated temperature with decreased relative humidity inside the glasshouse with the prolonged summer period and the restricted irrigation in growth substance. The mean of midday temperature, recorded with data logger (Ebro EBI 20 TH1) in the experiment area, was 37.5±5.6 °C, while air relative humidity was around 34.0±8.1%. The tensiometer readings were maintained between 30 and 42 cb. Slight wilting of fresh shoot tips at around 40±2 cb level indicated that the vines experienced mild stress conditions sometimes in midseason. The experimental grapevines were fertilized with the same amount of fertilizers during the summer season (approx. 30 g N, 20 g P, 30 g K, and equal mixture of microlements per vine).

The experimental grapevines were divided into four rows according to the treatments as 1) control (no pulverization), 2) micronized calcium, an organic produce containing 40% $CaCO_3$, (0.5%), 3) iron [Fe chelates (FeNaEDTA), 0.3%], and 4) mixture of calcite and iron. The first application was carried out just before flowering, followed by the second when the berry width was around 3 mm and the last one fifteen days later the second treatment according to the directions of manufacturer using a hand pressure sprayer.

Measurements and Analyses

The stomatal conductance (gs) and leaf temperatures (T_{leaf}) determinations were performed from 09:30 to 11:30 h (using twelve leaves (6th leaf) using six grapevines Sabir and Yazar, 2015), at three different dates with a porometer (SC-1 Leaf Porometer) (Zufferey et al. 2011) and was recorded as mmol H_2O m^{-2} s^{-1}. Near the central vein of the leaf blade (Stavrinides et al., 2010) of the newly expanded and sun-exposed leaves were selected for analysis (Johnson et al., 2009). The same surface of the leaves were analyzed (Miranda et al., 2013), as the gs might be heterogeneous on the large leaf like grapevines. Chlorophyll density of the same leaves was recorded, using a mobile chlorophyll meter (Minolta SPAD-502, Japan).

At around véraison, twelve mature leaves of each grapevines per treatment were harvested from the middle of the summer shoots (OIV, 1997) of each experimental vines in the early morning to obtain fresh and dry masses was obtained from (Tramontini et al., 2013). The fresh leaves were weighed with an analytical scale, with sensitivity of 0.0001 g to record fresh mass (FM). Afterwards, they were hydrated to about the highest turgor by dipping in distilled water for four two days to ensure full rehydration (Yamasaki and Dillenburg, 1999). Before the measurements, the water on the leaf was slightly wiping by using a tissue paper. After the rehydration period, the samples were weighed to record turgid mass (TM) and placed in an oven (Turner 1981), at 70 °C for 48 h to find dry mass DM. Values of FM, TM, and DM were employed to find RWC, using the equation of Gonzalez and Gonzalez-Vilar (2003):

RWC (%) = [(FM – DM)/(TM – DM)] × 100. Instantaneous air temperature, air relative humidity (using mobile data logger EBRO EBI 20) and light intensity (using light meter Lutron LX-105) inside the study glasshouse were obtained simultaneously with the leaf temperature (Hirayama et al., 2006).

Twelve representative clusters per treatment were harvested according to the norms of the O.I.V. (1983) when the grape

berries reached at least 16.5 °Brix grape juice (must) total soluble solid content (SSC) to find cluster and berry growth feature. The length, width and mass of the clusters were recorded. Sixty berries for each treatment were randomly collected from the middle of the clusters to find berry weight. The mass of the berry and clusters were recorded using an analytical scale. The must of the berries was obtained with hand press and filtered through cheesecloth and the supernatants were collected for the biochemical investigations. SSC was obtained with a hand-held temperature compensated refractometer (Atago 9313). Titratable acidity (TA) was analized by titrating 10 mL of the must with 0.1 N NaOH to an endpoint of pH 8.1 and recorded as the percentage of tartaric acid (Valero et al., 2006).

In the following winter, one year-old canes of each grapevine were weighed to obtain pruning residue mass for a logical comparison of vine vegetative development response to the treatments.

Statistical analysis

Numerical data from the analyzed features were subjected to analysis of variance. Comparisons of means were carried out by Tukey's multiple range tests at $P < 0.05$ significance level. The analyses were carried out with SPSS software package v. 15.0 for windows.

Results and Discussion

The stomatal conductance (gs) response of 'Italia' table grapevines in soilless culture was illustrated in Figure 1. In the first measurement, the gs values did not show significant variation ranging from 293.0 (calcite+Fe) to 307.4 mmol H_2O m^{-2} s^{-1} (control). The values are well adjusted to those of the previous investigations performed on different grapevine cultivars (Zsófi et al., 2014; Sabir and Yazar, 2015). This case indicates that the treatments did not adversely affect the gas exchange of the leaves. The second measurement was performed in one of the hottest (around 38 °C) and the driest days (at a relative humidity around 32%) of the experimental season to understand the effects of the treatments on leaf gs under extreme temperature conditions. General investigations on gs in this date indicated that the environmental extremes resulted in the imposition of the progressive decrease in gas exchange of the stomata. However, the gs in this case displayed significant variation from 100.7 (calcite) to 187.8 H_2O m^{-2} s^{-1} (calcite+Fe). The gs increase was most probably due to the Fe, because the Fe application alone gave the similar value 179.7 (H_2O m^{-2} s^{-1}) to that of combined application. The final measurement, when the shoot growth was near to terminate, the gs values across the treatments were at the lowest values with no significant variation.

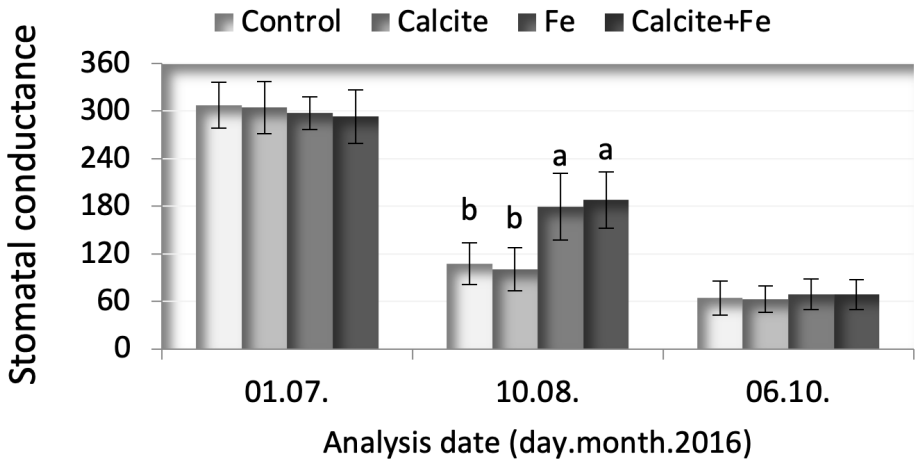

Figure 1. Seasonal changes in stomatal conductance (mmol H_2O m^{-2} s^{-1}) in response to Fe and calcite pulverizations. Values of bars indicated by different letters indicate significant difference ($P < 0.05$).

As depicted in Figure 2, the T_{leaf} did not significantly respond to the treatment throughout the vegetation period. In general, the T_{leaf} of overall grapevines displayed changes depending on seasonal ambient conditions. Nonetheless the obtained values on T_{leaf} were similar to those of literature investigations on grapevines (Düring and Loveys, 1996; Rogiers et al., 2009). Overall values across the experimental grapevines indicated that the range of T_{leaf} was within the recommended values for optimum photosynthesis (25-30 °C) as previously suggested by Greer (2012). This also implies that the treatment did not impair the T_{leaf}.

Chlorophyll concentration of mature leaf was significantly improved by the treatments (Figure 3). The positive effects of the treatments on chlorophyll concentration of the vines were evident from the first measurement to the end of the summer season, except for the Fe application alone in last investigation. Improvement in leaf chlorophyll concentration of grapevines in response to iron was also reported in the previous studies conducted on various cultivars (Chen et al., 2004), studying on the response of the vines to iron supply. Iron could induce the electron transport systems in mitochondria and chloroplasts (Bertamini and Nedunchezhian, 2005) which may ultimately improve the chlorophyll synthesis. Furthermore, Katyal and Sharma (1980) have already revealed that Fe regulates the plant growth through the chlorophyll synthesis as the photosynthetic chain reaction is related to plant Fe status.

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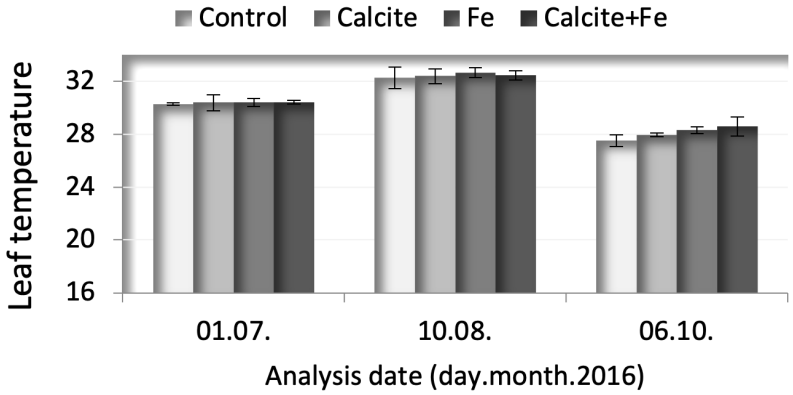

Figure 2. Seasonal changes in leaf temperature (°C) in response to Fe and calcite pulverizations.

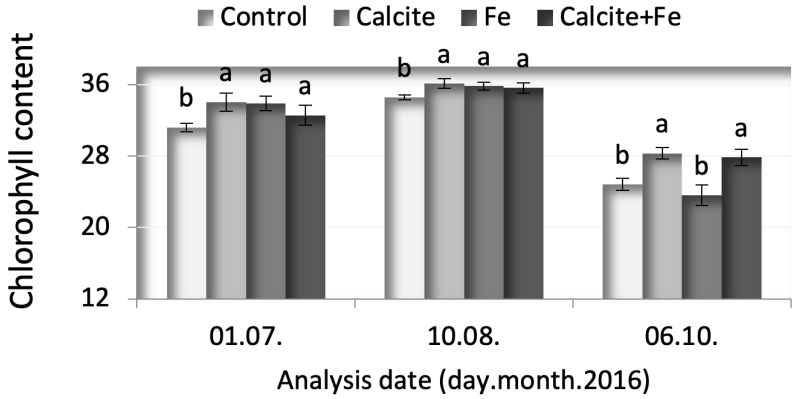

Figure 3. Seasonal changes in leaf chlorophyll content (SPAD meter readings) in response to Fe and calcite pulverizations. Values of bars indicated by different letters indicate significant difference ($P < 0.05$).

The leaf treatments led to significant improvement in relative water content of (RWC) the leaves as can be seen in Figure. 4. The highest RCW was determined in Calcite+Fe pulverization (91.2%) which was followed by Fe (89.3%) and calcite treatments (88.3%). The RWC values were quite similar to those previously determined in the leaves of various grapevine rootstocks (Karaca and Sabir, 2018). Significant increases in fresh and dry weights of the leaves were also determined in response to the treatments (Table 1). The highest improvement in both fresh and dry weights were recorded in vines received the calcite pulverization, followed by Fe application. On the other hand the lowest values were found in control vines.

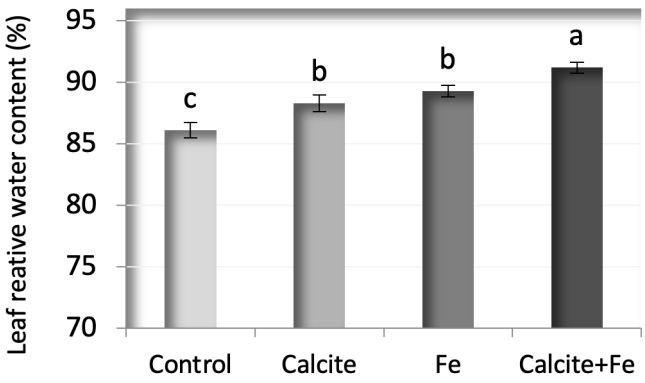

Figure 4. Changes in leaf relative water content (%) in response to Fe and calcite pulverizations. Values of bars indicated by different letters indicate significant difference ($P < 0.05$).

Table 1. Changes in leaf fresh and dry masses (g) in response to Fe and calcite pulverizations. Values of means indicated by different letters identify significantly different means (P < 0.05).

Treatments	Leaf fresh mass (g)	Leaf dry mass (g)
Control	1.73±0.07 c	0.55±0.05 c
Calcite	2.19±0.12 a	0.83±0.05 a
Fe	2.01±0.11 ab	0.72±0.06 b
Calcite+Fe	1.88±0.08 bc	0.64±0.06 bc
LSD	0.18	0.09

Means with different letters in a column are significantly different according to Student's t test (P<0.05).

Pruning residue mass, as a good determinant parameter for vegetative development of grapevines, was significantly affected by calcite (120.9 g per vine) and Fe treatments (116.6 g per vine) as can be found in Figure 5. The pruning residue mass in the vines of calcite and Fe pulverizations were 15.1 and 12.0%, respectively, higher than that of the control vines (102.6 g per vine), with the highest value obtained from the calcite pulverization. Electron-microscopical studies performed by Yamazki et al. (2008) have displayed that calcium is necessary for the construction of lamellar structures in cell organelles, a fact which might describe its indispensability for meristematic development in plants. A well-balanced vegetative development with early and complete cane maturity support the vines cope with winter freeze. This issue, in particular, is essential for the ecosystems characterized with cold winters like Konya province in Central Anatolia. Fe and calcite treatments induced the vegetative growth of the vines, which, in turn, may be anticipated to help the vines endure cold injuries.

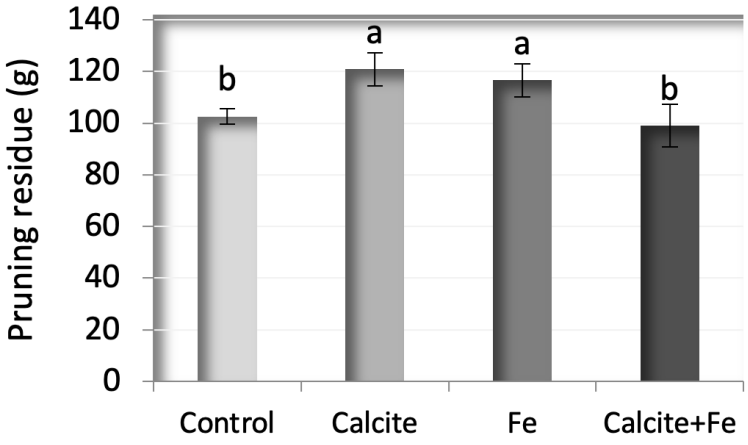

Figure 5. Changes in pruning residue (g) in response to Fe and calcite pulverizations. Values of bars indicated by different letters indicate significant difference (P < 0.05).

Combined application of calcite and Fe led to remarkable increase in berry mass, although single use of these substances were ineffective on berry development (Figure 6). Improvement in berry growth in response to calcite plus Fe treatment resulted in increased cluster weight as indicated in Table 2. The increase in cluster mass was 8.4% higher in vines of combined application that that of the control. This means a significant yield improvement emerging from this treatment, as the cluster number per vine (data not shown) was similar across the experimental vines. The greatest changes in length and width of the clusters were also found in the vines received the calcite plus Fe pulverization. Such remarkable increase in the agronomic characters would most probably be emerging from the vital role of Fe in photosynthesis reactions (Val et al., 1987), as well as its duty as constituents of essential enzymes associated with saccharide metabolism and photosynthesis (Bertamini and Nedunchezhian, 2005).

Changes in biochemical components of the grape must as influenced foliar treatments are presented in Table 3. Total soluble solid content, acidity and pH of the must were not manipulated by the treatments.

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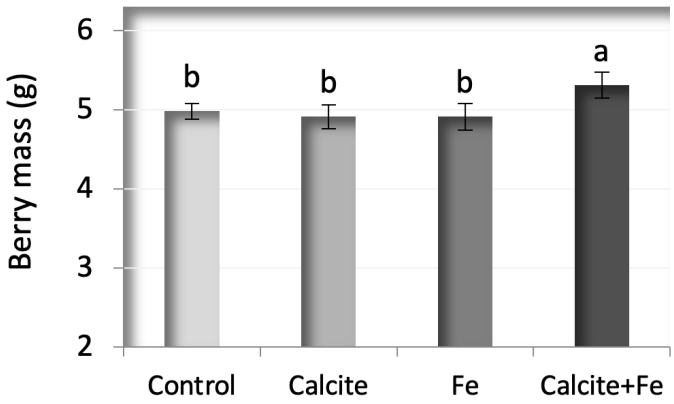

Figure 6. Changes in berry mass (g) in response to Fe and calcite pulverizations. Values of bars indicated by different letters indicate significant difference (P < 0.05).

Table 2. Changes in cluster mass (g), cluster length (cm) and cluster width (cm) in response to Fe and calcite pulverizations. Values of means indicated by different letters identify significantly different means (P < 0.05)

Treatments	Cluster mass (g)	Cluster length (cm)	Cluster width (cm)
Control	217.4±13.0 b	14.8±1.1 b	12.8±1.4
Calcite	212.2±5.9 b	15.5±0.9 b	13.9±0.8
Fe	225.9±4.7 ab	14.7±1.0 b	13.1±1.9
Calcite+Fe	237.4±2.4 a	17.7±0.2 a	13.4±0.6
LSD	14.26	1.56	ns

Means with different letters in a column are significantly different according to Student's t test (P<0.05). ns: not significant.

Table 3. Changes in SSC (°Brix), TA (%) and pH in response to Fe and calcite pulverizations. Values of means indicated by different letters identify significantly different means (P < 0.05).

Treatments	SSC (°Brix)	TA (%)	pH
Control	16.9±0.31	0.42±0.03	2.94±0.05
Calcite	16.9±0.17	0.47±0.01	3.10±0.09
Fe	16.6±0.32	0.46±0.00	3.07±0.03
Calcite+Fe	16.8±0.06	0.47±0.03	3.13±0.18
LSD	ns	ns	ns

ns: not significant.

Conclusion

Multidisciplinary studies indicate that climate change incidences are forecasted to affect agricultural productivity primarily due to rise in temperature and extremities in the droughts. Such stress factors cause crop limitation in lands with rain fed viticulture. Thus, the cultural practices to mitigate such constraints should be developed to match the food demands of the growing population. Iron and micronized calcite pulverizations, performed three times during the vegetation period, remarkable supported the physiology and growth of the soilless grown 'Italia' grapevines imposed to mild stress condition of elevated air temperature and decreased humidity. In the hottest period of the summer, Fe chelate increased the stomatal conductance which may provide a continuous photosynthesis reaction. The chlorophyll and relative water contents of the leaves were also improved by all the applications. Fe or calcite induced the vegetative development of the vines by increasing the leaf and pruning mass values. The cluster features were enhanced by combined application of Fe and calcite. Overall findings show that the use of Fe chelates supplemented with micronized calcite would be recommended to support grapevine development and to maintain grape quality on the face of global climate change events.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Effect of Vermicompost and Isopod (*Porcellio laevis*) Fertilizers on The Emergence and Seedling Quality of Lettuce (*Lactuca sativa* var. *capitata* cv. Wismar)

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Abstract

A great number of insects play an essential role in stabilizing and sustainability of ecosystems. The terrestrial isopods are also important members of soil macrofauna in many habitats, and they are considered as an integral part of the decomposition process. Although the role of isopod in decomposing organic matter and recycling nutrients is very well known, there is no available scientific research or information dealing with the utilization of fertilizer obtained from it as the plant and seedling growing media or soil conditioner in agriculture production. The main goal of this study was to evaluate the usability of fertilizer obtained from a terrestrial isopod (*Porcellio laevis*) and the effect of vermicompost added into the growing media in the growing of lettuce seedlings. By this purpose, 10 different mixtures containing 1, 5, 10 and 20% of each fertilizer [including agricultural soil (control) and peat] as seedling growth media were used. According to results the differences among growing media in terms of emergence and seedling characteristics were not significant. However, the leaf number, fresh-dry shoot weight, and fresh-dry root weight of seedling in all mixtures were equal or higher than control. Results from the study suggest that the isopod fertilizer for the evaluation of agricultural organic wastes which are sometimes regarded as pollutants could be confidently used.

Keywords: Lettuce, *Porcellio laevis*, Vermicompost, Seedling

Introduction

Lettuce (*Lactuca sativa* L.), the most commonly consumed salad vegetable, is rich in several minerals, vitamins and an important source of antioxidants. It is cultivated by the seedling in both the field and greenhouse. The lettuce is taking place in second place with a 13% share among commercial vegetable seedling production in Turkey (Yelboğa, 2014). Peat is widely using as the main growing medium for the production of commercial vegetable seedling in the container. Since the used peat is imported and not cheap, it constitutes a significant production cost. Other hand, the need to recycle wastes and the increasing environmental pressures against peat extraction leads to an increasing interest in utilizing low cost, environmentally friendly materials. Also, the organic wastes, which are considered as sources of pollution during agricultural production and crop processing, are not sufficiently utilized as organic fertilizer and soil conditioner (Hernandez et al., 2010). It has been stated that organic materials like compost, vermicompost can be a good alternative to peat-based substrates for the production of vegetable seedling (Ribeiro et al., 2007). Vermicompost, processed organic material by earthworm, which is increasingly used in agricultural practices, has high porosity, drainage, water-holding capacity, and microbial activity. It improves absorbability and retention of nutrient

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due to having a large surface area, and thus contains nutrients in forms that are readily taken up by the plants (Edwards and Burrows, 1988; Orozco et al., 1996; Atiyeh et al., 1999; Sharma and Banik, 2014). Because of these properties, vermicompost is considered as bio-fertilizer that played a significant role in soil biology, chemistry, and physics (Singh et al., 2010). There are many researches dealing with the positive influence of vermicompost on growth, productivity, and quality of vegetables in literature. For instance, Edwards et al., (2006) demonstrated that vermicompost enhanced the seed germination and growth of tomato and cucumber plants. In a study conducted by Kumar and Raheman (2012), the vermicompost has been added to the soil sand mix (in equal proportion) at 20, 25, 33.33 and 50% by volume to produce the seedlings of tomato, eggplant, and chili pepper. The high-quality seedlings of all vegetables were obtained from a mix of 25% vermicompost and 75% sand and soil. However, in some research related to the effects of vermicompost on seed germination and seedling quality are reported inconsistent results or adverse effects depending on the mixing ratio used, the main organic material of the vermicompost, the species of earthworm, the used soil properties, etc. For instance, in a study conducted to evaluate the effect of compost on lettuce growth parameters, it was determined that yield was not affected by compost applications (Leon et al., 2012). Ievinsh (2011) determined that the vermicompost substitution inhibited the seed germination and seedling growth of several vegetable crops with an almost linear decrease of growth with increasing concentration of vermicompost in the substrate. By Tombion et al. (2016), the effect of substrates with different amounts of vermicompost (0, 20, 40%) added and a commercial mix of peat and perlite of the mixture on lettuce seedling quality has been assessed. As result, it was determined that the mixture containing 40% vermicompost gave the lowest leaf number, and the fresh and dry weight of leaf and root decreased (even lower than the control), while the seedling quality in which mix containing 20% vermicompost is higher than others.

Terrestrial isopods (Isopoda: Oniscidea), a member of soil macrofauna, are invertebrate species that playing an important role in the decomposition of agriculture and livestock waste material (Drobne, 1997; Odendaal and Reinecke, 2003; Hussein et al., 2006; Loureiro et al., 2006). They are identified as an integral part of the decomposition proses, which recycles essential nutrients of the soil and maintains its fertility by the fragmentation of organic matter and stimulating and/or ingesting fungi and bacteria. Due to its high physiological adaptation capacity and exhibiting a broad distribution, they have become an important model organism for the monitoring of pollution and to test the hypotheses in global change biology (Kammenga et al, 2000; Zimmer, 2002). Among terrestrial isopods, particularly *Porsellio laevis*, since it is cosmopolitan and shows plasticity in physiological and life-history traits in response to different geographic–climatic conditions and can accumulate the heavy metals, it is utilized more intensively according to other species for the mentioned purpose (Powers and Bliss, 1983; Castañeda et al., 2004; Bacigalupe et al., 2007; Lardies and Bozinovic, 2008; Dailey et al., 2009; Folguera et al., 2009; Da Silva Junior et al., 2014).

Although the important role of isopods in the decomposition of organic materials, and the soil ecosystem is known very well, there is no available scientific research or information dealing with the utilization of fertilizer obtained from isopod as the plant growing media in agriculture production. The main objective of this study was to evaluate the usability of terrestrial isopod (*Porcellio laevis*) fertilizer added to growing media as a substrate component in different proportions for lettuce seedling production which is having an important place in vegetable production. Also, the effect of vermicompost added at a different ratio to the growing media has been tested.

Materials and Methods

The trial was conducted at the unheated greenhouse of the experimental field of the Department of Horticulture, Faculty of Agriculture, Tekirdağ Namik Kemal University, Turkey (40.99 N°, 27.58° E). By taking into consideration the results of the previous studies, the following seedling growing mediums were tested (Edwards and Burrows, 1988; Atiyeh et al., 1999; Ali et al., 2007; Hosseinzadeh et al., 2017).

1. Agricultural soil (collected from the top 30 cm depth)
2. Agricultural soil mixed with 1% vermicompost (v/v)
3. Agricultural soil mixed with 5% vermicompost (v/v)
4. Agricultural soil mixed with 10% vermicompost (v/v)
5. Agricultural soil mixed with 20% vermicompost (v/v)
6. Agricultural soil mixed with 1% isopod fertilizer (v/v)
7. Agricultural soil mixed with 5% isopod fertilizer (v/v)
8. Agricultural soil mixed with 10% isopod fertilizer (v/v)
9. Agricultural soil mixed with 20% isopod fertilizer (v/v)
10. Peat

Cow manure vermicompost, processed by red earthworm (*Eisenia foetida* L.), and peat that are using for vegetable seedling production were provided from commercial firms. The salinity and pH value of vermicompost used for the experiment were 3,46 dS/m and 6.8 (respectively). It had a 42,8% organic matter, 56,4% moisture, 1,40% N, 1,2% P_2O_5, 0.71% K_2O, 8.02% CaO. The peat recommended for the production of vegetable seedlings was used (Klasmann Potgrond H, Doktor Tarsa Inc., Antalya, Turkey). It had pH of 6.0, EC-value of 0.72 dS/m 160-260 ppm N, 180-280 ppm P_2O_5, 200-300 ppm K_2O and 80-150 ppm Mg. To prepare isopod fertilizer, the plastic pot having dimension 40-50x40 cm was used (Figure 1.) Firstly, three-liter agricultural soil was put into the pot and watered with tap water until the water drains. Then, two kg dry cow manure (old cow dung) that was obtained from the research farm of our university, and lettuce and carrot waste, wheat straw (each one 0.5 kg) were added. The mixture was stirred thoroughly and again was moistened. Three hundred adult isopods (*Porcellio laevis*) per pot was released into the pot and covered with fine mesh and left for 3 months at room temperature. During this period, it was mixed and moistened to provide the appropriate decomposition medium when it is necessary.

Figure 1. Preparation of isopod fertilizer: a) isopods, b) mixture, c) used pots and decomposing

The basic chemical properties of the agricultural soil and isopod fertilizer used in the experiments are presented in Table 1.

The agricultural soil had the clay loam texture (35.13% clay, 24.40% silt, 40.47% sand). Seeds of commercial lettuce (*Lactuca sativa* L.), iceberg type, 'Wismar' (Vilmorin-Anadolu Vegetable Seeds, Istanbul, Turkey) were sown at a depth of approximately 1 cm in multi-cell trays with cell volume 30 cc filled with media mentioned above. The trays were placed on benches inside the greenhouse. In order to clearly see the effect of the growing media, no additional fertilizer was applied during the experiment period. Regular watering by hand to keep the soil with adequate water supply was made. The minimum and maximum temperatures recorded during the growing period were given in Figure 2. In this period, the relative humidity ranged from 46% to 95%.

Table 1. The basic chemical properties of the agricultural soil and isopod fertilizer (If) were used in the experiments.

	pH	Salt (%)	Lime (%)	Organic matter (%)	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Soil	7.74	0.13	4.72	1.73	0.025	32.31	95.37	531.41	56.20	0.43	1.37	0.71	16.07
If	7.02	0.74	1.71	6.52	0.33	480.61	9591.45	7763.27	2528.88	6.89	3.18	42.43	21.59

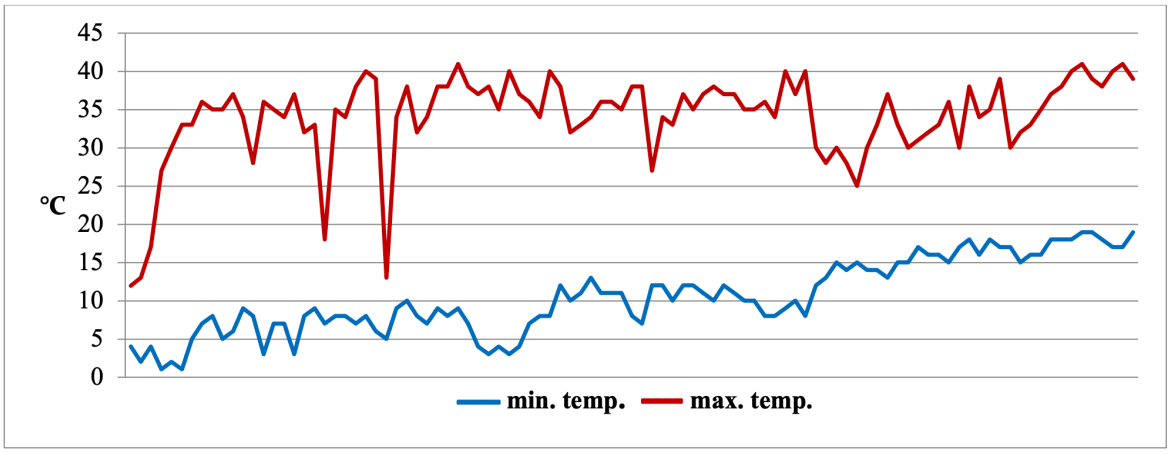

Figure 2. Daily minimum and maximum temperatures during the experiment period.

Emergence was daily recorded until there were no newly emerged seedlings for 3 days. The seedlings with fully opened cotyledons were recorded as 'emergence', and mean emergence time (MET) was calculated by using the following formula (Demir and Okcu, 2004):

Mean emergence time $=\Sigma nd/\Sigma n$

Where n = Number of seeds which emergence on day d, d = Number of days counted from the beginning of the emergence

Thirty-five days after sowing, 10 seedlings selected randomly for each replication were carefully removed from trays and the media adhering to the roots of seedlings was washed using tap water. were carefully removed from trays and the media adhering to the roots of seedlings was washed using tap water. After counting the leaves and measuring the stem diameter, seedlings were cut to separate the root and stem portions for determining the fresh and dry weight. The dry weight of shoot and root was determined by drying in an oven at 65°C for 24 hours. The weight of the root and shoot of

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each seedling were taken using an electronic weighing balance (least count=0.01 g).

The experimental design was a randomized complete block with three replications. All data were subjected to analysis of variance (ANOVA) and mean values were compared with the LSD ($P \leq 0.05$) test.

Results and Discussion

It is desirable to germination at a high rate and in a short time in the vegetable seedlings production. Also, stem diameter, weight, etc. are useful data tools for the identification of plants with desirable horticultural characteristics and the ideal a seedling should have a substantial weight, thick stem, adequate leaf area, etc. (Song and Tan, 1989). According to the results of this study shown in Table 2, there was no statistical difference among different growing media regarding the germination and the seedling characters of lettuce. Besides the differences among the growing media concerning germination percentage were not important statistically, the highest germination percentage (64%) was obtained from seeds sown in peat. A good growing media should be porous for root aeration and drainage besides capable of retaining water and nutrients (Kumar and Raheman, 2012). The fresh and dry weight of shoot and the fresh and dry weight of root of seedling grown in peat, and in mixtures containing vermicompost or isopod fertilizer in different proportions were higher than control (soil). The shoot and root growth of seedlings grown in soil were poor probably because of poor aeration and insufficient of some plant nutrients. As similar to the results of our study, it has reported an increase in the weight of tomato seedlings when vermicompost and compost were substituted in the growth medium up to 20% by volume (Subler et al., 1998; Atiyeh et al., 2000). Kumar and Rahemann (2012) revealed that the best growth media was a mix of 25% vermicompost and 75% soil for three vegetable seedlings (tomato, eggplant, and chili peppers). Bachman and Metzger (2008) have stated that additional vermicompost in the growth medium very little affected the growth of pepper seedlings. In a study conducted to analyze substrate changes according to vermicompost doses and evaluate the effect of the mixtures on lettuce seedling quality, the substrates with different amounts of vermicompost added and a commercial mix of peat and perlite had been used (Tombion et al., 2016). As a result, the addition of vermicompost resulted in a linear increase in pH, electrical conductivity, nutrient level, bulk density and total porosity of the substrate and a 20% increase was determined in seedling size. In a study conducted by Ribeiro et al. (2007), substrates containing compost at different ratio was used as an alternative to peat-based substrates for the production of vegetable seedlings. While the growth of tomato seedlings was higher on the substrates containing compost, both the emergence and growth of lettuce seedlings were not affected by the presence of compost.

Table 2. The effect of different growing media on the emergence percentage (%), mean emergence time (days), number of leaves, stem diameter (mm), shoot fresh weight (FW, g), shoot dry weight (DW, g), root fresh weight (FW, g) and root dry weight (DW, g) of lettuce seedling*.

	Emergence percentage (%)	MET (days)	Number of leaves	Stem diameter (mm)	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)
Soil	48.6	12.1	3.89	1.74	1.23	0.25	0.27	0.14
1% Vc**	48.6	13.0	4.00	1.60	1.32	0.27	0.32	0.15
5% Vc	54.9	11.7	3.89	1.86	2.49	0.42	0.49	0.23
10% Vc	49.3	11.7	4.22	1.96	1.53	0.31	0.36	0.20
20% Vc	46.5	12.0	3.89	2.02	1.49	0.31	0.36	0.17
1% If***	50.0	11.8	3.89	1.70	1.40	0.27	0.31	0.16
5% If	48.6	11.5	4.11	1.75	1.44	0.29	0.34	0.17
10% If	58.3	11.7	4.11	1.93	2.33	0.39	0.51	0.20
20% If	55.6	11.4	4.22	2.05	2.14	0.32	0.47	0.17
Peat	64.6	11.5	3.99	2.14	2.32	0.43	0.40	0.18

*According to the analysis of variance, the differences among mean values of the emergence and seedling parameters were not significant.
**Vc: Vermicompost, ***If: Isopod fertilizer

Conclusion

When the vermicompost and isopod fertilizer was added to the seedling growing medium, the differences seen in terms of emergence and seedling characteristics among applications were not found statistically significant. This may be due to very short of a lettuce seedling growing time to see the effect of vermicompost or isopod fertilizer adding. However, fresh and dry weights of seedlings in isopod fertilizer and vermicompost containing media were higher than those grown in soil. Indeed, the same growing media was used in lettuce cultivation and by adding vermicompost and isopod fertilizer into the soil, the increase of yield and quality was provided (data not presented).

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Leaf-litter inhabitant weevils (Coleoptera: Curculionidae) in a small forest refuge fragment among hazelnut orchards at Trabzon

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Abstract

The leaf-litter weevil diversity in a natural forest refuge fragments among in hazelnut orchards is investigated at eastern Black Sea Region. Ten weevil species *Curculio nucum* (Linnaeus, 1758), *Curculio glandium* Marsham, 1802, *Acalles caucasicus* Reitter, 1891, *Anoplus roboris* (Suffrian, 1840), *Anchonidium caucasicum* (Motschulsky, 1845), *Plinthus osellai* Meregalli, 1985, *Ceutorhynchus picitarsis* Gyllenhal, 1837, *Coeliodes transversealbofasciatus* (Goeze, 1777), *Pseudomyllocerus schneideri* (Schilsky, 1911) and *Trachodes hystrix* Gyllenhal, 1836 are found by litter reducer. Of these, *Trachodes hystrix* is new record for the fauna of Turkey.

Keywords: Leaf-litter, Weevil diversity, New records, Trabzon, Turkey

Introduction

Leaves, twigs and pieces of bark that have fallen to the ground make up leaf litter. Leaf litter is an important component of healthy soil and also serves as great nesting material, hiding places and protected habitats for animals. This dead organic material provides the perfect habitat for organisms, including insects as well as beetles. For this reason, leaf litter is considered very biodiverse (Lin, 2012).

The beetle fauna of leaf litter represents a particularly rich, diverse, and largely specialized community (Owens and Carlton, 2015). Leaf-litter beetles cover the breadth of terrestrial niches, including herbivores and detritivores, and they are key decomposers in terrestrial systems (Burghouts et al., 1992). A propensity to endemicity results from a broad range of dispersal abilities. Litter-beetle diversity has received some attention in the comparative diversity studies of forest types (Caterino et al., 2017). One of the best samples regarding leaf-litter weevil fauna is to a monograph by Anderson (2010) who described 94 new species in a single genus *Theognete* Champion (Coleoptera: Curculionidae) from Middle America.

The hazelnut *Corylus* L. (Betulaceae: Coryloideae) genus contains a wide diversity of deciduous shrub and tree species that are important components of many temperate forests across the Northern Hemisphere, all bearing edible nuts (Molnar, 2011). *Corylus* species distributed in Japan, Korea, and China, through Tibet, India, northern Iran, Turkey, the Caucuses, Europe, and in North America, comprises anywhere from 9 to 25 species depending on the authority (Molnar, 2011; Yoo and Wen, 2007). According to Turkish Plants Data Service (TÜBİVES), three species *Corylus avellana* L., *C. colurna* L. and *C. maxima* Miller are distributed in Turkey. Of these, *C. avellana* is the most widely distributed and *C. maxima* is cultivated one. As primary hazelnut producer in the world, Turkey is a homeland of hazelnut and cultivated in the

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northern Anatolia mainly eastern Black Sea Region (İslam, 2018). Many insect and mite species associated with *Corylus* in Turkey, six species are considered as serious pest and three of them from weevils (Curculionidae), *Curculio nucum* is the most important species (Tuncer and Ecevit, 1997).

In this paper, the litter weevil findings at hazelnut orchards and small patch of natural forest refuge among them in a village of Trabzon are presented.

Material and Methods

The specimens were collected by litter reducer (Ento Sphinx) (Figs 1C, D) at natural forest refuge fragment (Figs 1A, B) among hazelnut gardens in Arsin district, Trabzon province in eastern Black Sea Region of Turkey between 2014-2019. The shifted litter was spread out on a white sheet, waited about 1-2 hours at open area getting sun light, walking specimens on litter were collected by aspirator or hand. The individuals were killed using ethyl acetate and were mounted on a paper card with water soluble glue in the laboratory. Photographs were taken with a Canon DSRL 70D camera attached to Leica Z6APO Macroscope managing Canon EOS Utility software. The digital images were combined to stack using Adobe Photoshop CS 6.0. software.

Results

***Acalles caucasicus* Reitter, 1891**

Material examined. Turkey: Trabzon Prov., Arsin District, Fatih neighborhood, 40°56′36”N, 39°55′41”E, 125 m, 21.06.2014, 1♂, 2♀ L. & N. Gültekin leg.; 14.07.2019, 1♀, N. Gültekin leg.

Distribution. Armenia, Georgia, Russia, Turkey (Alonso-Zarazaga et al., 2017).

Habitat. Litter under *Castanea sativa* Miller, *Quercus pontica* C. Koch, *Fraxinus angustifolia* Vahl., *Laurocerasus officinalis* Roemer and *Fagus orientalis* L. at small patch of natural forest refuge among hazelnut orchards.

Remarks. This genus weevils were captured on dead tree branched, especially from *Quercus* and *Fagus* (Scherf, 1964).

***Anchonidium caucasicum* (Motschulsky, 1845) (Fig. 1F)**

Material examined. Turkey: Trabzon Prov., Arsin District, Fatih neighborhood, 40°56′36”N, 39°55′41”E, 125 m, 21.06.2014, 2♂, 2♀ L. & N. Gültekin leg.; 14.07.2019, 1♂, 1♀, N. Gültekin leg.

Distribution. Bulgaria, Romania, Russia, Turkey, Ukraine, Georgia (Alonso-Zarazaga et al., 2017).

Habitat. Litter under *Castanea sativa* Miller, *Quercus pontica* C. Koch, *Fraxinus angustifolia* Vahl., *Laurocerasus officinalis* Roemer and *Fagus orientalis* L. at small patch of natural forest refuge among hazelnut orchards.

Remarks. This species recently transferred to the genus *Anchonidium* by Savitsky (2018).

***Anoplus roboris* (Suffrian, 1840)**

Material examined. Turkey: Trabzon Prov., Arsin District, Fatih neighborhood, 40°56′36”N, 39°55′41”E, 125 m, 21.06.2014, 3♂, 2♀ L. & N. Gültekin leg.; 14.07.2019, 2♂, 3♀, N. Gültekin leg.

Distribution. Belgium, Czech Republic, Denmark, Estonia, Finland, France, Great Britain, Hungary, Ireland, Italy, Latvia, Lithuania, Luxemburg, Norway, Poland, Romania, Russia, Slovakia, Sweden, Switzerland, Turkey (Alonso-Zarazaga et al., 2017).

Habitat. Litter under *Corylus maxima* Miller at garden of hazelnut and *Alnus glutinosa* (L.) in at small patch of natural forest refuge among hazelnut orchards.

Remarks. This leaf mining species is known as hazelnut pest in Black Sea Region of Turkey (Işık et al. 1992; Ecevit et al., 1993) and live in leaves of *Alnus glutinosa* (L.) Gaertner in France (Hoffman, 1954; Scherf, 1964).

***Ceutorhynchus picitarsis* Gyllenhal, 1837**

Material examined. Turkey: Trabzon Prov., Arsin District, Fatih neighborhood, 40°56′36”N, 39°55′41”E, 125 m, 14.07.2019, 1♀, N. Gültekin leg.

Distribution. This species widely distributed Europe, North Africa, Transcaucasia, Iran, Turkmenistan and Turkey (Alonso-Zarazaga et al., 2017).

Habitat. Litter under *Alnus glutinosa* (L.) Gaertner.

Remarks. This species feeds on Brassicaeae (Colonnnelli, 2004), known as vegetable crop pest, host plants include *Brassica napus, B. rapa, B. oleracea* (Scherf, 1964).

***Coeliodes transversealbofasciatus* (Goeze, 1777)**

Material examined. Turkey: Trabzon Prov., Arsin District, Fatih neighborhood, 40°56′36”N, 39°55′41”E, 125 m, 14.07.2019, 1♂, 1♀, N. Gültekin leg.

Distribution. Europa, Morocco, Azerbaijan, Armenia, Cyprus, Georgia, Iran, Syria (Alonso-Zarazaga et al., 2017).

Habitat. Litter under *Quercus pontica* C. Koch at small patch of natural forest refuge among hazelnut orchards.

Remarks. The host plant is *Quercus sessilis* and larva lives in female flowers (Scherf, 1964)

***Curculio glandium* Marsham, 1802**

Material examined. Turkey: Trabzon Prov., Arsin District, Fatih neighborhood, 40°56′36”N, 39°55′41”E, 125 m, 21.06.2014, 1♂, 2♀ L. & N. Gültekin leg.

Distribution. This species widely distributed Europe, North

Africa, Transcaucasia, Middle East, Russia (Alonso-Zarazaga et al., 2017).

Habitat. Litter under *Quercus pontica* C. Koch, at small patch of natural forest refuge among hazelnut orchards.

Remarks. Larvae develop inside acorns of *Quercus robur*, *Q. sessilis* (Hoffmann, 1955; Scherf, 1964).

***Curculio nucum* (Linnaeus, 1758)**

Material examined. Turkey: Trabzon Prov., Arsin District, Fatih neighborhood, 40°56′36”N, 39°55′41”E, 125 m, 16.06.2018, 2♂, N. Gültekin leg.

Distribution. This species widely distributed Europe, Turkey, Morocco (Alonso-Zarazaga et al., 2017).

Habitat. Litter under *Corylus maxima* Miller at garden of hazelnut.

Remarks. It is one of the most important *Coryllus* pest in Turkey (Tuncer and Ecevit, 1997).

***Plinthus osellai* Meregalli, 1985 (Fig. 1E)**

Material examined. Turkey: Trabzon Prov., Arsin District, Fatih neighborhood, 40°56′36”N, 39°55′41”E, 125 m, 21.06.2014, 2♂, 2♀ L. & N. Gültekin leg.; 14.07.2019, 1♂, 1♀, N. Gültekin leg.

Distribution. Endemic for Turkey (Alonso-Zarazaga et al., 2017).

Habitat. Litter under *Castanea sativa* Miller, *Quercus pontica* C. Koch, and *Laurocerasus officinalis* Roemer at small patch of natural forest refuge among hazelnut orchards.

Remarks. It is an endemic species for Turkey (Alonso-Zarazaga et al., 2017) and was described from Sümela-Trabzon (Meregalli, 1985). This is the first finding specimens after description and second location.

***Pseudomyllocerus schneideri* (Schilsky, 1911)**

Material examined. Turkey: Trabzon Prov., Arsin District, Fatih neighborhood, 40°56′36”N, 39°55′41”E, 125 m, 14.07.2019, 3♂, 2♀, N. Gültekin leg.

Distribution. Azerbaijan, Armenia, Georgia, Russia (South European territory), Turkey (Alonso-Zarazaga et al., 2017).

Habitat. Litter under *Alnus glutinosa* (L.) Gaertner L. at small patch of natural forest refuge among hazelnut orchards.

Remarks. This species was recently recorded from Armenia, Azerbaijan and Turkey, collected on *Alnus glutinosa* at Artvin (Korotaev et al., 2015).

***Trachodes hystrix* Gyllenhal, 1836: 513 (Fig. 2)**

Material examined. Turkey: Trabzon Prov., Arsin District, Fatih neighborhood, 40°56′36”N, 39°55′41”E, 125 m, 21.06.2014, 1♂, 1♀ L. & N. Gültekin leg.; 14.07.2019, 1♂, 2♀, N. Gültekin leg.

Distribution. Azerbaijan, Georgia, Iran, Russia (South European territory), Turkmenistan (Alonso-Zarazaga et al., 2017). Turkey, present finding and new record.

Habitat. Litter under *Fagus orientalis* L., *Castanea sativa* Miller, *Quercus pontica* C. Koch and *Laurocerasus officinalis* Roemer at small patch of natural forest refuge among hazelnut orchards.

Remarks. This species *Trachodes hystrix* (Fig. 2) is closely related to *Trachodes hispidus* (Linnaeus, 1758) and not easy to differentiate them always. Body size, smaller (2.6-2.8 mm) than *T. hispidus* (3.3-3.5 mm). Both species have leaf-like erect scales on pronotum and in a row on 1., 3., 5., 7., interstriae; apex of scales is almost straight on *T. hystrix* for majority of scales while roundish on *T. hispidus*. Postocular lobes less developed than *T. hispidus*. First interstria of *T. hispidus* narrower than *T. hystrix* One of the most important discriminative character between these two species is the shape of ridge between lower margin of metepisternum between upper margin of metaventrite which bears a row miniature vertically sorted leaf-like scale. This metepisternal ridge sinuous on *T. hispidus*, 0.88x as long as diameter of eyes, number of vertical scales less than 20 (usually 17-18); metepisternal ridge straight on *T. hystrix*, 1.1x as long as diameter of eyes, number of vertical scales more than 20 (usually 22-23). Median ventral tooth of fore tibia robust on *T. hispidus,* moderate size on *T. hystrix*.

According to EPPO report on forest pest in the former USSR, *T. hystrix* develops in trunks of *Fagus* and *Juglans* at Transcaucasus. In a study conducted in Ukraine, this weevil was collected in forest of *Pinus pallasiana* (Lamb.), *Fagus orientalis* and *Quercus petrea* (Mattuschka) Liebl. (Yunakov and Nazarenko, 2003).

Discusssion

In this present paper, the litter weevils finding is originated solely a single village at Trabzon where first author’ village. Ten weevil species were captured in a rather small restricted forest refuge land approximately two decares among hazelnut orchard. Of these, one of them is new records for fauna of Turkey, one of them is rare endemic species known only from type locality. It is obviously seen that this small natural forest refuge fragment is how so rich with comparatively with presence only two pest weevil species at hazelnut orchard. Presence of this kind natural forest fragments as natural reserve refuge habitats in cultivated agricultural land is great value and having rather importance to survive biodiversity components.

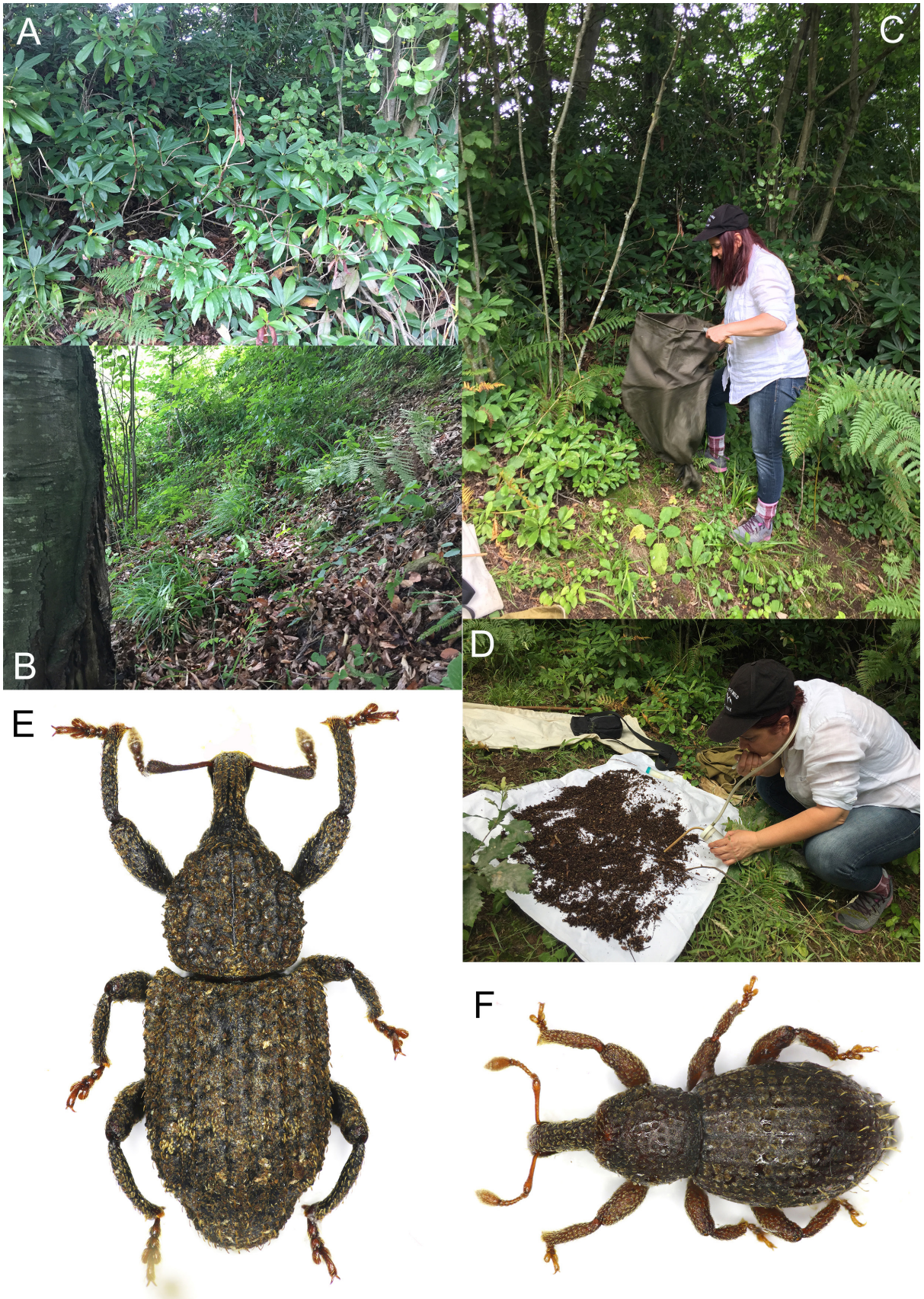

Figure 1. Leaf-litter habitat and collecting with litter reducer with two litter inhabitant weevils. A, *Laurocerasus officinalis* Roemer; B, litter under *Fagus orientalis* L.; C, hazelnut garden margin in natural forest fragment and collecting equipment litter reducer; D, collecting weevils from litter, E, *Plinthus osellai* Meregalli; F, *Anchonidium caucasicum* (Motschulsky).

Figure 2. Habitus of *Trachodes hystrix* Gyllenhal, 1836, dorsal view.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

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Data availability

Not applicable.

Consent for publication

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Effect of varying water applications on growth, yield and water use efficiency of okra under drip irrigation in Akure, Ondo state, Nigeria

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Abstract

The conservation of water is crucial to sustainable agricultural production during dry season when there is little or no rainfall to improve crop production. Thus this study is aimed to estimate the effects of different levels of water applications on the growth, yield and water use efficiency of Okra under drip irrigation system during dry season. The sixteen plots consist of four treatments (2 m x 2 m) with four replicates in a complete randomized block design to determine the water consumptive use of Okra. The growth, yield and water use efficiency of Okra under four different irrigation management i.e irrigation water applied at 100FIT (full irrigation treatment), 80FIT, 60FIT and 40FIT were measured. Results showed highest growth parameters being observed at 100FIT and the least at 40FIT. The difference in the growth parameters in all the experimental blocks were not significant (p=0.05) for all the stages of development of okra. Using standard bar errors the yield obtained at 60 and 100FIT was not significantly different but significantly different when compared to other treatment blocks and it ranges from 2.05-3.60 tons/ha. The Irrigation Water Use Efficiency (IWUE) ranges from 0.024-0.041tons/ha.mm while the Crop Water Use Efficiency (CWUE) ranges from 0.0088-0.0139 tons/ha.mm. Okra crop irrigated with drip irrigation at 60FIT recorded the highest IWUE, CWUE and about 73% more yield than the 40FIT of 0.041 tons/ha.mm, 0.0139 tons/ha.mm and 3.56 tons/ha respectively. It was concluded that Okra crop irrigated at 60FIT should be adopted in order to save 40% water to irrigate additional land. Drip irrigation is encouraged during dry season for farmers to produce okra all year round.

Keywords: Okra yield, Water Use Efficiency, Irrigation, Drip irrigation

Introduction

Agricultural sector consumes about 83 per cent of water whereas, about 50-70 per cent of water is wasted through conveyance, evaporation, field application and distribution losses in conventional method of irrigation. These losses can be reduced by adopting drip irrigation method with efficient water management practices (Dahiya et al., 2005). Recently water supply has become major hindrance to crop production due to competing water demand from other sector of the economy such as rapid industrialization and high population growth (Konyeha and Alatise, 2013). Water is very essential in the growth and production of crop. It needs, thus, to ensure an appropriate growth of vegetable crops throughout the year most especially during dry season when there is little or no rainfall for production. This can be achieve by adopting the irrigation water management strategy to produce more crops per drop of water with the use of drip irrigation system (Panigrahi and Sahu, 2013). Properly managed irrigation increases crop

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yields, increase product value, reduce pest infestation, and precisely deliver and manage nutrients.

Okra (*Abelmoschus esculentus*) is one of the most well-known and utilized species of the family *Malvaceae* and originated from Africa (Abid et al., 2002). It is also a chief vegetable crop grown for its immature pods that can be consumed as a fried or boiled vegetable or may be added to salads, soups and stews (Kashif et al., 2008). Okra is a vegetable that has a lot of stored nutrient. The pods of okra plants should be harvested while young; as long as they are nearly 10cm long they are ready for picking. Okra plays, thus, an important role in human diet by providing carbohydrates, protein, fat, minerals and vitamins that are commonly deficient in basic foods. In spite of the high economic value of the crop and the available potentials for its high production in Nigeria, okra is extensively cultivated by farmers in the country during raining season. This is possible in the south-western Nigeria because of high rainfall for about two-third of the months of the year. Unlike tropical countries where a lot of researchers have studied the production of okra under different irrigation management (Abid et al., 2002; Panigrahi et al., 2011). The few researchers who worked on the production of okra in Nigeria (Ijoyah et al., 2010; Akanbi et al., 2010 and Iyagba et al., 2012). Akanbi et al, 2010 have studied on the response of okra to organic and inorganic source of nitrogen fertilizers using pot and field experiment. The researchers concluded that, small concentration of fertilizer being added to okra increases its yield. Ijoyah et al. (2010) also evaluated the response of okra to different intra-row spacing and they concluded that 30 cm intra-row spacing gives the highest yield. According to the few research carried out in Nigeria, response of okra to irrigation management have not been taking into consideration which implies that water has been added without proper consideration and this might have led to wastage of water most especially dry season.

In planning for irrigation system, it is important to calculate both the seasonal and peak water requirements of crop which is to be irrigated. Okra requires adequate water application and relatively humid soil throughout the growing season in order to have high yield. The flowering and the fruiting stage of okra are considered to be the most sensitive period in the entire growing season (Al-Harbi et al., 2008). Water shortage at this stage reduces the yield of okra. Therefore, to avoid reduced yield there is need for controlled irrigation system. According to Al-Harbi et al. (2008), controlled irrigation is important for high yields in okra field due to the sensitivity of the crop to both over and under irrigation. Therefore, the objective of this research is to evaluate the effects of water applications on the growth, yield and water use efficiency of okra under drip irrigation system in Akure South-Western city of Nigeria.

Materials and Methods

Description of the Study Area

The study was conducted during dry season of October 2017-March 2018 at Training and Research Farm of the Department of Agricultural and Environmental Engineering, Federal University of Technology, Akure. Akure is located within the humid region of Nigeria at Latitude $7^0$16'N and Longitude $5^0$13'E. Akure has a land area of about 2 303km^2 and is situated within the Western upland area. The study area has elevation between 300 and 700 meters above the mean seas level and mean rainfall ranges between 1300 mm to 1500 mm.

Soil Physical and Chemical Properties of the Experimental Site

The descriptive statistics of the soil properties at the experimental site before and after the experiment are presented in Table 1. The site has a mean soil texture (USDA method) of sandy clay loam in the top soil which forms mainly the agricultural layer required for the cultivation of shallow rooted crops. The soil is mainly sandy and it allows downward flow of water, which requires constant irrigation. Bulk density of the soil was determined by core method using 20 cm long by 4.4 cm diameter of cylindrical can. The soil moisture content was determined once in 2 weeks at 20 and 40 cm depth using gravimetric method. The soil chemical properties and particle size distribution were determined in the laboratory using standard procedures. Rainfalls were measured during the experiment with the aid of automated rainguage installed at the experimental site. The mean bulk densities of soil at the experimental site at depths 0-20 cm and 20-40 cm are 1.32 and 1.44 g cm^{-3} respectively.

Table 1. Physical and chemical properties of the soil at depth 0-40 cm

Parameters	Before planting	After planting
pH	4.76	5.07
P(mg/kg)	8.76	6.98
Na(cmol/kg)	0.53	0.43
Ca(cmol/kg)	3.10	2.90
Mg(cmol/kg)	1.30	1.10
K(cmol/kg)	0.60	0.24
CEC(cmol/kg)	12.14	9.26
O.C.(%)	1.21	0.88
O.M.(%)	2.08	1.52
N(%)	0.12	0.08
Sand(%)	52.80	52.80
Clay(%)	31.20	33.20
Silt(%)	16.00	14.00

P=Phosphorus; Na=Sodium; Ca=Calcium; Mg=Magnesium; K=Potassium; N=Nitrogen
C.E.C= Cation Exchange Capacity; O.C=Organic Carbon Content; O.M=Organic Matter Content

Experimental design and layout

An area of 10 m x 60 m was first slashed, ploughed and harrowed to ensure good soil tilth for crop growth and 10m x 10m of the prepared bed was divided into sixteen seed beds using drip irrigation system. A variety of Okra *(Abelmoschus esculentus)* was planted at equal distance of 90cm within rows and 50cm between rows. Cultural practices such as thinning, weeding and controlled of pests and diseases were carried out appropriately. The sixteen plots consist of four treatments (2m x 2m) with four replicates each in a complete randomized block design as shown in Figure 1. Each drip line was installed at a spacing of 90cm on the treatment blocks making a total of three drip lines on each treatment blocks. Each of the treatments blocks was connected to a main pipe which supplies water from the reservoir placed at higher elevation on the field. Catch-cans (2600 cm^3) were arranged in each treatment to harvest water from the emitter on the drip lines. The volume of water harvested over each treatment area with respect to the time considered was measured using a measuring cylinder. The irrigation water amount of 670.81 cm^3 was applied at irrigation interval of 2 days with average evapotranspiration of 4 mm/day. The irrigation time of 90 minutes was recorded for 100% FIT while 72 minutes, 54 minutes and 45 minutes were recorded for 80% of FIT (or 0.80 FIT), 60% of FIT and 40% of FIT respectively.

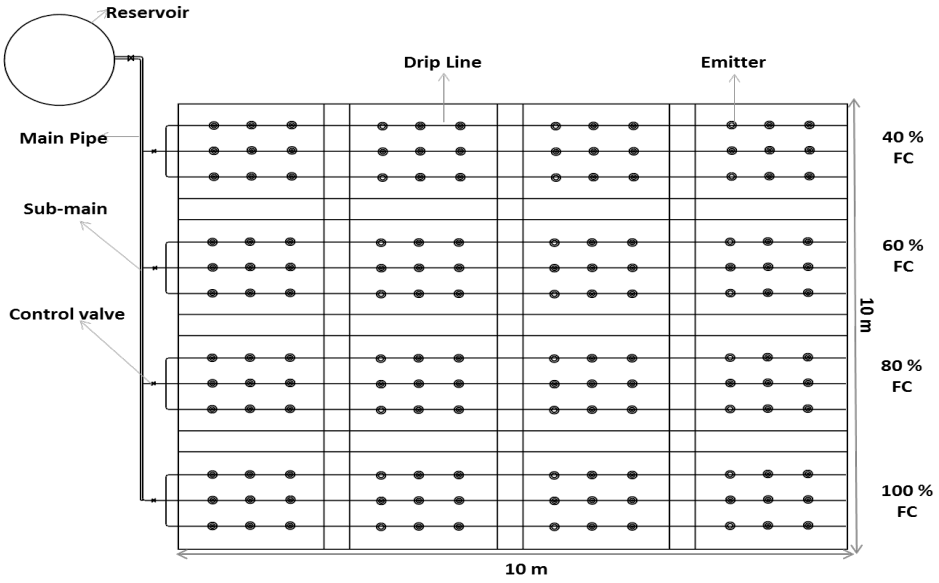

Figure 1. Experimental layout

Okra yield Measurement

Okra pod yield was determined at maturity. The yield was harvested manually in batches from the field and weighed using weighing balance of good precision of 0.01g. Water consumptive use was determined using soil water balance method (Hillel, 1998) and water use efficiencies were also calculated.

$$ET = I + P \pm \Delta S - R - D \quad (1)$$

Where;

ETc=evapotranspiration (mm)

P=precipitation i.e. rainfall (mm); I=water applied by irrigation (mm)

DP=deep percolation (mm); R=runoff (mm); ΔS = change in soil water storage (mm)

Runoff and deep percolation were assumed negligible because of occasional rainfall were observed been inadequate enough to cause runoff and deep percolation into soil and only crop water requirement at the required depth was applied through drip irrigation system.

Water use efficiency/ Irrigation Water Productivity

Water use efficiency is defined as a ratio of biomass accumulation, which is usually expressed as carbon dioxide assimilation, total dry matter yield, or crop grain yield, to water consumed, expressed as transpiration, evapotranspiration, or total water input to the system. Water use efficiency can be divided into irrigation water use efficiency (IWUE) and crop water use efficiency (CWUE). Which is calculated using Equations 2-3;

$$IWUE = \frac{yield}{\text{amount of irrigation water applied}} \quad (2)$$

$$CWUE = \frac{yield}{\text{total crop evapotranspiration}} \quad (3)$$

Results and Discussion

The site has a mean soil texture (USDA method) of sandy clay loam in the top soil which forms mainly the agricultural layer required for the cultivation of most shallow rooted crops. The soil is predominantly sandy and it allows downward movement of water, which will require constant irrigation when use for cultivation. The source of irrigation water at the experimental site was from a borehole. The borehole has a capacity

to supply irrigation throughout the growing season. The result analysis of agronomic response, crop and water productivity are discussed in this chapter.

Soil moisture content variation

It was observed that the soil moisture increases downward in the soil profile as shown in Figures 2. The fluctuations on the soil moisture might be attributed to different application of irrigation water, rainfall at different days after planting and evaporation at the soil surface (Konheya and Alatise, 2013). Figures 2 shows the relationship between the variations of soil moisture content stored under drip irrigation system in each treatment blocks at depth of 0-20cm and 20-40cm and their replicates. The moisture content at field capacity was high because the soil was fully saturated before planting okra. The reduction in the soil moisture content up to 40 days after planting was due to high demand of water by the crop during the flowering and fruiting stage when water is most required. At maturity and harvesting stage small amount of water is required by the crop thereby increases the moisture content of the soil up to 70days after planting. Figure 2a-2d shows that the highest moisture content was observed at first day and lowest at 40 days after planting for both depth (20 cm and 40 cm) for 100FIT, 80FIT, 60FIT and 40FIT treatments.

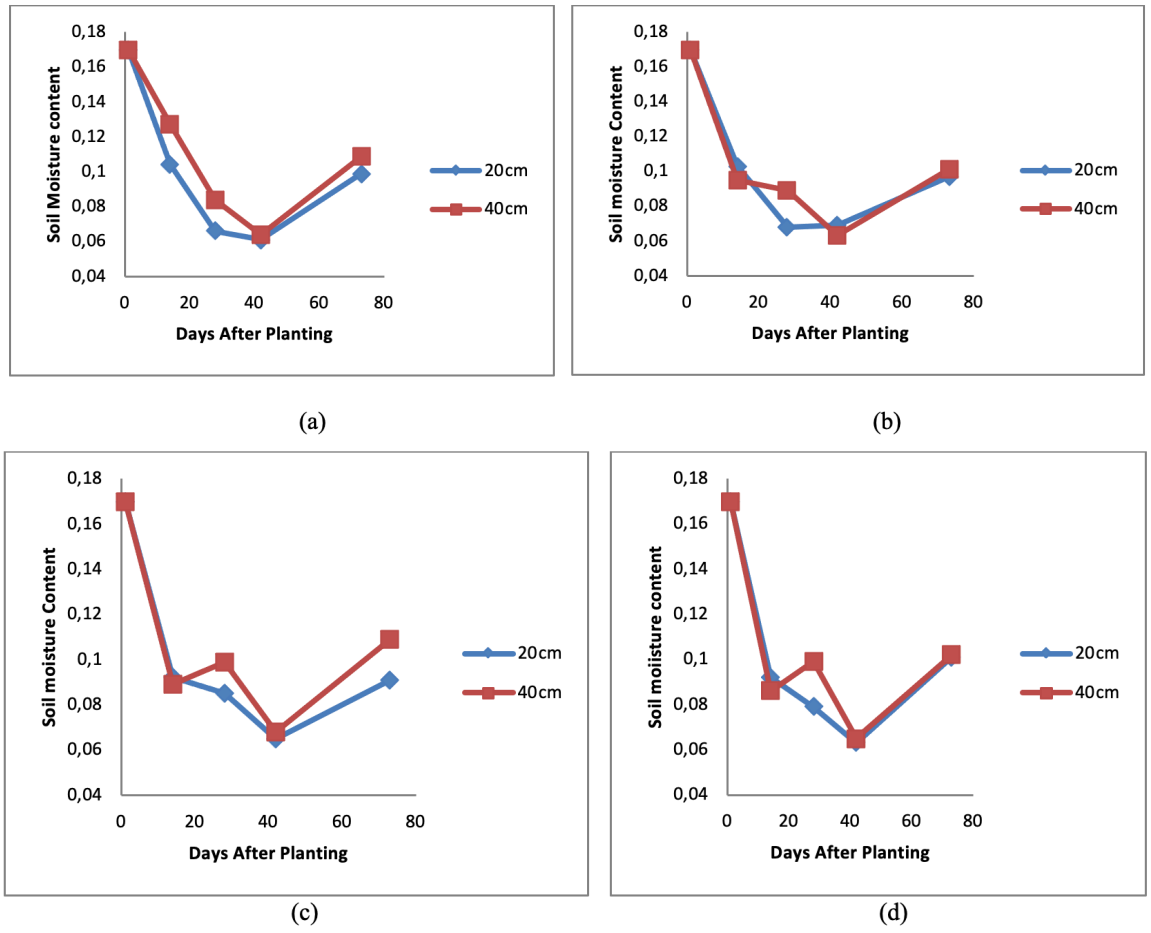

Figure 2. Variation of soil moisture content (a) 100FIT (b) 80FIT (c) 60 FIT and (d) 40FIT treatments block under drip irrigation system.

Agronomic response of okra

The relationship between the growth parameters with respect to days after planting in all the treatment blocks are shown in Figure 3. It was observed that there was no rapid increase in the mean of all the growth parameters during the emergence and the growing season of the crop until it gets to fruiting stage which was 35days after planting. This rapid increases were observed until the crop reaches maturity at 60days after planting when it no longer develop and the leave started dropping to add fertility to soil.

Figure 3a shows relationship between the mean stem girths with respect to days after planting. It was observed that at the emergence there was no different in the mean stem girth of all the treatment blocks. There was a rapid increase in the mean stem girth after the emergence stage to fruiting stage of the crop which occurs at 14 to 49 days after planting which later remain constant till maturity. From Figure 3b it was observed that there were no rapid increase in the mean height of the leaf during the emergence and the growing season of the crop until it get to fruiting stage which was 35days after planting. It was observed that there was no increase from 49days till maturity stage when the okra leaves started dying out and dropping

on soil surface and add nutrient to the soil. Figure 3c shows the graph relationship between the mean numbers of leaves with respect to days after planting. It was observed that at the emergence and growing season there was no increase in the number of leaves in all the treatment blocks. There was a rapid increase in the number of leaves from the flowering to fruiting stage of the crop which occur at 28 to 49 days after planting. At maturity the leaves started dropping and add fertility to the soil. Figure 3d shows the graph relationship of leaf area against days after planting. It can be seen clearly from the graph that the control treatment (100FIT) which received adequate water supply has the highest leaf area. Figure 3e shows the relationship between leaf area index against days after planting. It can be seen clearly from the graph that the control treatment (100FIT) which received adequate water supply has the highest leaf area index. The leaf area index tends to increase up to fruiting stage in all the treatment blocks

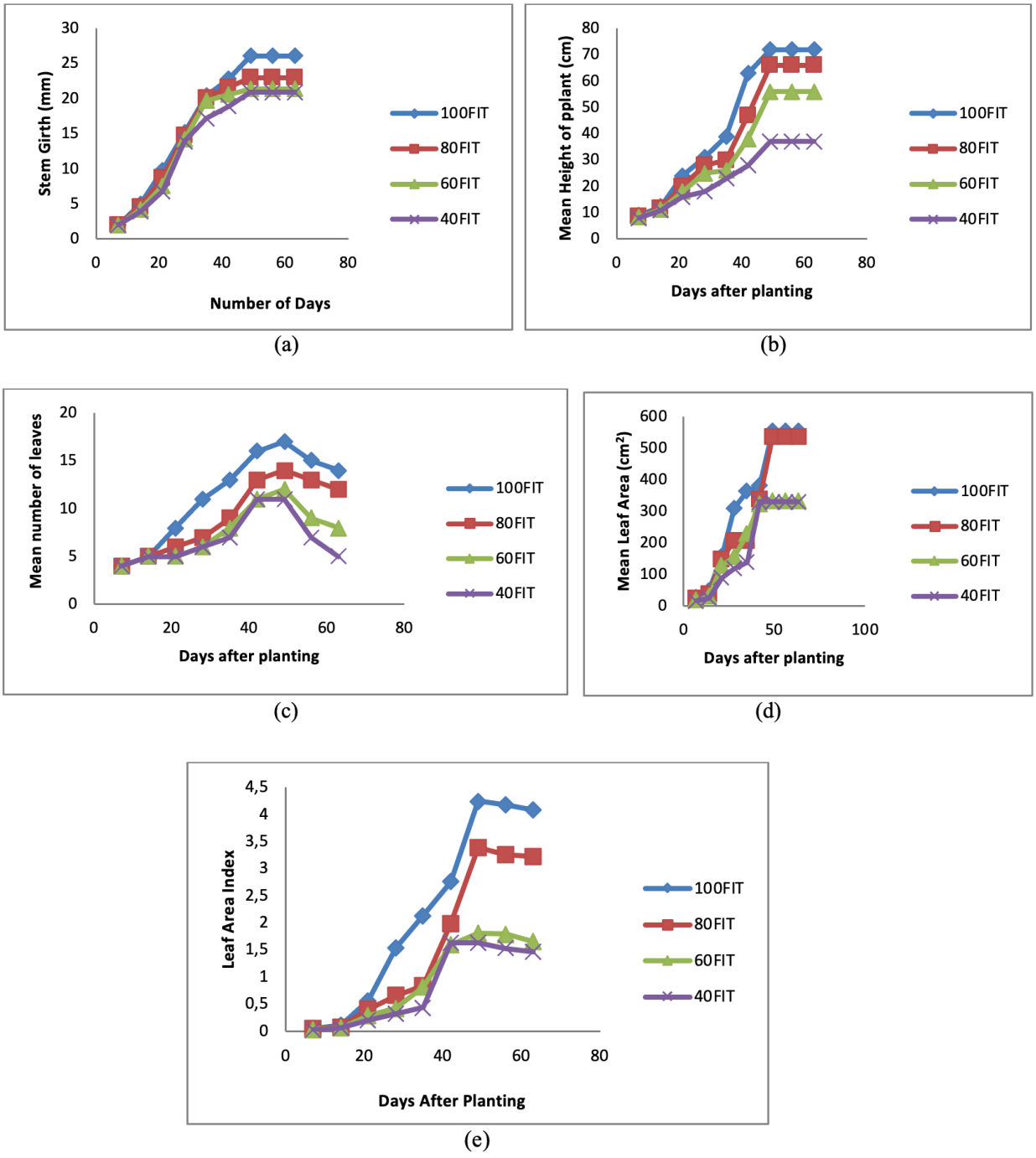

Figure 3. The graph of mean (a) stem girth (b) height (c) number of leaves (d) leaf area (e) leaf area index with respect to days after planting in all treatment blocks

Okra pod yield

The results of okra pod yield in tons per hectare is given in Figure 4. The average values of okra pod yield obtained was highest in the 100FIT treatment and lowest in the 40FIT treatment. The higher yield can be obtained due to the efficient application of water and proper aeration in the root zone which helps for the favorable conditions for growth of the plant. Standard error bars shows that there is no difference in the mean of the yield obtained in 100FIT, 80FIT and 60FIT. This implies that 40% reduction in crop water requirement has no negative effect on the yield of Okra. The yield ranges from 2.05 to 3.60tons/ha which is within the range obtained from that of Babu et al., 2015. Poor yield obtained in 40FIT was due to insufficient water application to meet crop growth requirement.

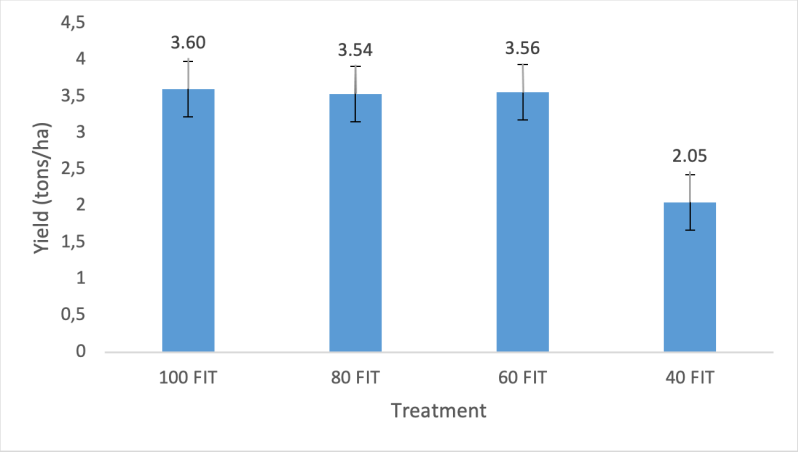

Figure 4. Yield response of Okra in all the treatment block.

Water use efficiecny/ Irrigation Water Productivity

Both the irrigation water use efficiency (IWUE) and crop water use efficiency (CWUE) were calculated and recorded in Table 2 and 3. Results shows that treatment 60FIT has the highest IWUE and CWUE of 0.041t/ha.mm and 0.00139t/ha.mm respectively compared to other treatment blocks and it was considered to be the best among all other treatments. There was significant difference in the IWUE at 5% levels of significance and this was caused by different irrigation water applied which in turn result to different yield being obtained. Reduction in the IWUE and CWUE for 80 and 100FIT shows that addition of water has no positive effect on the yield of Okra rather it only leads to wastage of water since the crop water requirement has been fully met at 60FIT. The arithmetic average (mean) of water applied in treatment block 100FIT, 80FIT, 60FIT and 40FIT are 25.32mm, 23.59mm, 21.60mm and 18.94mm at a time interval of 90mins, 72mins, 54mins and 36mins being considered for water application in each treatment blocks respectively.

Table 2. IWUE (tons/ha.mm) in all the treatment blocks

Treatments	IWUE (tons/ha.mm)				Average
	R1	R2	R3	R4	
100FIT	0.015	0.028	0.024	0.029	0.024
80FIT	0.017	0.031	0.029	0.040	0.029
60FIT	0.037	0.037	0.047	0.042	0.041
40FIT	0.031	0.035	0.031	0.039	0.034

Table 3. CWUE (tons/ha.mm) in all the treatment blocks

Treatments	CWUE (tons/ha.mm)				Average
	R1	R2	R3	R4	
100FIT	0.0072	0.0138	0.0115	0.0141	0.0116
80FIT	0.0069	0.0129	0.0118	0.0162	0.0120
60FIT	0.0127	0.0127	0.0159	0.0145	0.0139
40FIT	0.0081	0.0090	0.0080	0.0100	0.0088

Conclusion

A field experiment was carried out to investigate the effects of varying water application on the growth, yield and water use efficiency of okra. The yield obtained from all the treatment blocks was significantly different at 5% level of significance using standard bar errors and this was as a result of different levels of water applications. The irrigation water amount of 670.81 cm^3 was applied at irrigation interval of 2 days with irrigation time for full capacity for 90 minutes (100 FIT). The average evapotranspiration was 4 mm/day. The highest IWUE and CWUE were recorded at 60FIT as 0.041 and 0.0139 respectively. The yield obtained at 60FIT was 3.56tons/ha which has no significant difference to the yield obtained at 100FIT using standard bar errors. From this research; it was observed that Okra crop irrigated at 60FIT gives the highest thereby 40% of water to irrigate additional land. For farmers this research findings can help in planting and scheduling during dry season using drip irrigation if the amount of irrigation water amount and evapotranspiration are known.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

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

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Research Article

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Types of waste in the context of waste management and general overview of waste disposal in Turkey

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Abstract

In Turkey, there has been an increase in waste management applications within the context of sustainability activities recently. The most important reason for that was the legal requirements for recycling and disposal of the waste generated by the producers. Developing nations still utilize the storage method, while developed countries recycle almost all produced waste to produce raw materials and energy. Although there is an increase in the number of work conducted on waste management in Turkey, the attained levels are still far from satisfactory. This is mainly due to waste recycling or recovery costs. In order to convert the problem of waste into economic benefits, it is of utmost importance to recycle the waste, avoid the transfer of waste to landfills and to conduct waste management. Thus, an environmentally and economically sustainable productivity would be achieved. In the present study, waste management in Turkey was scrutinized. In this context, types and volume of the waste produced in Turkey and utilized disposal methods were discussed. The present study also provided a general overview on waste disposal business processes such as sanitary landfills, composting and recycling in Turkey. Thus, the study aimed to shed light on future studies on waste management.

Keywords: Waste management, Types of waste, Disposal methods, Sanitary landfills, Composting, Recycling

Introduction

It was estimated that the world's population exceeded 7 billion by 2015 (Ağdağ, 2009) and the number of individuals who lived in urban areas doubled or tripled as a result of rapid urbanization. Urbanization, which is a natural consequence of rapid population growth, is not a problem in itself, however it leads to several environmental problems such as damages to public spaces and wetlands, air and water pollution and solid waste generation as a result of random and unplanned growth (Demir et al., 2006).

The need for natural resources and energy increases with global economic growth and population increase, and the indiscriminate use of resources has become a serious environmental threat. Similar to elsewhere in the world, waste management is of great importance to eliminate these threats in Turkey. The environmental damage caused by humanity increases with globalization. The most important among these damages is the rapid consumption of natural resources, associated with the increasing raw material use and production of waste. Thus, it is necessary to separate and assess the waste at the source. This could be achieved by initially changing the waste perception of individuals. The perception of waste, which neglects economic and environmental aspects of waste where it is considered as a financial burden and a form of garbage, should be transformed into an asset that contributes to the economy and the environment (Kocak, 2018). Thus, with the popularity of waste management, the economy and ecology would be considered as a whole, optimum utilization

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of resources would be realized and the harmony between humankind and nature would be sustained both for living individuals and to allow the right of future generations to live in a healthy environment.

International waste management philosophy is described as systematic product and process design and management to prevent and reduce the toxicity in waste and products, to protect and preserve all resources, and to prevent waste incineration and burial. The environmental and waste management philosophy of the Environmental and Urban Development Ministry in Turkey is to review the causes of waste to prevent or minimize waste and separate collection and recycling of the waste at the source (Republic of Turkey Env. U.M., 2016).

It is important to design products that could be recycled safely and economically, that are reusable, compostable or could be converted into biogas in order to prevent the waste in production and businesses. This approach where the waste is classified at the source, collected adequately and waste production is prevented would lead to sustainability of natural resources. In the present study, 1) waste management in Turkey 2) the types of waste produced in Turkey 3) and disposal of these waste was investigated and certain personal recommendations were presented to improve the conditions in Turkey and improve the ease of application and efficiency.

Waste Management in Turkey

Waste is significant for both human and environmental health as well as the economy in Turkey similar to the rest of the world. There is a need for a planned waste management in order to remove the waste that should be regularly collected, transported, stored and disposed in urban areas to protect the environmental health before causing major problems (Yılmaz and Bozkurt, 2010).

Since the early years of the Republic, solid waste management, conducted as sanitation and public health services by the Ministry of Health, was evolved into an environment-based approach in the 1970s as a result of the increasing interest in environmental problems in the world and led to the inclusion of waste management in the realm of Ministry of Environment with its establishment in 1991. Developed countries completed the solid waste management process in the '80s, and began to seriously discussed phenomena such as sustainable waste management, waste ethics, when discussing the serious cases such as waste management, ethics, and waste management ethics, the topic was started to be perceived as a problem that needs to be managed in the following years in Turkey due to the impetus created by international developments, although the developments in waste management in Turkey were rather slow (Drinking, 2012).

In Turkey, where the traditional method of landfills is used to dispose the waste, the explosion that occurred at Ümraniye landfill on April 28, 1992, killing 39 people, was a cornerstone of a new era in solid waste disposal (Turan et al., 2009).

Due to these requirements, collection and removal of solid waste are conducted within the context of Solid Waste Control Regulations and related directives (Regulation on Control of Medical Waste, Regulation on Control of Hazardous Waste, Regulation on Control of Packaging Waste, Battery Waste Control Regulation, Oil Waste Control Regulation, Excavation Soil, Construction and Demolition Waste Control Regulation) issued on 14.03.1991 (No: 20814) based on the Environmental Law no: 2872. Municipalities are authorized and responsible for the collection, transportation, storage, recycling and disposal of waste under the Municipal Law No. 5393 and the Metropolitan Municipality Law no. 5216 (Yılmaz and Bozkurt, 2010).

Recent studies reported the annual waste collection volume in Turkish cities as 25.28 million tons, while only 27.8% of this volume was recycled with disposal methods and the problems associated with the remaining part were attempted to be solved with landfills (Ağdağ, 2009). These findings demonstrated that there were developments in disposal of solid waste in Turkey, however the performance was not satisfactory.

Several recycling projects are implemented in developed countries and developing countries including Turkey. Although these projects are attractive, several political, social and economic factors are required to achieve success. A determined government, financial support for municipalities and the private industry, maximum collection and recycling, market development and promotion, training and informative studies are required for recycling projects. The operations in recycling facilities are not efficient in Turkey due to low recyclable waste volume, the effect of street collectors, the high plant capacities and inefficient collection operations (Şen and Kestioğlu, 2007).

The most common and implemented separation method in Turkey is separation in the field. However, this method is conducted by waste sorters at landfills under unhealthy and unsafe conditions. Similarly, the waste collectors collect paper and metal waste from the containers on the streets. Although 20% waste recycling is achieved in these processes, unfortunately the rate is quite limited. The most important reasons for that include high operating costs, mistakes in feasibility studies, economically unproductive waste sorting and recycling units, leading to a short operating life (Leblebici, 2001).

Types of Waste in Turkey

In order to assess waste management, initially waste characterization should be determined. In Turkey, 34% of organic waste is used in compost production, 16%, which includes paper and cardboard products, are used as paper pulp, 2%, which includes plastic waste, is used as granules and burrs, 6%, which is glass waste, is used as cullet, 1%, which includes metal waste, can be converted into raw materials such as iron and aluminum (Turkey Env. U.M., 2016).

The phenomenon of waste is defined in various literature and regulations. Demir et al. (2006) defined waste as solid substances with economic value that are not wanted by the owner and should be collected and disposed based on artistic and scientific rules, scientific and engineering principles for the benefit of the society, while in the Regulation on Solid Waste Control, it was defined as solid substances and sewage sludge that are considered trash by the owner and should be disposed of regularly for social peace and in particular, to protect the environment.

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The waste is defined by Palabıyık and Altunbas as substance produced by domestic, commercial and/or industrial activities and disposed of by the owner due to lack of use, and should be disposed regularly due to environmental and human health reasons as well as other social benefits (Palabıyık, 1998), and waste is categorized in four categories (Drinking, 2012): Domestic Waste, Industrial Waste (Hazardous and Non-Hazardous Waste), Medical Waste, and Special Waste.

Although waste is a general concept that includes all waste products except hazardous waste, liquid waste and atmospheric gases, urban waste is caused by residential, commercial, institutional, construction-demolition and urban services (Badram et al., 2006). It is possible to classify the methods used to dispose the waste that occurs as a result of human activities as dump sites, sanitary landfills, composting, reuse, recycling, recovery and incineration (Palabıyık and Altunbaş, 2004).

Review of annual waste collection by the municipalities in Turkey revealed that the volume has increased every year. This waste include the domestic waste produced by daily activities and could be categorized as paper, glass, cardboard, plastic, tin and fuel waste. The vegetable market, school, office, agricultural, tree and land waste that are not produced at homes, but collected by the municipalities as garbage, is also considered as domestic waste (BKAI, 2016). The mean municipal waste per capita and municipal waste collected in Turkey are presented in Figures 1 and 2.

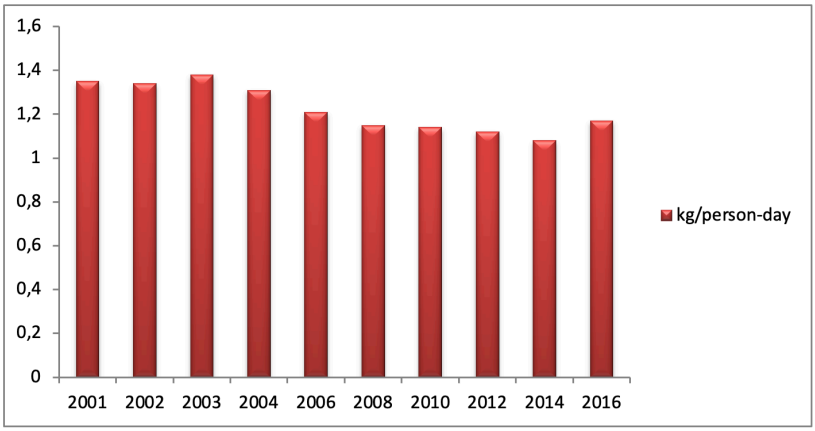

Figure 1. The mean municipal waste per capita in Turkey

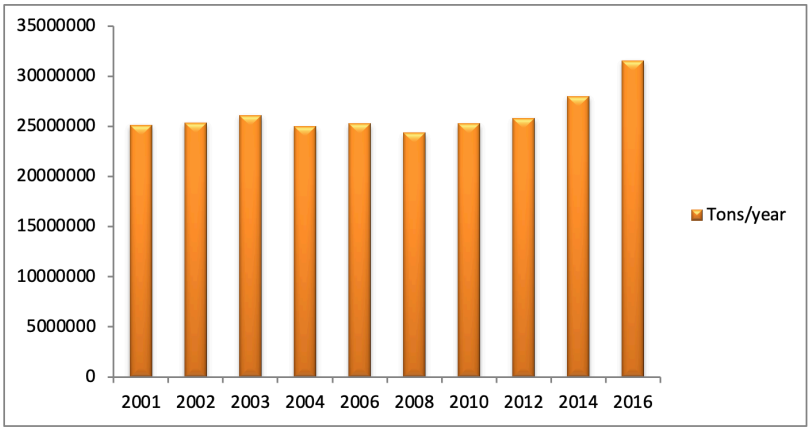

Figure 2. Waste collected by the municipalities in Turkey

Waste Disposal Methods in Turkey

It is possible to classify the methods used to dispose the solid waste produced by human activities as dump sites, sanitary landfills, composting, reuse, recycling, recovery and incineration.

Sanitary landfills

Based on the costs, sanitary landfill method is widely used for waste disposal in Turkey. The management of leachates and gases produced in landfills is extremely important in order to classify a landfill as sanitary landfill. In other words, measures should be taken for collection, removal, treatment, disposal and reuse of the leachate and gases.

The objective of sanitary landfills is to remove the solid waste from residential areas and prevent the damages caused the solid waste that could not be economically recovered for reuse through mechanical, chemical and biological processes or produced by these processes and hazardous for human health or for other living organisms and damage the environmental aesthetics (BKAI, 2016).

Sanitary landfills that are built in accordance with technical standards, such as suitable site selection and environmental protection measures are the most effective method to dispose of the waste. The sanitary landfill waste volume in Turkey is presented in Figure 3.

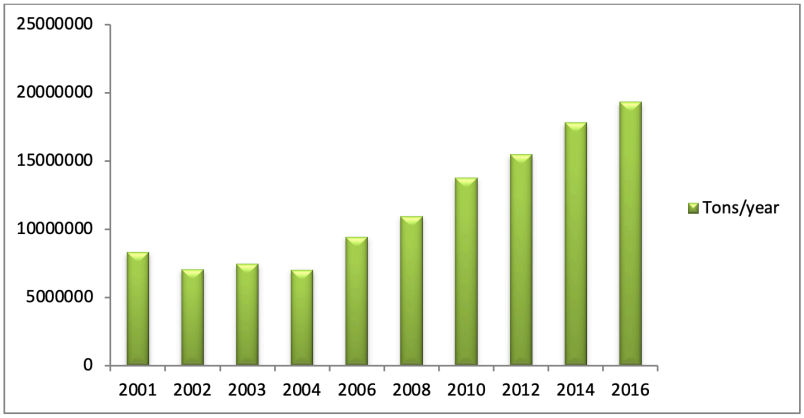

Figure 3. Sanitary Landfill Volume in Turkey

Unsanitary landfills

It is a method used in undeveloped or developing countries where solid waste is removed from the human environment by randomly disposing the waste to open spaces without any precautions. This method leads to serious problems such as formation of dust clouds due to wind in landfills, the air pollution caused by the formed gases, the environmental and visual pollution caused by the solid waste spread over a large area, and the infectious diseases caused by the animals that inhabit and feed in these areas.

The disposal of waste randomly in open spaces, seas or lakes leads to disturbing views and bad odor. The use of such unsanitary landfill methods that adversely affects soil, air, water and human health in Turkey almost ended in recent years. The volume of waste randomly disposed to rivers, streams and lakes in Turkey is presented in Figure 4.

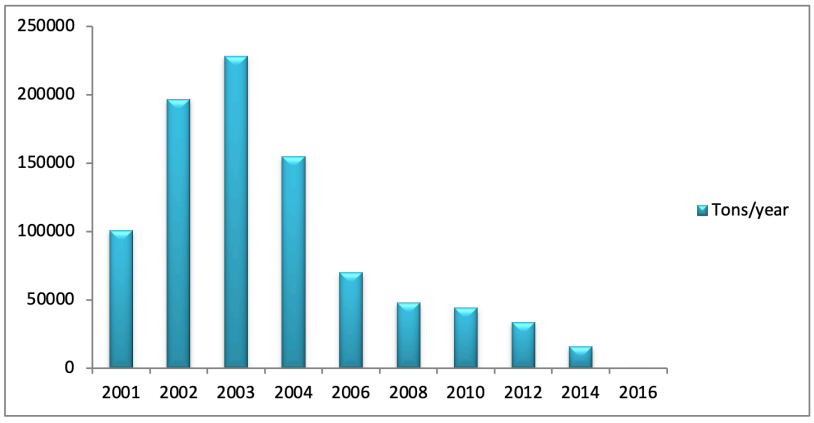

Figure 4. The waste disposed to rivers, streams and lakes in Turkey

Incineration

It is a method used to make solid waste hygienically harmless, to reduce their volume and to obtain energy from this type of waste when it is economical. In order to obtain effective results with this method, the waste must be rich in combustible material, while the toxic gases released during the combustion and the storage of highly hazardous residues after the incineration process lead to significant problems.

As a result of the waste incineration process, about 350 kg ash and 650 kg gas waste (6000 - 7000 m^3) are produced. Incineration method is generally preferred in developed countries with land shortage and incineration costs are very high. Open space waste incineration volume decreased in Turkey during recent years and it is presented in Figure 5 (He et al., 2009).

Recycling

This is the method that includes both reuse (reuse of waste without any process except cleaning for several times) and recycling concepts and conversion of waste into other products or energy via physical, chemical or bio-chemical processes using the properties of the waste (Gören, 2005). Recycling is the process of introducing the waste into the production process as secondary raw material after physical and/or chemical processes.

It is the process of converting waste into other products or energy using physical, chemical or biochemical methods based on the properties of the components of the waste. The recycling method contributes to the national economy in terms of raw materials. Pre-treatment is important in recycling processes and in the first step, waste volume is reduced. Certain equipment is used in the volume reduction processes that are utilized for temporary storage or transportation of waste.

First, it is possible to mention cullet crusher equipment. Its function is to crush container, bowl, bottle cullet waste, etc. into pieces of a few millimeters and to reduce the material volume.

Another equipment is the hydraulic plastic and paper press, whose function is to concentrate the material such as

cardboard, paper, plastic bottles and convert this waste into bales. 200-500 kg bales could be stored on top of each other. Such a press could be used where the paper, cardboard or plastic bottle waste volume is high.

Other important equipment is the fly press for metal beverage cans, and it could be used by the staff in cafeterias to raise awareness. Thus, utilized beverage cans could be compressed before disposal into separate collection boxes.

Finally, fluorescent bulb crusher equipment can be mentioned. This equipment can be used in places that frequently change fluorescence bulbs and has limited storage space. It contains a filter system that separates dust and mercury vapor. Thus, the environmental risks due to mercury/dust emissions that occur when the fluorescent bulbs are involuntarily broken during recycling could be avoided.

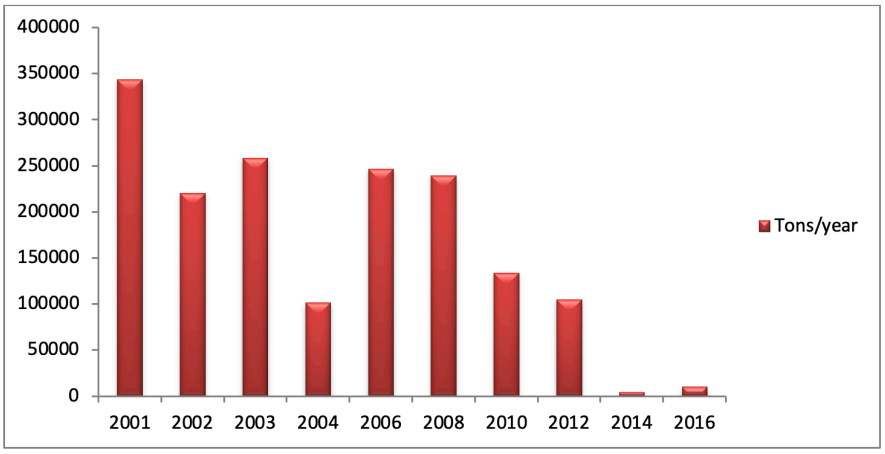

Figure 5. Open space waste incineration volume in Turkey

Composting

The other waste disposal method used in Turkey is compost production and composting is the transformation of the organic parts of the waste into mold by microorganisms in an oxygenated or oxygen-free environment. Composting takes place at 65-75°C. Raw waste is heated by the heat generated by bacteria metabolism, and then gradually cooled down and separated into pieces and transformed into soil. During the process, the water in the waste partially evaporates and partially discharged as leachate. A portion of the solid material is converted to heat, thus leading to a significant loss of substance. A ton of waste yields 200 - 400 kg compost (Republic of Turkey Env. U.M., 2016).

Compost processes have certain advantages and disadvantages. Open compost piles technique do not have initial investment costs, require very little labor, the capacity is flexible and occupies little space. Disadvantages include visual pollution, odor and pest problems when not operated well, lack of protection against rain and sun, and long composting process.

In single compost box technique, the investment cost is low, it is suitable for small capacity facilities, there is no need to mix and move the compost, it provides protection against rain and sun and requires little labor. Disadvantages include lack of a domestic manufacturer in Turkey, visual pollution, long composting process, odor problem when not operated well, and lack of irrigation requirement.

In double, triple or quadruple compost box line technique produced with recycled plastic, the investment cost is low, processing volume is flexible, it is suitable for mid-size buildings, it provides protection against rain and sun, contributes to plastic recycling, and requires little labor. Disadvantages include lack of a domestic manufacturer in Turkey, visual pollution, long composting process, odor problem when not operated well, and lack of irrigation requirement.

In single or multiple compost box technique produced with waste pallets, the investment cost is low, processing volume is flexible, it is suitable for mid-size buildings, it provides protection against rain and sun, contributes to wood recycling, and requires little labor. Disadvantages include visual pollution, long composting process, irrigation requirement, and odor problem when not operated well.

Finally, the technique of composting machine could be automatically operated to compost different volumes and deployed anywhere, creates no odor and pest problem, and it is convenient and rapid for large buildings. Disadvantages include high cost, requirement for trained technicians, leachate discharge and treatment. It only produces pre-compost. The annual waste volume disposed to composting facilities is presented in Figure 6.

Within the context of the disposal methods generally described above, the number of plants in Turkey and the tons of waste disposed in these plants are presented in Table 1.

Review of waste recycling facilities demonstrates that there has been an increase in the number of facilities and waste conversion column in 2016 when compared to 2014.

The distribution of sanitary landfill, unsanitary landfill, composting and recycling facilities in Turkey and the waste recycled in these facilities in tons are presented in Figure 7.

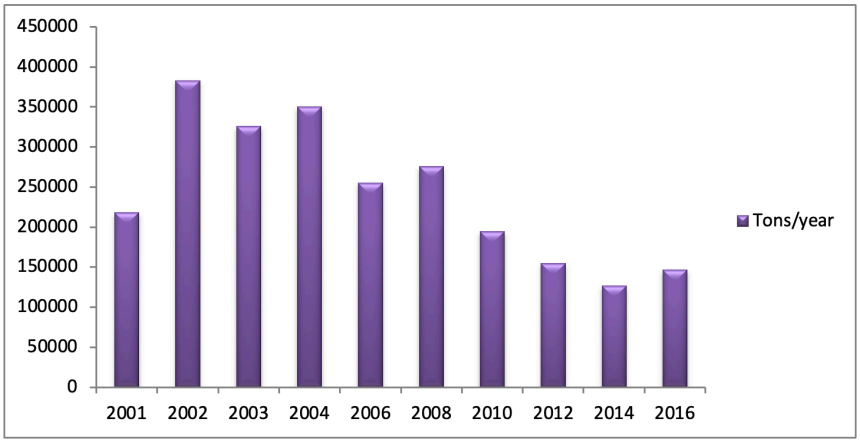

Figure 6. The annual waste volume disposed to composting facilities in Turkey

Table 1. Waste volume in Turkey based on the disposal method

Years/Disposal Method	2014		2016	
	# of plants	Waste volume (ton)	# of plants	Waste volume (ton)
Sanitary Landfill Plant	113	41.281.755	134	43.815.135
Incineration Plant	4	42.882	6	310.127
Waste Recycling Plant	868	19.724.241	1.558	33.083.400
Compost Plant	4	94.019	7	140.467
Co-incineration plant	39	532.343	35	738.908
Other Recycling Plants	825	19.097.879	1.516	32.204.025

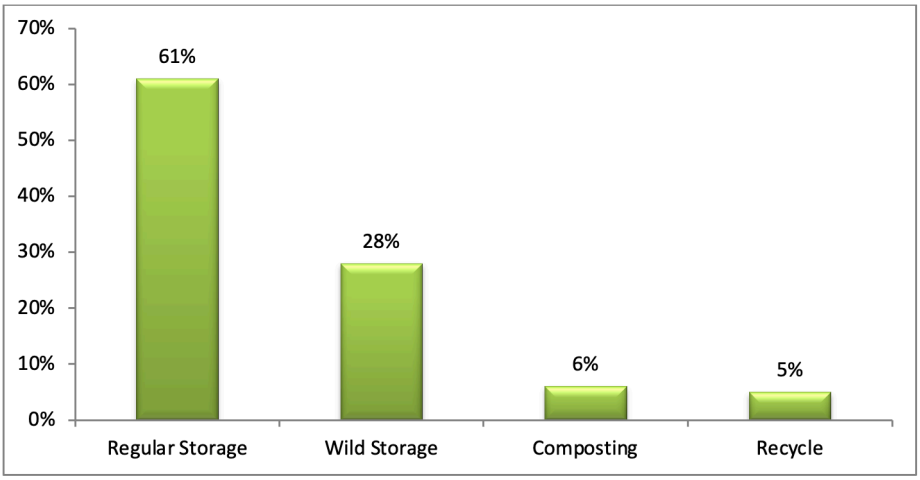

Figure 7. The distribution of waste disposal processes in Turkey

Conclusion

In the present study, waste management in Turkey, types and volume of waste produced in Turkey, as well as waste disposal methods were discussed. The study also provided information on waste disposal methods such as sanitary landfill, composting and recycling processes and the general status of these processes in Turkey to provide a basis for future studies on waste management.

The review of the types of waste produced in Turkey and waste disposal systems identified certain significant findings on waste management in Turkey. As is known, several municipalities and licensed facilities, collect the mixed waste from both homes and businesses, separate part of this waste in sorting facilities and compost the rest. Although these processes are attempted to be conducted in an organized manner, composting processes are not widespread in Turkey

and conducted only in a preliminary level.

General review of waste disposal processes in Turkey would demonstrate that 61% of the waste is disposed in sanitary landfills. This is followed by unsanitary landfills, which are no longer utilized in developed countries and where 28% of the total waste is disposed in Turkey. 8.100.000 tons of waste has been accumulated in open spaces due to unsanitary landfills and poses an environmental risk. This needs to be remedied in accordance with the relevant laws and regulations. This type of open space unsanitary landfills should be replaced by sanitary landfills. Among the waste disposal methods, only 6% of the total waste is disposed with composting processes. As mentioned-above, the percentage of this application, where the waste is transformed into the environment in the most efficient way, is still at the beginning level in Turkey and it is open to development. The last in waste disposal methods implemented in Turkey is the recycling operations with a share of 5%, which is extremely low.

Although important studies have been conducted on waste in Turkey, these studies are far from sufficient. This is mainly due to the high recycling costs that are considered as a major burden. In the long term, this type of practices would provide much more than the initial costs due to the national development they would lead to. Thus, both public spaces and private corporations would produce more, providing economic growth and development on one hand, and on the other, when they adopt an environment-friendly sustainable approach, they would guarantee their existence in the future.

Local governments often have to opt for external financing for high cost solid waste services, since their own resources are usually insufficient. The related institutions and organizations should be made aware that waste management could lead to savings and profits in the long run, despite the initially high recycling or recovery costs. Training and seminars should be conducted to raise awareness and to lay the foundation for sustainability studies to create a more habitable environment for future generations.

In medium and long term, solid waste management should be conducted based on socio-economic, technical, demographic and geographical conditions in Turkey and active participation of ministries and other central government institutions and organizations, and local governments, municipalities, businesses, NGOs, associations and individuals is required.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

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

Ethical approval

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Data availability

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Consent for publication

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Numerical response of green lacewing *Chrysoperla carnea* on different preys (*Aphis fabae* and *Acyrthosiphon pisum*)

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Abstract

Aphids can caused significant economic losses in agricultural productions because they have the ability of fast reproduction. In other hand, they are vector of several plant diseases and viruses. Therefore, in the scope of biological control studies, it is important to reveal the numerical responses of the species to determine the effect of green lacewing predator acting on aphids. In this study, the numerical response of *Chrysoperla carnea* on two aphids (*Aphis fabae* and *Acyrthosiphon pisum*) was tested, and the reproductive abilities of the green lacewing were determined. In the experiments, aphids at different densities (5, 10, 20, 40, 80 and 160) were offered to the development periods of the predator insect separately, and their development was recorded. The experiments were carried out in the laboratory at 26±1°C and 60±5% Relative Humidity (RH). The development of predator, fecundity and numerical response parameters (Efficiency of Conversion of Ingested Food (ECI), Prey Usage Efficiency) were significant differences according to prey density. Finally, the ECI values were 142.15, 160.58, 184.99, 213.91, 229.48 and 199.44 for *C. carnea* fed on *A. fabae*, respectively; and 146.43, 173.09, 200.05, 214.04, 226.01 and 205.26 for *C. carnea* fed on *A. pisum*, respectively.

Keywords: *Chrysoperla carnea,* Green lacewing, *Acyrthosiphon pisum, Aphis fabae*, Numerical response

Introduction

The family of Chrysopidae, which are distributed worldwide, is predator of aphids, thrips, and whiteflies (Ridgway and Jones, 1968; McMurtry et al., 1970; Jeppson et al., 1975; Mansell, 1983; Stark and Whitford, 1987). The Chrysopidae family is important in all and biological control studies because their presence in the natural ecosystem, ease of production in the laboratories and the field for scientific studies, and they have high search and consumption power to the pests mentioned above (Jeppson et al., 1975; Obrycki et al., 1989; Bozsik, 1995). *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) is a very common polyphagous species and observed in field and agricultural areas (McMurty et al., 1970; Jeppson et al., 1975; Varma and Shenhmar, 1983; Stark and Whitford, 1987; Jokar and Zarabi, 2012). Green lacewing plays an important role as a biological control agent in greenhouses and the fields (Venkatesan et al., 1997). The larvae of *C. carnea* start feeding after hatching immediately on a wide range of pests. *C. carnea* has fed on lepidopteran larvae, mites, mealybugs, crustaceans, thrips, aphids and whiteflies mature and nymph stages (Lingren et al., 1968; Ridgway and Jones, 1968; Lingren and Green, 1984; Hagley and Miles, 1987; Syed et al., 2005; Sattar et al., 2007; Sattar, 2010; Jokar and Zarabi, 2012; Batool et al., 2014).

From the literature, aphids cause economic losses on the different plant families in the field and need to be controlled. They cause damage to plants into two directions, first, the direct damage by feeding on host plants and the second by indirect damage transmission of plant viruses. The aphid can produce high reproduction in short time and chemical pesticides are

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intensively used to control (Lodos, 1982; Catherall et al., 1987; Kovalev et al., 1991; Elmalı and Toros, 1994). Some control methods have not negative effects on nature and human health should be emphasized to avoid the negative effects of these chemicals. For this reason, it is very important to find alternative way to control aphids, the numerical response of the species to determine the potency of green lacewing *C. carnea*, for the effective way against aphids and also subjected of this study.

Materials and Methods

Production of Broad Bean (*Vicia faba*)

The Broad beans were used as host plants in the experiment. The plants were grown in the production room under controlled conditions 26±1ºC and 60±5% Relative Humidity (RH) at the Plant Protection Department, Agriculture Faculty, Yozgat Bozok University. For this purpose, broad bean seeds were planted to in the small plastic pots (1 liter) and paper cups filled with soil mixture (1:1:1 ratio of soil: peat: perlite mixture) and seedlings were left under the light conditions (16L: 8D) after grown. When the height of the cultivated plants became suitable (4-6 leaves) for the infestation of aphid and left to let the aphid reproduction for two days, they which were used in this experiment. The experiment was repeated periodically as long as the experiments continued. The aphid cultures were weekly maintenance on the plants.

The Culture of Aphids (*Aphis fabae* and *Acyrthosiphon pisum*)

In this study, *Aphis fabae* and *Acyrthosiphon pisum* individuals were used as food for *Chrysoperla carnea* predator that were obtained from Biological Control Research and Application Center, Faculty of Agriculture, Isparta University of Applied Sciences and mass production was carried out at the Plant Protection Department, Agriculture Faculty, Yozgat Bozok University. The broad beans were reared in cages (50x50x50 cm) in the controlled environment 26±1ºC and 60±5% Relative Humidity at the Plant Protection Department, Agriculture Faculty, Yozgat Bozok University. The host plants were infested with both species of aphid *A. fabae* and *A. pisum*.

The Culture of *Chrysoperla carnea*

The predator green lacewing *Chrysoperla carnea* that used in the study were collected from clover fields around Isparta and Yozgat in Turkey by using net and mouth aspirator. The collected predator were brought to the laboratory and then placed in plastic containers covered with tulle cloth. The predator was feed on yeast extract + honey + water mixture and was placed in containers (Kişmir and Şengonca, 1981; Tireng et al., 1999). The tulle cloths were replaced with strips cloth piece to let *C. carnea*'s females laying eggs. All experiments were carried out in cages at laboratory conditions 26±1ºC and 60±5% Relative Humidity.

Numerical Response Trials

The experiment was conducted in the laboratory after the predator was laid eggs in mass-produced. The larvae of green lacewing were located into separate petri dish (one larva per petri-dish) after emerged. A number of aphids (5, 10, 20, 40, 80, and 160) at 2nd and 3rd nymph's stage were given to each larva in per petri dish. The number of aphids were consumed by the larva and recorded every day and the missing of aphid number was added. The experiment was continued until the predator became pupa, and then emerged to the adult. The male and female of predators were marked separately by using the paint that does not damage the insect body according to the number of preys given per petri-dish. Marked predators were mating in petri-dish and after one day were placed into separate petri dish. The new predator was feed on the different number of aphids according to the literature; while the number of eggs that laid by predator was recorded. Numerical response trials were arranged separately for each period and consist of 50 repetitions. The experiments were carried out at the laboratory conditions which were 26±1ºC and 60±5% Relative Humidity.

Numerical response data were calculated using depends on the equation of Holling (1959) and graphics were arranged. In addition, the relationship between the number of eggs left by the predator and different prey densities were described on graphics. The experiment was conducted in a completely randomized design (CRD). The numerical response of predatory larvae of green lacewing to various prey densities of two species of aphids was express by fitting the data to the Omkar and Pervez (2004) and Omkar and Kumar (2013) equations.

ECI (Efficiency of Conversion of Ingested Food) =(Number of eggs laid)/(Number of consumed food) ×100 (Omkar and Pervez, 2004).

Prey Usage Efficiency(%)=(Number of consumed food)/(Number of preys given) ×100 (Omkar and Kumar, 2013).

Statistical Analysis

SPSS (Ver. 17), Minitab (Ver. 16) programs were used in the statistical analysis of the data that obtained from the numerical response of the predator in laboratory studies. Using the data that obtained to calculate the life table parameters of varying prey densities of the predatory insect were obtained. The age-related life table of the predator on two different foods were created based on the Euler-Lotka equation ($\sum e^{(-rm.x)}.lx.mx=1$) (Birch, 1948). All parameters were calculated using the RmStat-3 (Özgökçe ve Karaca, 2010). The data were subjected to the Tukey multiple comparison test in order to compare the results of the trials.

Results and Discussion

The results indicated that the numerical response values of *Chrysoperla carnea*, increased on two different aphid species (*A. fabae* and *A. pisum*) with different densities (5, 10, 20, 40, 80 and 160) that given to each development instar of *C. carnea*. It was observed that the amount of consumption increased as the density of aphids increased in all larval periods of *C. carnea* feed on *A. fabae*. Similarly, the results showed that increased in consumption of aphids by green lacewing between different instars feeding at the same number of aphids that given to the green lacewing. Moreover, the total of consumption number of aphids decreases when the number of prey density was low ($F_{Instar1}$:841.98; $P_{Instar1}$:0.001 / $F_{Instar2}$:1931.32; $P_{Instar2}$:0.001 / $F_{Instar3}$:3311.71; $P_{Instar3}$:0.001 / F_{Total}:174.02; P_{Total}:0.001). From

the results obtained from this study observed that the amount of consumption was increased as the aphids density increased in all larval instars of *C. carnea* feeding on *A. pisum*. The prey consumption rate increased with rise of aphids number such as the 1st instar of green lacewing consumption rate 14.89 while the 3rd instar consumption was 52.85 when gave 160 aphids per instar. The predicted number of aphids eaten when 80 were given was 12.63 for the 1st instar of green lacewing and 44.39 for the initial 80 aphids for the 3rd instar of the predator, while, the lowest initial prey density 5 and 10 aphids the 1st instar of *C. carnea* was 4.70 and 4.99 respectively of offered 5 aphids and 8.53 and 9.99 respectively of offered 10 aphids from the species *A. fabae*. The results showed the cumulative consumption of green lacewing feeding on *A. pisum* density at different number of aphids that no significant consumption rate between 1st, 2nd and the 3rd instars 4.70 when offered 5 aphids while there was a significant consumption rate when offered 80 and 160 prey number (Table 1). When the total consumption rate determined the consumption was the highest prey density, while consumption decreases when the prey density decreases. In addition, a statistical similarity was observed between 80 and 160 preys in the second larval period and total consumption ($F_{Instar1}$: 1222.81; $P_{Instar1}$: 0.001 / $F_{Instar2}$: 2657.66; $P_{Instar2}$: 0.001 / $F_{Instar3}$: 5453.36; $P_{Instar3}$: 0.001 / F_{Total}: 183.69; P_{Total}: 0.001) (Table 1).

Table 1. Daily consumption amounts of different biological periods of Chrysoperla carnea on Aphis fabae and Acyrthosiphon pisum

Biological stages	Prey Densities (*Aphis fabae*)					
	5	10	20	40	80	160
Instar1	4.70±0.037 f	8.53±0.077 e	11.70±0.104 d	14.07±0.200 b	12.63±0.112 c	14.89±0.203 a
Instar2	5.00±0.000 f	9.79±0.42 e	16.34±0.131 d	21.76±,0.221 c	29.68±0.253 b	33.09±0.439 a
Instar3	4.99±0.007 f	9.99±0.011 e	19.26±0.060 d	33.33±0.310 c	44.39±0.387 b	52.85±0.557 a
Total	52.76±3.22 f	102.44±6.43 e	169.70±11.00 d	291.00±10.30 c	379.80±12.70 b	435.50±19.60 a
Biological stages	Prey Densities (*Acyrthosiphon pisum*)					
	5	10	20	40	80	160
Instar1	4.54±0.039 e	8.62±0.058 d	11.16±0.094 c	13.06±0.154 b	12.75±0.126 b	14.35±0.116 a
Instar2	5.00±0.000 e	9.73±0.034 d	15.38±0.133 c	22.39±0.189 b	30.38±0.309 a	31.04±0.294 a
Instar3	5.00±0.005 f	10.00±0.005 e	19.16±0.075 d	36.60±0.205 c	44.93±0.358 b	50.38±0.412 a
Total	54.12±3.01 e	106.42±5.56 d	167.20±10.30 c	303.60±10.10 b	378.60±13.70 a	410.10±16.80 a

Different letters on the same line show that there is a statistically differences between the averages according to the Tukey test ($p<0.05$).

In this study, the prey was used to determine the efficiency of *C. carnea* on two different aphids and according to the calculation made in the equation of Omkar and Kumar (2013), the low-density preys were consumed all by different stages of predator instars, while high-density preys were consumed less because they were high number than the predator can eat (Figure 1).

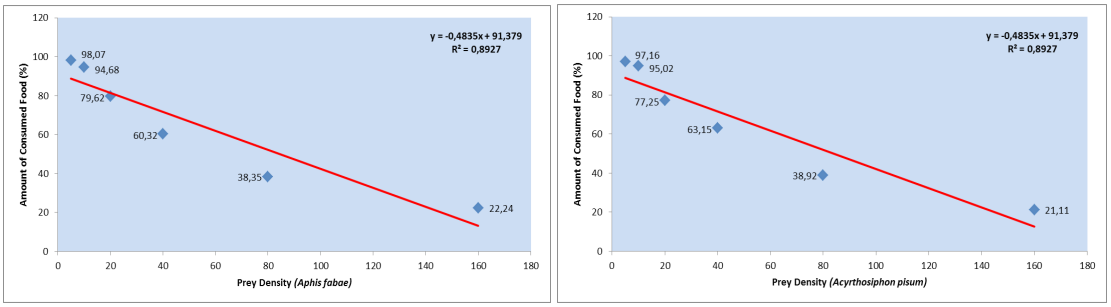

Figure 1. Amount of consumed food on different prey at different prey densities

Atlıhan et al. (2004) indicated that offered different number of prey which were 5, 10, 20, 40, 80, 180 and 250 of *Hyalopterus pruni* (Hemiptera: Aphididae) as food; the amount of consumed rate were calculated as 55.8, 92.7, 134.6, 215.9, 341.6, 411.3 and 404.9 respectively in the pre-adult periods according to prey densities. Similar to our results that were obtained for both preys and their densities. Batool et al. (2014) provided *Citotroga cerealella* (Lepidoptera: Gelechiidae) eggs to *C. carnea* in different densities which were 20, 30, 40, 50, 60, 70, 80, 90 and 100 to determine the consumption rate that increased with increasing prey density. Although the preys given to the predator were different in our study, it has the same results compared with the literature. El Zahi (2017) was used *Aphis gossypii* as a food for *C. carnea* regardless of prey density and determined the prey consumption amounts of the predator. According to our data that obtained from the

experiment, showed that the amount of consumption rate increased as the periods of larvae developed (Table 1). In our study, a similar result is noticeable at the highest density of both preys (*A. fabae* and *A. pisum*) compared with other studies. Rana et al. (2017) were used different preys (*Aphis craccivora*, *Myzus persicae* and *Aphis fabae*) as food for *C. carnea* regardless of prey density and calculated the average number of preys consumed in pre-adult periods. And their data that obtained from their study similar results with our study.

On the other hand, the eggs that given by the female of predator *C. carnea* fed on different preys at different prey densities were recorded. Accordingly, the number of eggs that given by the predator increased when feed on both of aphid species and the number of eggs depending on the prey density; it was shown that egg number decreased slightly when offer 160 prey density. The results indicated that the number of eggs laid by the predator fed on *A. fabae* was calculated and recorded 75, 164.5, 314, 622.6, 871.5 and 868.5, respectively depended on the prey density; and also the number of egg laid by predator were counted when *C. carnea* fed on *A. pisum* and the number of eggs were 79.3, 184.2, 334.4, 649.9, 855.7 and 841.8, respectively (Figure 2).

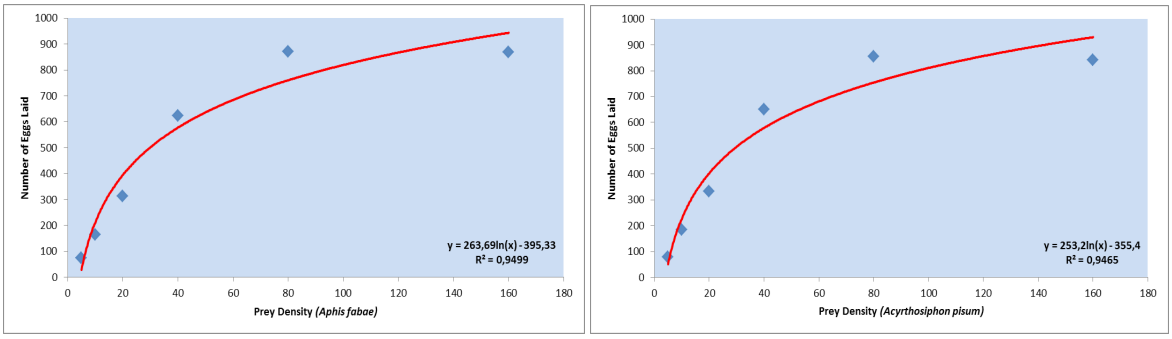

Figure 2. Number of eggs laid by *Chrysoperla carnea* on different preys at different prey densities

ECI (Efficiency of Conversion of Ingested Food) rate were determined based on the data obtained after calculated the number of eggs that laid by the predator fed on different preys at different densities depended on the number of preys that consumed. Omkar and Pervez (2004) equation was used to calculate the number of eggs that laid by *C. carnea* after offered different number of aphids from both species (R^2= 0.8486 for *A. fabae* and R^2= 0.7321 for *A. pisum*). According to the statistical analyzes, the results determined that ECI rate increased depending on the increasing of prey density (Figure 3). Khan and Zaki (2008) referred to the functional and numerical response of *C. carnea* (Stephens) on *Aphis fabae solanella* and when the numerical response data were analyzed, it was determined that there was a linear rised in the graph that created based on the density of the aphid. However, our data which obtained were agreed with Khan and Zaki (2008) results.

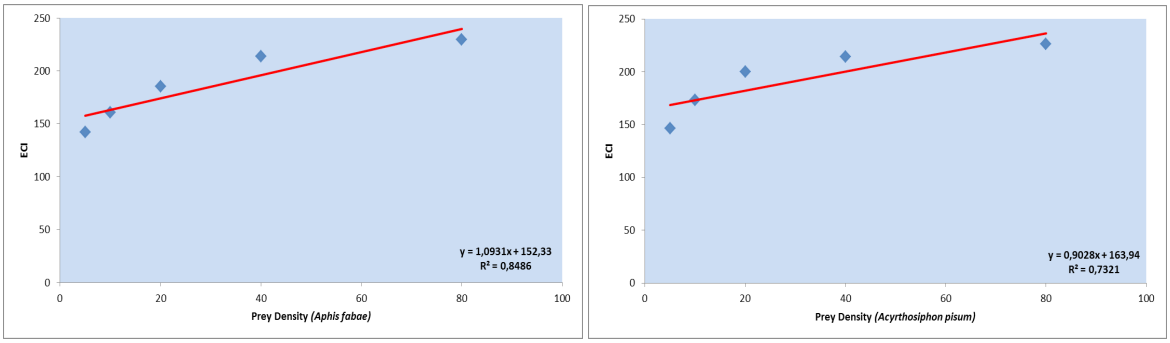

Figure 3. Values of ECI of *Chrysoperla carnea* on different preys at different prey densities

Whereas, the immature stages of adult development times and life table parameters of *C. carnea* were calculated depending on the prey and their density. The results indicated that the pre-adult development times of the predator fed on *A. fabae,* the total development time decreased with the increase significantly in prey density (F_{Total}: 6.40; P_{Total}: 0.001). There is no statistical difference among the development periods of predator (eggs, instar1, instar 2, instar3 and pupa), while a difference significant was observed in the second instar depending on the aphids density of *A. fabae* (F_{Egg}: 0.21; P_{Egg}: 0.960) ($F_{Instar1}$: 0.35; $P_{Instar1}$: 0.879) ($F_{Instar2}$: 3.30; $P_{Instar2}$: 0.007) ($F_{Instar3}$: 2.13; $P_{Instar3}$: 0.063) (F_{Pupa}: 3.17; P_{Pupa}: 0.015) (Table 2). The results determined that the total development time for *A. pisum* decreased with the increase statistically significantly in prey density (F_{Total}: 5.52; P_{Total}: 0.001). Besides, the values obtained from development periods were shown in Table 2 (F_{Egg}: 0.22; P_{Egg}: 0.955) ($F_{Instar1}$: 0.28; $P_{Instar1}$: 0.921) ($F_{Instar2}$: 4,97; $P_{Instar2}$: 0.001) ($F_{Instar3}$: 2.57; $P_{Instar3}$: 0.028) (F_{Pupa}: 3.65; P_{Pupa}: 0.007).

Table 2. Development times of immature stages of *Chrysoperla carnea* on different preys and different prey densities

P.D.	Development Times (Day) on *Aphis fabae*											
	N	Egg	N	Instar1	N	Instar2	N	Instar3	N	Pupa	N	Total
5	45	3.38±0.07 a	45	3.47±0.11 a	35	4.63±0.18 a	34	4.94±0,15 a	3	10.33±0.33 a	3	28.67±0.33 a
10	44	3.34±0.07 a	44	3.39±0.12 a	34	4.44±0.21 ab	33	4.73±0.18 a	4	10.25±0.48 a	4	28.25±0.25 a
20	45	3.33±0.07 a	45	3.31±0.11 a	35	4.29±0.20 ab	34	4.71±0.18 a	5	9.60±0.51 a	5	27.00±0.45 ab
40	39	3.41±0.09 a	39	3.28±0.14 a	39	3.80±0.21 b	39	4.41±0.16 a	9	8.89±0.54 a	9	26.00±0.65 ab
80	37	3.41±0.09 a	37	3.32±0.14 a	37	3.95±0.17 ab	37	4.32±0.21 a	16	8.13±0.36 a	16	24.81±0.43 b
160	36	3.42±0.09 a	36	3.28±0.14 a	36	3.83±0.17 b	36	4.25±0.21 a	15	8.13±0.39 a	15	24.80±0.46 b
P.D.	Development Times (Day) on *Acyrthosiphon pisum*											
	N	Egg	N	Instar1	N	Instar2	N	Instar3	N	Pupa	N	Total
5	46	3.37±0.07 a	46	3.39±0.11 a	37	4.65±0.78 a	34	4.95±0,14 a	3	9,75±0.25 ab	3	27.50±0.87 ab
10	44	3.34±0.07 a	44	3.23±0.13 a	37	4.49±0.17 ab	33	4.46±0.18 a	4	10.00±0.32 a	4	27.80±0.58 a
20	45	3.36±0.07 a	45	3.24±0.11 a	37	4.24±0.19 abc	34	4.69±0.17 a	5	9.60±0.51 ab	5	27.00±0.45 abc
40	40	3.33±0.08 a	40	3.25±0.14 a	40	3.73±0.19 c	39	4.33±0.20 a	9	8.83±0.44 ab	9	25.75±0.57 abc
80	36	3.42±0.09 a	36	3.22±0.13 a	36	3.86±0.15 bc	37	4.17±0.22 a	16	7.94±0.37 b	16	24.38±0.47 bc
160	36	3.42±0.09 a	36	3.28±0.14 a	36	3.78±0.17 bc	36	4.17±0.22 a	15	8.00±0.35 ab	15	24.67±0.44 c

Different letters in the same column show a statistical difference between the averages according to the Tukey test ($p<0.05$). (N: Number of individuals, P.D.: Prey Densities)

Atlıhan et al. (2004) were offered *Hyalopterus pruni* (Hemiptera: Aphididae) to *C. carnea* at different densities (5, 10, 20, 40, 80, 180 and 250) in their study and the results of development times of immature stages of predator were recorded according to their prey density. There was no significant difference on the 1st instar, 3rd instar and pupa periods in terms of development times according to their data that obtained, and the total of development times were decreased as the prey density increased. Batool et al. (2014) were given *Citotroga cerealella* to *C. carnea* in their study and they determined that the immature stages of development period of *C. carnea* were shortened due to the prey density. Similar results were observed in our experiment for both aphid species compared with the past literature. Alghamdi and Sayed (2017) were calculated the immature stages of development times of *C. carnea* fed on *A. fabae* and *Ephestia kuehniella* depend on prey density. According to the data, the immature stages (1st instar, 2nd instar, 3rd instar and pupa) and the total development times of *C. carnea* fed on *A. fabae* were 3.50, 5.00, 6.63, 10.63 and 25.75 days, respectively. Our results showed that the development times of predator fed on both preys increased according to the periods and the total development times were similar to the past literature. Kasap et al. (2003) were used *Aphis pomi* and *Tetranychus urticae* as preys regardless of prey density, and determined that larvae of predator were fed on *A. pomi* developed faster during the immature of development periods than fed on the mite *T. urticae* and more adult of predator were obtained. The development periods of *C. carnea* pre-adult periods (1st instar, 2nd instar, 3rd instar and pupa) and the total of development times of *C. carnea* fed on *A. pomi* were 3.08, 3.54, 4.72, 10.82 and 25.68 days, respectively. In our study, the results referred to the development times that obtained from fed on both aphid species were close to their finding. Takalloozadeh (2015) calculated the immature stages of development times and adult periods of *C. carnea* that feed on different aphid species (*Aphis gossypii*, *Myzus persicae*, *Aphis punicae*, *Aphis fabae*, and *Aphis craccivora*) based on the prey density. The development times of *C. carnea* larvae fed on *A. fabae* were (1st instar, 2nd instar, 3rd instar and pupa) and total development times were calculated 4.31, 3.75, 3.94, 10.34 and 22.35 days, respectively. This finding was agreed with the data in the previous literature compared with our finding.

The emerging values of *C. carnea* after adult periods were examined, and the duration of preoviposition and postoviposition days were calculated similarly in both preys and their densities. (For *A. fabae*= $F_{Preovipozitioon}$: 2.00; $P_{Preovipozition}$: 0.096 / $F_{Postovipozition}$: 0.07; $P_{Postovipozition}$: 0.996) (For *A. pisum*= $F_{Preovipozition}$: 2.76; $P_{Preovipozition}$: 0.028 / $F_{Postovipozition}$: 0.47; $P_{Postovipozition}$: 0.794). The oviposition times (day) were calculated, and determined that the time was increased with the increase of prey density (For *A. fabae*= $F_{Ovipozition}$: 80.27;

$P_{Ovipozition}$:0.001) (For *A. pisum*= $F_{Ovipozition}$: 94.16; $P_{Ovipozition}$: 0.001). The average daily number of eggs were determined at last two aphids densities (80 and 160) and a statistical difference was found compared to other aphid densities when *C. carnea* fed on *A. fabae* ($F_{D.N.E}$: 155.19; $P_{D.N.E}$: 0.001) (D.N.E: Daily Number of Eggs). The average daily number of eggs was determined in the last three aphid densities (40, 80 and 160) and a statistical difference was found compared to other aphid number that offered to *C. carnea* fed on *A. pisum* ($F_{D.N.E}$: 130.22; $P_{D.N.E}$: 0.001). The total average of eggs was determined at the last two prey denisties (80 and 160) and a significant difference was found compared to other prey densities when *C. carnea* was fed on *A. fabae* ($F_{T.N.E.}$: 261.45; $P_{T.N.E}$: 0.001) (T.N.E: Total Number of Eggs). The total avarage of eggs was determined at most in the last two prey densities (80 and 160) and a statistically significant difference was found compared to other prey densities when *C. carnea* was fed on *A. pisum* ($F_{T.N.E}$: 199.72; $P_{T.N.E}$: 0.001) (Table 3).

Table 3. Development times and number of daily and total eggs of adults of *Chrysoperla carnea* on different preys at prey densities

		Aphis fabae			*Acyrthosiphon pisum*		
	Prey D.	N	Mean±SE		N	Mean±SE	
Preoviposition times	5	3	7.67±0.88	a	4	7.50±0.50	a
	10	4	7.00±0.58	a	5	7.20±0.49	a
	20	5	6.00±0.45	a	5	6.00±0.45	a
	40	9	6.00±0.33	a	12	6.08±0.26	a
	80	16	6.06±0.23	a	16	6.06±0.23	a
	160	15	6.07±0.25	a	15	6.00±0.26	a
Oviposition times	5	3	17.33±0.67	c	4	17.50±0.50	c
	10	4	18.25±0.63	c	5	18.00±0.55	c
	20	5	22.20±0.66	c	5	22.00±0.55	bc
	40	9	26.78±0.55	b	12	25.83±0.64	b
	80	16	35.56±0.70	a	16	32.63±0.71	a
	160	15	35.47±0.74	a	15	35.53±0.74	a
Postoviposition times	5	3	3.00±0.00	a	4	3.25±0.25	a
	10	4	3.00±0.00	a	5	3.00±0.00	a
	20	5	3.00±0.00	a	5	3.00±0.00	a
	40	9	2.89±0.20	a	12	2.92±0.15	a
	80	16	2.94±0.11	a	16	3.00±0.13	a
	160	15	2.93±0.12	a	15	2.87±0.13	a
Daily number of eggs	5	3	2.69±0.32	d	4	2.79±0.27	d
	10	4	5.83±0.31	d	5	6.54±0.30	c
	20	5	10.11±0.41	c	5	10.81±0.37	b
	40	9	17.50±0.40	b	12	18.68±0.51	a
	80	16	19.62±0.41	a	16	19.20±0.44	a
	160	15	19.58±0.40	a	15	19.02±0.46	a
Total number of eggs	5	3	75.00±8.14	d	4	79.25±8.84	d
	10	4	164.50±8.01	d	5	184.20±8.18	d
	20	5	314.00±5.86	c	5	334.40±8.44	c
	40	9	622.56±9.59	b	12	649.90±20.10	b
	80	16	871.50±16.00	a	16	855.70±18.20	a
	160	15	868.50±18.30	a	15	841.80±18.70	a

Different letters in the same column and in the same parameter indicate a statistical difference between the averages according to the Tukey test ($p<0.05$). (D.:Densities; SE: Standart Error)

Atlıhan et al. (2004) stated indicated that different prey densities did not effect of *C. carnea*'s oviposition, postoviposition and total lifespan during feed on *Hyalopterus pruni*, while they calculated the number of eggs that given by adult females of predator increased at the high of prey density. In our study, it was observed that the duration of oviposition was increased when aphid density was increased for both preys, and preoviposition and postoviposition periods did not change. The total number of eggs increased daily depending on prey density. El Zahi (2017) determined that the number of eggs laid by females of *C. carnea* fed on *A. fabae* and *E. kuehniella* were 373.75 and 481.75, respectively; this finding was agreed with our study. It was found that predator fed on *A. fabae* can give more eggs than previous literature. Kasap et al. (2003) showed that calculated preoviposition, oviposition and postoviposition times of *C. carnea* females fed on *A. pomi* were 7.56, 45.22, 2.67 days, respectively. In our study, it was seen that these durations of preoviposition and postoviposition

were close to the both of preys with the highest prey density. The number of eggs left laid by *C. carnea* female was lower than the number of we obtained, but it was observed to be a close value to the finding (Kasap et al., 2003).

Life table parameters were calculated separately for both of preys at different prey densities according to Euler-Lotka equation. Predator fed on *A. fabae*, intrinsic rate of increase (r_m) were calculated and were 0.010, 0.041, 0.067, 0.103, 0.121 and 0.118 females/female/day at different prey densities; whereas, the predator fed on *A. pisum* the intrinsic rate of increase were 0.032, 0.052, 0.069, 0.112, 0.122 and 0.119 females/female/day, respectively (Table 4). The other values that obtained (R_0, T_0, GRR, λ) were increased depending on the increasing of prey density when all the data are examined, but these values decreased at 160 prey density (Table 4). Atlıhan et al. (2004) in their studies on *H. pruni*, it was determined that different prey densities were effective on *C. carnea*'s life table parameters (Reproduction rate, Intrinsic rate of increase). According to the past literature, both r_m and R_0 values were increased by the prey density increased. This finding was similar to the literature when our study is evaluated on this subject.

Table 4. Life table parameters of *Chrysoperla carnea* fed on *Aphis fabae* and *Acyrthosiphon pisum* at different densities

Prey Densities	Life Table Parameters (*Aphis fabae*)					
	r_m	R_0	T_0	GRR	T_2	λ
5	0.010	2.143	78.522	32.143	71.414	1.010
10	0.041	5.982	43.827	65.800	16.983	1.042
20	0.067	17.444	42.551	157.700	10.316	1.069
40	0.103	64.650	40.510	280.400	6.735	1.108
80	0.121	207.929	44.273	482.814	5.750	1.128
160	0.118	187.188	44.165	451.237	5.851	1.126
Prey Densities	Life Table Parameters (*Acyrthosiphon pisum*)					
	r_m	R_0	T_0	GRR	T_2	λ
5	0.032	3.938	43.499	45.619	21.998	1.032
10	0.052	9.514	43.277	83.727	13.316	1.053
20	0.069	18.578	42.458	167.375	10.072	1.071
40	0.112	106.355	41.567	356.591	6.174	1.119
80	0.122	202.838	45.537	458.559	5.681	1.130
160	0.119	181.427	43.855	437.993	5.845	1.126

r_m: Intrinsic rate of increase, R_0: Net reproductive rate, T_0: Mean generation time, GRR: Total productivity rate, T_2: Doubling time, λ: Daily maximum reproductive value.

Conclusion

Aphids are pests that cause economic losses in the worldwide. They reproduce very quickly in their environment and also cause indirect damage on plants because they are a disease vector. For this reason, it is very important to reveal the numerical response of the species in determining the impact power of a predator that has an effect on aphids.

In this research, the numerical response of *C. carnea* on two different aphid species (*A. fabae* and *A. pisum*) was determined. The reproductive abilities of the green lacewing at varying prey densities were also determined. The obtained data were evaluated and it was observed that *C. carnea* was effective on two different aphids (*A. fabae* and *A. pisum*) under laboratory conditions. It has been determined that the aphid population was intensive and the predator is more effective at the high density of aphids than lower aphid densities. It is thought that the data obtained in this study will help the researchers who want to produce mass production of *C. carnea* in the laboratory. However, it was concluded that similar experiments should be carried out in the field conditions in order to determine the information related to the numerical response of the species more clearly.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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In "Technical and Economic Features of Tractors in the Second Hand Market in Sanliurfa Province" (Int J Agric Environ Food Sci 4 (3): 384-393 (2020)) the Author noted one eror.

The Author name (Nusret Mutlu) has been changed from:
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The affiliation for Mustafa Vatandas has been added from:

Ankara University Faculty of Agriculture Department of Agricultural Machinery and Technologies Engineering, Ankara, Turkey

The correction will appear in the online version of the paper on the nternational Journal of Agriculture, Environment and Food Sciences (Int. J. Agric. Environ. Food Sci.) website on December 21, 2020, together with the publication of this correction notice.

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Technical and Economic Features of Tractors in the Second Hand Market in Sanliurfa Province

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Abstract

In this study, it was aimed to determine various parameters of tractor use in agricultural production in Şanlıurfa province and the tendency of the farmers in the region towards tractor use by analyzing the collected data and obtaining generalizable results. In this context, an information gathering form was prepared to determine the technical and economic characteristics of tractors in the second-hand tractor market. The 450 information gathering forms were filled in face to face with the tractor dealers in the province, the dealer (broker) trading tractors and the vendors in the tractor market. The collected data were analyzed with the help of different statistical analysis programs. According to the results, the average rated engine power for tractors in the second hand market was determined as 57 kW. In addition, the average annual usage time of tractors in the second-hand market has been determined as 432.8 h, and other operating parameters related to the use of tractors include age, rated engine power, engine, gearbox, hydraulic system, cabin/hood and tires, and average annual usage. duration, estimated selling price, depreciation characteristics, etc. parameters were determined for tractors and the obtained data were evaluated by multiple regression analysis and the results were examined.

Keywords: Tractor, Operating parameters, Agricultural mechanization level, Sanliurfa

Introduction

The use of tractors in agricultural enterprises is an issue that needs to be examined in technical and economical terms. Scientific approach in making decisions about the tractor is a requirement of a rational management. Because, tractor investment has an important place in the fixed investment expenditures made in the enterprise. On the other hand, the part stemming from the tractor constitutes the leading part of the operating expenses. For these reasons, it is necessary to determine the operating parameters related to the use of tractors in order to make correct investment and management decisions.

Operating parameters of tractors related to use include age, rated engine power, engine power per unit area, loading rate, fuel and oil consumption, maintenance and repair costs, condition of engine-gearbox-hydraulic system-cabin/bodywork and tires, average annual service life, market value, depreciation characteristics, scrap value etc. parameters can be counted (Bowers 1975, Witney 1988b, Hunt 2001, Basol 2006). The values of these parameters may vary depending on the business and the user. The process, which initially started with the decision to choose suitable tractors for the business, ends in many different ways depending on the usage characteristics. From this point of view, determining the usage characteristics of tractors is important in terms of the appropriateness of the selection and the operational performance.

The aim of this study is to examine the various parameters of tractor use in agricultural production in Sanlıurfa-Harran

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region (age, rated engine power, engine, gearbox, hydraulic system, cabin/hood and tire condition, average annual usage time, estimated sales price, depreciation characteristics, etc.) obtains generalizable results by determining and analyzing the data collected for tractors. In this way, it is aimed to determine the tendency of the farmers in the region towards tractor use.

Material and Method

Material

The research material consists of the tractors found in the tractor dealers in Sanlıurfa Province and its districts and in galleries (brokers) that trade and exchange tractors. In order to reach the determined sample size, data of 450 tractors in total, which are offered for sale in 31 separate galleries, have been compiled. The general agricultural production characteristics of Sanlıurfa and its districts and the general soil structure of the districts, climate characteristics, the use of agricultural land, agricultural products production, business size and land distribution determine the types of tractors preferred in the plain.

Method

Method used to determine the sample size

An information gathering form was prepared to determine the technical and economic characteristics of tractors in the used tractor market. The 450 information gathering practices consisting of 20 questions were carried out by meeting face to face with the tractor dealers in the province, the dealer (broker) trading tractors and the vendors in the tractor market. An information gathering form was filled in for each tractor subject to sale in the market.

Conducting interviews and information gathering method

The questions in the information collection form used in the second hand tractor market research were prepared for the purpose of determining the current situation in the region in accordance with the purpose of the research. Some of the questions in the information collection form reflect the views of the authorized person interviewed on the issue. These questions were answered in line with the personal opinion of the authorized person and the general structure of the used tractor market. Before preparing the information gathering form, information about the general, technical and economic characteristics of tractors was reviewed. Particular attention was paid to selecting the most prominent and relevant questions on the subject. Some of the questions in the form include the date of the interview, the place where the interview was held, the address information of the interviewee and the company. Most of the questions in the form are related to the technical and economic characteristics of the tractor. These are engine, cabin-hood, gearbox, hydraulic system and tire condition.

Methods used in data analysis

The questions in the information collection forms showing the technical and economic characteristics of tractors were filled out separately for each tractor. The information collection forms obtained as a result of the research were grouped and numbered primarily on the basis of districts. The answers to the questions in the information collection forms were processed in a workbook created in the Microsoft Excel program. The data obtained were analyzed with the help of various statistical analysis programs (SPSS, MINITAB).

Regression relations between variables were determined by creating means, proportional values and tables with the data obtained by evaluating the answers to the questions in the information collection forms. The data classified on the basis of two or more characteristics of the tractors within the scope of the study were evaluated. Accordingly, the degree of affinity between two or more tractors of tractors was determined. By applying statistical analysis to these variables, the model giving the highest degree relation was determined.

Later, in determining the relationship between the estimated sales price of tractors in the second-hand market and other variables, the LIMDEP package program was used for model approaches based on data transformations, tabulation, linear and nonlinear regression analysis (Gül et al. 2001). In the multiple regression analysis model used within the scope of the program, the estimated sales price (ESP) parameters for the tractors in the second hand market were used as the dependent variable.

Tractor brands (New Holland, Uzel (MF), Türk Traktör (FIAT) and Ford), tractor age, number of cylinders, rated engine power, total service life, engine condition, engine revision, cabin-bodywork condition, gearbox-differential, the parameters related to the hydraulic system and tire conditions were included as independent variables in the models. By correcting the variation of error terms in the model, each observation has the feature of having equal variation.

The following formula was used for calculating the elasticity coefficients of the variables in the developed linear models (Cinemre and Ceyhan 1998).

$$e = \frac{\partial Y}{\partial X} * \frac{\overline{X}}{\overline{Y}} \quad \text{.....(1)}$$

where,

e: Elasticity coefficient is used to calculate what percentage change in the independent variable creates a percentage change on the dependent variables of supply or demand (Anonymous, 2002),

$\frac{\partial Y}{\partial X}$: First order derivative of the dependent variable with respect to X (coefficient of X),

$\overline{X}$: Average value of the explanatory variable,

$\overline{Y}$: Shows the average value of the dependent variable.

In order to achieve meaningful results in this model, the elasticity coefficients were determined and evaluated in order to reveal the percentage change on the tractor purchase price (TPP), which is the dependent variable of the other independent variables (AGE, TUT and REP), which are not only dummy variables (expressed as unit 1 or zero).

Results and Discussion

Among the technical features of the tractors subject to research, the characteristics of the traction condition come first. The 87% of the second hand tractors offered for sale in

the market consist of standard structured tractors called single wheel drive. Double wheel drive tractors have a proportional share of 13%. This situation encountered can be said that the enterprises in the plain have turned to the use of double-wheel drive tractors with the polyculture product pattern implemented with irrigation.

The 91% of the tractors in the second hand market have 4 cylinders. Here, it is observed that the number of cylinders of medium power tractors (50-65 kW), which is the dominant group, stands out.

One of the most important operating parameters that come to the fore in researches on tractors is the duration of use. Because the operating time of the tractor directly affects the operating costs. In Figure 1, the total service life (TSL) values of the tractors in the second hand market with reliable data are given depending on the age. The lineer equation obtained as a result of the regression analysis was placed on the scatter plot of the data.

A probability level of p <0.0001 was determined with a coefficient of indication of R2 = 0.477 in the linear model between the age and TSL parameters for tractors in the second hand market. The equation for the linear model is y = 432.8 * x. From the first derivative of the regression equation obtained, the annual average usage time can be calculated as 432.8 h (Figure 1).

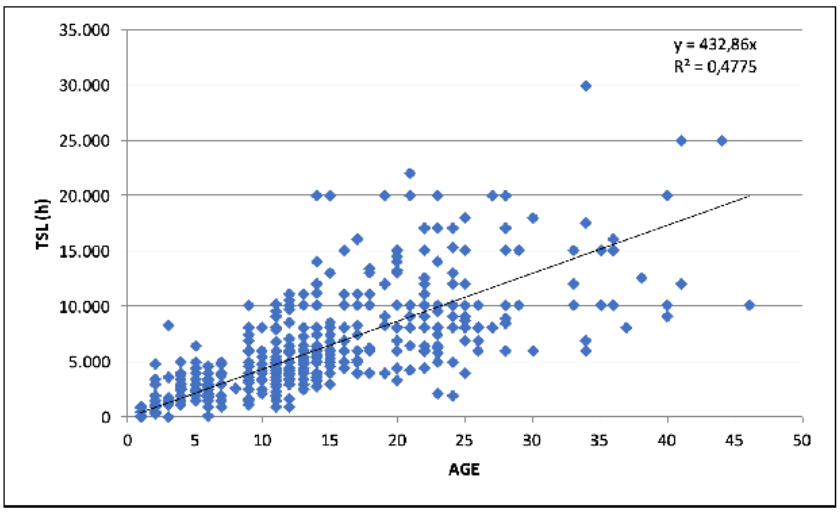

Figure 1. The change between tractor age and total service life (TSL) in the used tractor market

The graph shown in Figure 2 is obtained by taking the average of the total usage periods of tractors of the same age from the data in Figure 1. It has been determined that the observation values in the graph are suitable for a model (cubic model) that can be expressed with a third order equation with the coefficient of determination of R^2 = 0.7876 at the probability level of p <0.0001. The equation of the determined model is; y = 0.0537´3-8.0937x2 + 596.45´-65.509.

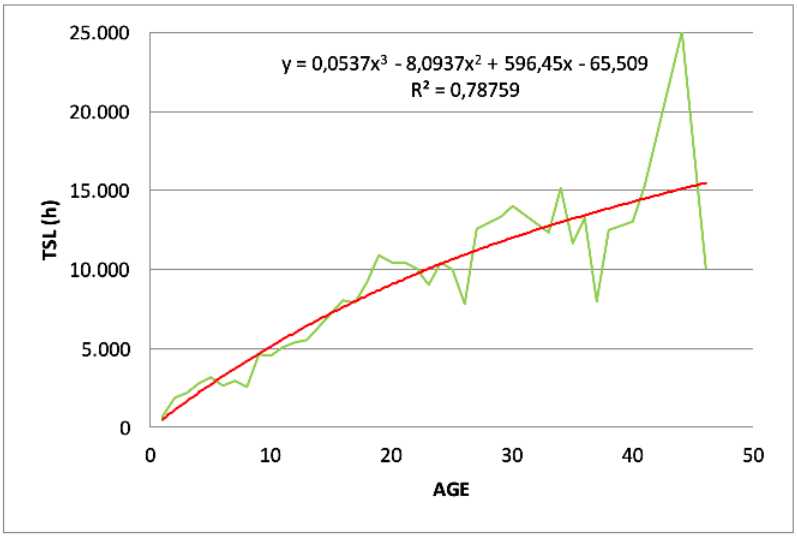

Figure 2. Change of age and total usage time values obtained according to average values

The average annual usage time of tractors in the second hand market is 432.8 hours. These values are based on the average annual usage time of tractors (365 h/year) determined for Harran District in the study conducted by Isik (1998) in Sanlıurfa-Harran Plain, and the average annual usage time determined by Sumer et al. (2008) for used tractors in Canakkale (377 hours/year) and Akinci and Canakci (2000) for the enterprises with the highest number of enterprises in the Antalya region with a land size of 5.1-10 ha values found for tractors and some in Business Administration (550.6 h/year) again, given robust and Akdemir in the literature (2002) by the average annual usage period set for Turkey's north west (479.32 h/year) high and tractors in the second hand market. The values found for (432.8 h/year) are lower than the values in the same literature.

Considering the literature information such that the hourly total costs of tractors decrease with the increase of annual usage time, hourly tractor costs are lower for enterprises with large production areas (Henderson and Fanash 1984) and the tractor usage time should be over 800 hours economically (Saral 1982), at least 650 h/year should be used and that the usage between 850-1000 h/year is the range where the tractor is used effectively (Demirci 1986), it is seen that the tractor usage time is quite low under the conditions of the Harran Plain.

The most important technical parameter of agricultural tractors is the rated engine power. This value significantly affects the machine size and operating costs that the tractor can operate. The rated engine power of tractors encountered in businesses varied between 40 kW and 86 kW. The rated engine power of tractors encountered in the second hand market varied between 35 kW and 89 kW. Examining the tractors in the used tractor market, it is understood that the power distribution is in the 50-65 kW range, which can be described as the medium power group.

It can be said that the proportional increase in the amount of tractors for sale with a nominal engine power of 50-55 kW in the second-hand market depends on the lack of power. With the widespread use of irrigated agriculture in the Harran Plain, it is observed that the tractors and the equipment operated with them have diversified and their capacities have increased in parallel with the applied product pattern and the tractors with a rated engine power below 45 kW are decreasing in the region. Accordingly, it is observed that there is an increase in the use of high powered tractors. In the example taken from the second hand tractor market, the average rated engine power value of all tractors was calculated as 57 kW.

However, the average tractor power for Turkey was 44 kW according to Agricultural Machinery and Equipment Manufacturers Association (TARMAKBIR) report for 2007 (Anonymous 2007). Sabanci et al. (2003a) Evcim et al. (2010) reported it as 43,6 kW and 43 kW, respectively. According to these values, the average power determined to track tractors scope of work in the second hand market (57 kW) seems higher than the average in Turkey. However, it is lower than the average tractor engine power (74 kW) determined for EU countries (Anonymous, 2007).

In order to determine the average annual service life of tractors in the second hand market, the total usage time (TUT) value was proportioned to the tractor age (AGE). The data obtained are given in Figure 3 depending on the rated engine power. The results of both the shape examination and the statistical analysis revealed that the average annual usage time is independent of the rated engine power for tractors in the second hand market.

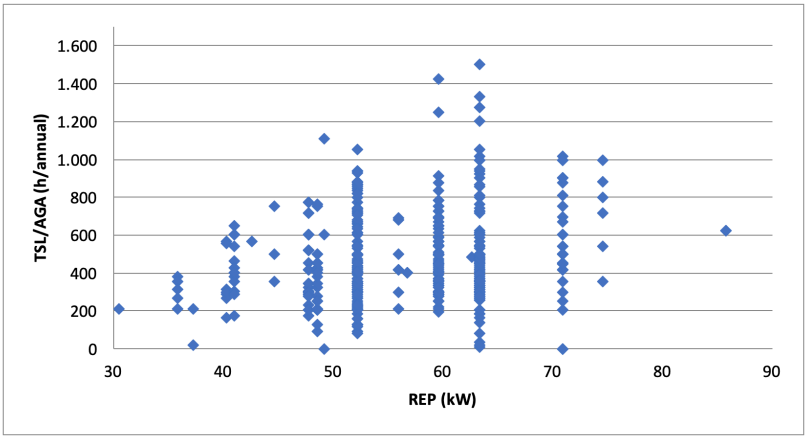

Figure 3.Variation of the average annual service life of tractors in the second hand market depending on the rated engine power (REP)

The 30% of the total tractors evaluated within the scope of the study are tractors that have not undergone engine revision before (0 revision number). Considering that the average age of the tractors in the example is approximately 15, it is seen that the remaining tractors have undergone at least one engine overhaul. On the other hand, by examining the number of revisions depending on age, it is seen that there is no tractor that underwent revision before the age of 8. In the 8-10 age range, it is observed that the first or the second engine revision took place. Tractors between the ages of 10-18 have at least 1 engine revision, and this number can go up to 5. If the age of 18 and over is, the average number of engine revisions is 2.6 per tractor.

It is noteworthy that the engine, gearbox and hydraulic system repair requirements of tractors in the used market show age-related repetitive changes. This situation, which shows

periodic changes, is thought to stem from the use and the importance of these three organs for the tractor. In other words, it is absolutely necessary to eliminate the malfunctions of these organs in terms of the tractor's function.

However, the change obtained in terms of the cabin/body repair requirement is different from these. This need for repair arises both at a later age and is considered to be a delayed repair requirement from the farmer's perspective. We have the impression that the reason for the proportional highness of 28-year-old tractors in the second-hand market in terms of the need for cabin/hood repair is due to the fact that for the first time such a requirement arises at the mentioned age is a result of the said delay. While there are periodic renewals that can be encountered at an early age due to use in the tire renewal requirement of tractors in the used tractor market, a proportional increase at the age of 14 and 22 is noteworthy. The reason for this is thought to be due to the general aging of the tractor.

Since the tractors encountered in the second hand market have not been sold yet, the monetary values demanded by the sellers have been named as the estimated sales price and used in order to determine the value of the tractor. In determining the estimated sales price, the opinions of the experts of the organizations operating in the market were also used. Figure 4a and 4b shows the distribution of estimated sales price (ESP) values depending on the model year of the tractors in the example. Figure 5 shows the distribution of estimated sales price values depending on the age of the tractor.

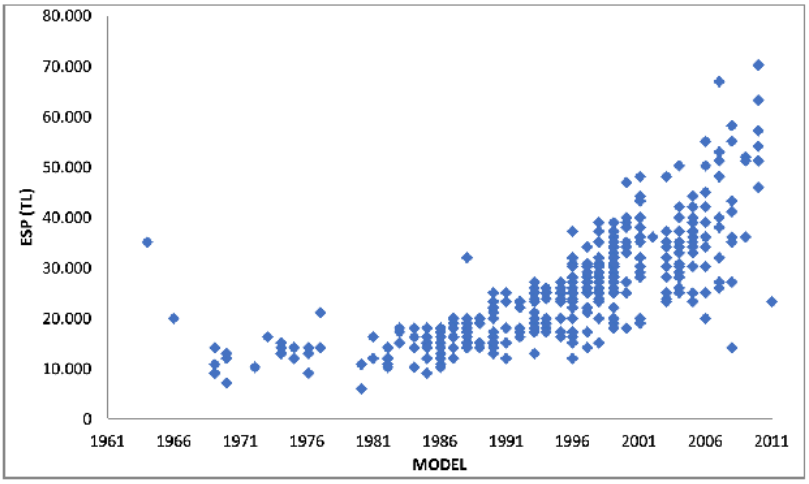

Figure 4a. The change of the estimated sales price (ESP) of tractors in the second-hand market depending on the model year.

As a result of the analyzes made by taking the average for the purpose of modeling the distribution, it was determined that the age-related estimated sales price values correspond to a third order (cubic) function at a high degree ($R^2 = 0.961$) and at the probability level of $p < 0.01$. The equation of the cubic model obtained is $y = -0.495x3 + 60.06x2-2562x + 49932$. Figure 5 shows the average values and the graph of the cubic model obtained depending on them.

The decrease in the estimated sales price (ESP) of tractors with the increase in age in tractors in the second hand market is similar to the results given in the literature (Isik et al. 1995, Guher 2008, Basol 2006).

As a result of the statistical analysis made with the average estimated sales price values of tractors with the same usage hours, the distribution of the estimated sales value of tractors in the second-hand market based on the total operating time (TOT) is second-order ($R^2 = 0.4858$, $p < 0.01$).) a relationship has been identified. The equation of the model obtained is $y = 0.000005x2-2.4593x + 39792$ (Figure 6).

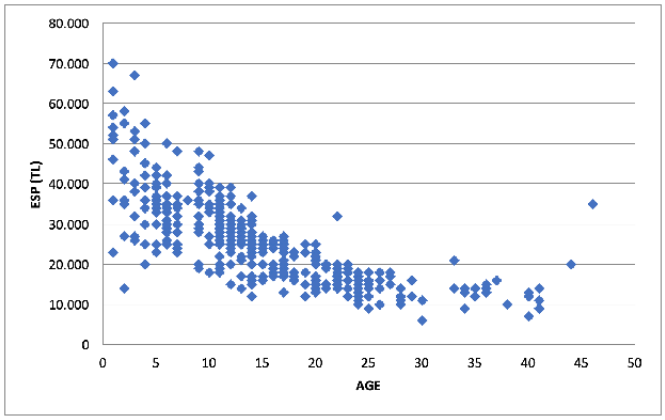

Figure 4b. Age-related change in the estimated sales price (ESP) of tractors in the second hand market

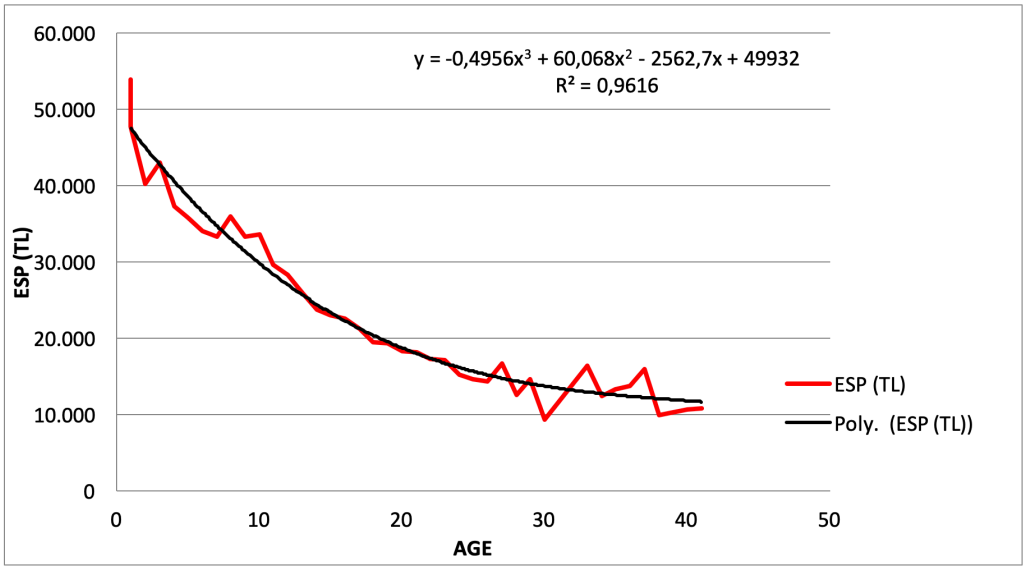

Figure 5. Changes in the age-estimated sales price (ESP) values of tractors in the second-hand market according to average values.

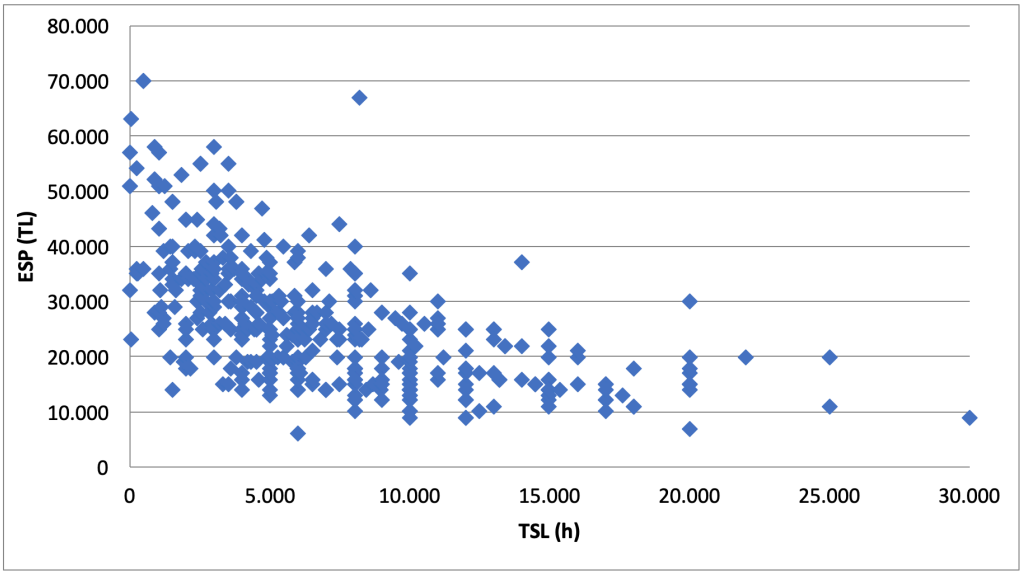

Figure 6.Variation of the estimated sales price (ESP) of tractors in the second hand market depending on the total service life (TSL)

In the study, the average annual life of the tractor was obtained by proportioning the total usage time (TUT) read from the tractor meter to the age. Figure 7 shows the distribution of estimated sales price (ESP) values depending on the TUT/AGE parameter.

Statistical analysis revealed that there was no relationship between the TUT/AGE parameter and ESP that could be subject to the model. The estimated sales price (ESP) of the tractor in the second-hand market is determined by the effects of various factors. The first factor that comes to mind among these is the tractor's rated engine power (REP).

As a result of the analyzes made for the purpose of modeling the relationship between the estimated sales prices of tractors in the second-hand market and the nominal engine power, the estimated sales price values depending on the nominal engine power can be converted to a third-order (cubic) function (R^2 = 0.821, p <0, 01) level.

The equation of the cubic model obtained is y = 0.163x3-13.94x2-532.5x + 11242. The result obtained here shows that the rated motor power factor has a high effect, approximately 82%, on the estimated sales price (Figure 8).

On the other hand, it was previously determined that the age-related estimated sales price values fit a third-order (cubic) function with a high degree (R^2 = 0.961), at the level of p <0.01. Accordingly, the age parameter has a higher effect on the estimated sales price than the nominal motor power parameter. These values are similar to the values given in the literature (Basol, 2006). As a result of the analyzes aimed at determining the effects of both parameters on the market value together, the following equation has been obtained:

ESP (TL) = 20109,187 - 835,793 ´ *AGE* + 325,623 ´ *REP* (kW)

In this equation, $R^2 = 0.637$ and $p < 0.01$, while the standard error of the estimation made using the equation is ± 6719.26 TL. The data obtained within the scope of the study are in great agreement with the results obtained in the study of Isık et al. (1995) in the literature and previously reported.

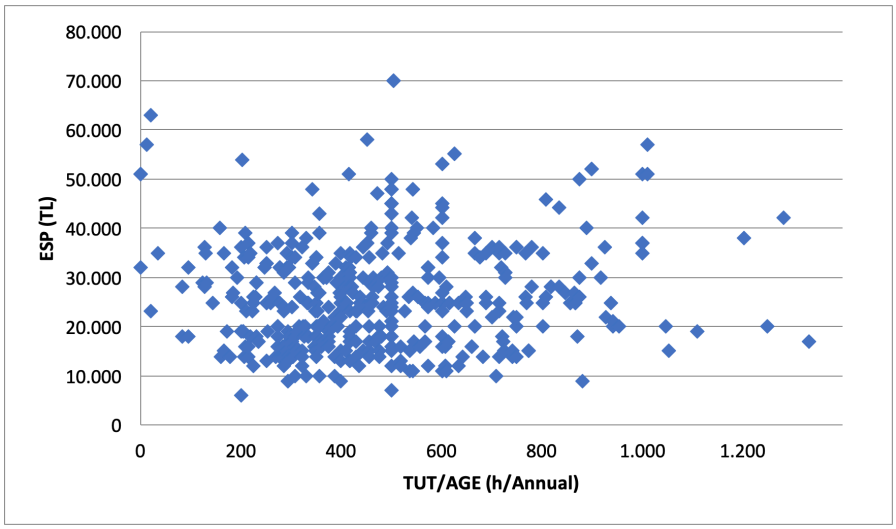

Figure 7. Variation in the estimated sales price (ESP) of tractors in the second hand market depending on the average annual usage period (TUT/AGE)

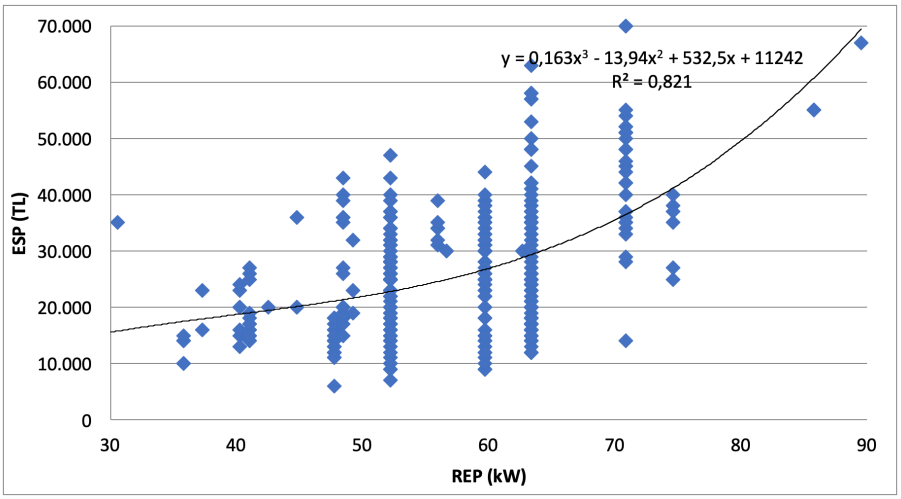

Figure 8. Variation of the estimated sales price of tractors in the second hand market depending on the average rated engine power.

Likewise, when the estimated sales price of the unit rated engine power of tractors is shown with the ratio of ESP/REP, the change of these values depending on the age is seen in Figure 9. In this graph, the average of the ESP/REP values of different tractors of the same age was obtained, and the observation values in Figure 10 were obtained. It was determined that the observation values in this way fit a third-order model with an indication coefficient of $R^2 = 0.563$ and a probability level of $p < 0.01$.

The equation of the cubic model obtained is y = 0.020x3-1.364x2 + 16.07x + 470.9. In other words, it is observed that due to the increase in tractor age, the estimated sales price (ESP) for unit power decreases within the framework of the determined model.

In order to have information about the depreciation characteristics of tractors in the second-hand market, used tractors, of which new ones are still produced and sold, were also analyzed. For this purpose, the ratio of estimated sales price (ESP) values to the sales price (SP) of the new one of the same tractor was used as the analysis parameter. The change of the values obtained is given in Figure 11.

Analyzes for tractors in the second-hand market highlighted two different model types. The first of these is the linear model and it has a coefficient of indication of $R^2 = 0.483$ and a

probability level of p <0.01. The equation for the linear model is y = -1,352X + 64.11. The second model is the cubic model with a third order equation. In this model, R^2 = 0.856 and p <0.01. The equation for the cubic model is y = -0.017X3 + 0.879X2-13.34X + 89.66. Analyzes made by Hunt (2001) for US conditions based on the age-dependent value (the value of the tractor at the beginning of the year - the depreciation amount for that year) also showed a very strong (R^2 = 0.924, p <0.001) cubic model between these two parameters. revealed that it was found. A similar result can be obtained with the values given by Witney (1988b).

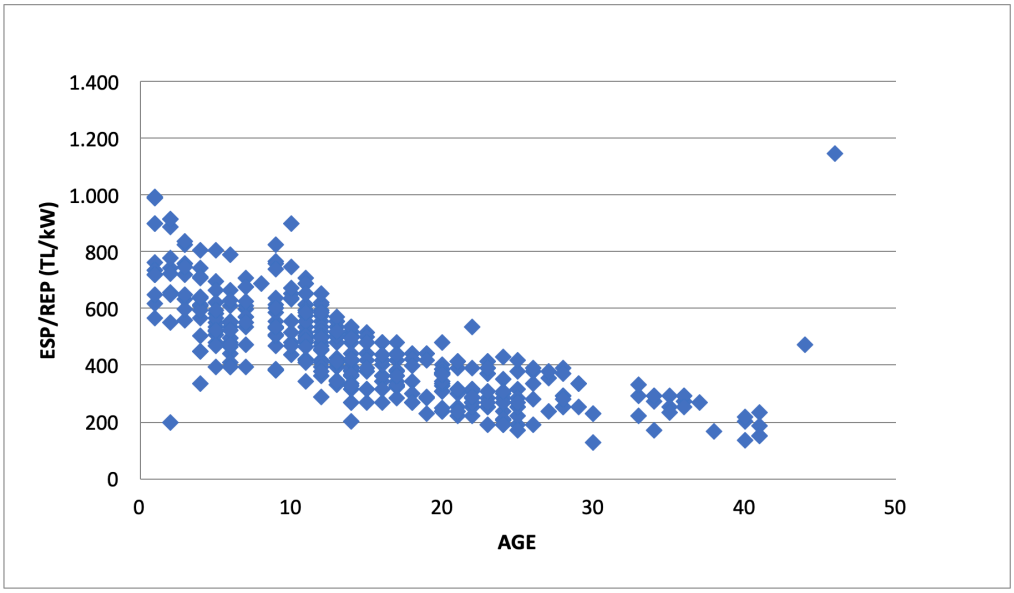

Figure 9. Age-dependent variation of the estimated purchase price (ESP/REP) of the unit rated engine power of tractors in the second hand market

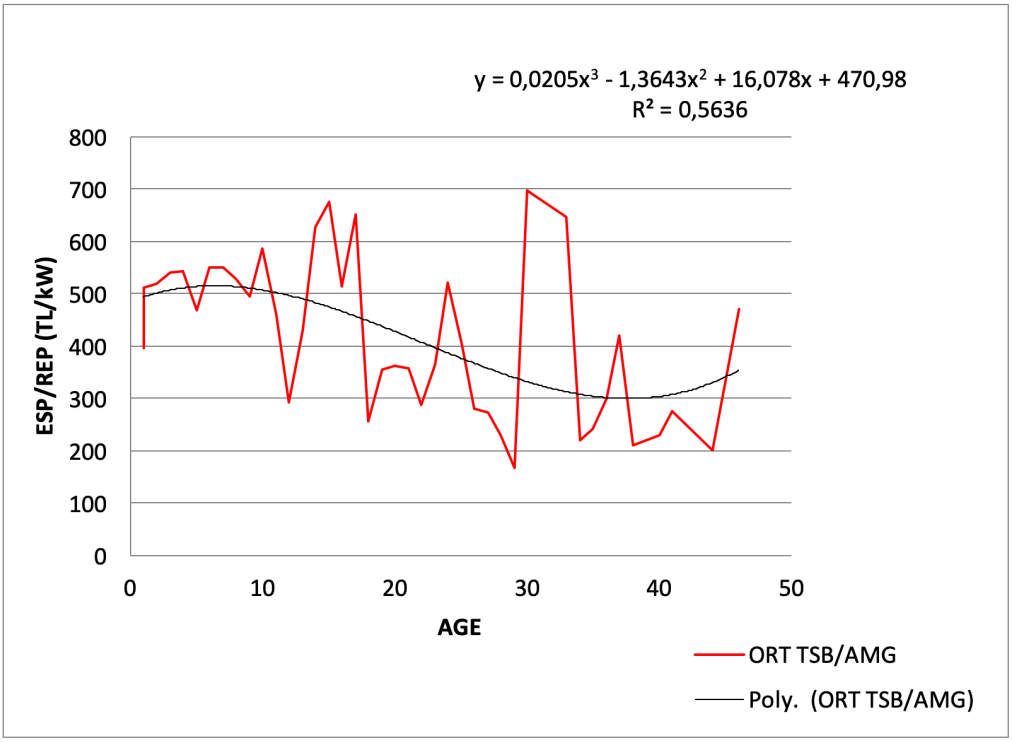

Figure 10. The change of age-related ESP/REP values obtained according to the mean values and the graph of the cubic model obtained accordingly

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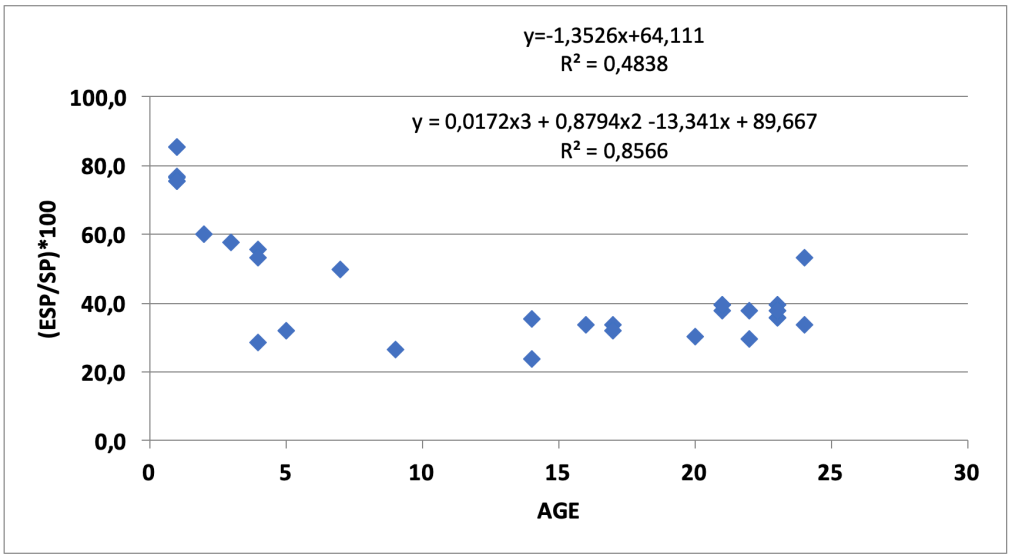

Figure 11. Age-related ESP /SP values of tractors in the second hand market

Results and Conclusions

With this study, the properties of used tractors for sale in Sanlıurfa Harran Plain (Central, Harran and Akcakale Districts) were tried to be determined. These features and some other factors that are thought to be effective were analyzed and their degree of influence was investigated. Studies conducted have revealed the results summarized below. According to this:

1) The average rated engine power of tractors in the second hand market in the research area is 57 kW, while the rated engine power of the tractors varies between 35-89 kW, while the power distribution is in the range of 50-65 kW, which is also described as the medium power group.

2) The average age of tractors in the second-hand tractor market is approximately 15, and tractors in the 0-15 age group constitute approximately 60% of the total tractors and 30% in the 16-30 age group. It is seen that 43% of the tractors in the second hand market are 15 years old and older tractors. Considering the 15-year economic life given in the literature for tractors (Sabancı et al. 2003a, Sabancı et al. 2003b), this situation reveals that the use of tractors that have completed their economic life is quite significant. However, when we look at the sales reasons for the tractors in the second hand market, 48% of the tractors are due to model aging, 2% to wear and again 2% to lack of capacity. This shows that more than half (52%) of second-hand tractors in the market have been put up for sale for upgrades; this reveals the renewal potential of the tractor park in Şanlıurfa Harran Plain.

3) The average annual usage time of tractors in the second hand market is 432.8 hours. When the product projections proposed for the region in both the GAP Master Plan (Anonymous 1989) and the GAP Regional Development Plan (Anonymous 2001b) are implemented, these values for the average tractor usage periods are calculated as the tractor used economically (650 h/year) or effectively (850-1000 h / year). years). In order to increase this period, it is necessary to increase the use of agricultural machinery foreseen by irrigated agriculture depending on the variety of product patterns of the enterprises.

4) As a result of the analysis of the regression analysis between the total usage time (TUT) of the tractors in the second hand market and the age parameters, it was observed that the total usage time of the tractor increased depending on the age of the tractor.

5) It has been observed that there is a strong negative relationship between the age-related market value of tractors in the second-hand market and the age-related estimated sales price. The price determination for used tractors is based on market conditions. Average prices are determined by model (age) and rated engine power and brand. Price values, which are highly affected by the general economic situation, are adopted by almost all market players. Other features of the tractor may have little effect on the actual price.

6) For tractors in the second hand market, it has been determined that the average annual service life is independent of the rated engine power.

7) Approximately 49% of the variation in the estimated sales price (ESP) of tractors in the second hand market can be explained by the variation in the total usage period.

8) It has been revealed that there is no relationship between the average annual service life (ASL/AGE) parameter of tractors in the second hand markets and the estimated sales price (ESP) that can be subject to the model. It is thought that the low annual average usage time of tractors is effective on this result.

9) The 82% of the variation in the estimated sales price of tractors in the second-hand market can be explained by the change in the rated engine power (REP) of the tractors. In this context, it has been determined that the rated engine power (REP) is very effective in price formation, especially for tractors in the second hand market.

10) The 56% of the change in unit power price (ESP/REP) in tractors in the second hand market is due to the age factor.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

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Consent for publication

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