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Evaluation of the chemical composition, genotoxic and cytotoxic effects of cocklebur (*Xanthium strumarium* L.) seed oil on human blood cells

Hatice BAS[1] Halil Erhan EROGLU[1] Hulya DOGAN[2]* Tansu USKUTOGLU[3] Belgin COSGE SENKAL[3] Cuneyt CESUR[4]

[1]Yozgat Bozok University, Department of Biology, Yozgat, Turkey
[2]Yozgat Bozok University, Yozgat Vocational School, Department of Plant and Animal Production, Yozgat, Turkey
[3]Yozgat Bozok University, Department of Field Crop, Yozgat, Turkey
[4]Karamanoglu Mehmetbey University, Department of Energy Systems Engineering, Karaman, Turkey
***Correspondence:** hulya.dogan@bozok.edu.tr

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Abstract
The cocklebur (*Xanthium strumarium* L.), is a wild plant, plant's seed contains up to 25% crude oil. It is obvious that the oil rate can be increased if this plant is breeding and cultivated. This study will evaluate whether this plant can be cultivated and used as an edible oil plant. In this study, cocklebur seeds were collected from nature and grown under cultural conditions. The oil was obtained from cold press, and different doses of oils were determined to malondialdehyde (MDA) and superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and acetylcholinesterase (AChE) activities in human erythrocytes. Its effects on mitotic index (MI), micronucleus (MN) and replication index (RI) parameters in human peripheral lymphocyte cultures were examined. According to the results, seeds of cocklebur averaged 30% oil content. The highest fatty acid composition was linoleic acid at 72.624%. The genotoxicity results showed a genotoxic effect to the dose-dependent (0.16, 0.32, and 0.50 µL mL^{-1}). Cocklebur seed oil caused significant decreases in MI and RI values (0.16, 0.32, and 0.50 µL mL^{-1}). Afterwards, MDA level increase, SOD, CAT, GST, GPx and AChE activities of human blood cells significantly decreased in increasing doses of oil compared with not-treated cells, especially from the 0.08 µL mL^{-1}. Consequently, cytotoxic and genotoxic effects are detected in high concentrations, cocklebur oil can be evaluated at lower doses. Antioxidant results also support this opinion.

Key words
Xanthium strumarium, fatty acid, genotoxicity, cytotoxicity

Introduction
Vegetable oils can be used both as a nutrient source and for industrial purposes (Ardabili et al. 2011). Also, they have an important role in human healthy as well as in human nutrition. Oils, which are a major metabolite such as carbohydrates and proteins, are an important food source for humans and animals. Seeds and fruits of oil crops, which can benefit economically, contain a high amount of oil. Cocklebur (*Xanthium strumarium* L.), which belongs to the Asteraceae family, is seen as weed in many parts of the World. Cocklebur, which can be used in many areas from traditional medical treatment to modern pharmacology, can be recommended such as an alternative oil crop. It is commonly growing in marginal areas (warm, cold, and arid) and it is an annual herb, up to 1 m in height, with a short, stout, hairy stem (Ruan et al. 2012). The plants fruit are 1.0-3.5 cm long, brown, hard, woody hook-shaped and thorny. Each fruit contains two seeds (Eymirli and Torun 2015). Its seed contains 25% to 40% crude oil, which is approximately 77% linoleic acid (Chang et al. 2013), and it has important potential in terms of fatty acids compositions. Fatty acid composition of cocklebur is similar to sunflower oil (Cesur et al. 2018). Standard safflower oil contains about 6–8 % palmitic acid, 2–3 % stearic acid, 16–20 % oleic acid and 71–75 % linoleic acid. The vegetable oil ratio and fatty acid composition obtained from the oil crops are very important, because they determined their economic importance. Not only physical and chemical properties of oils but also plants growing condition are very important for oil quality. For example; Plants are synthesized less linoleic acid but more oleic acid with increasing temperatures. In cold climates and high altitudes, mostly unsaturated fatty acids are formed (Samancı and Özkaynak 2003, Uppstrom 1995). There is a difference among species in regards to fatty acid composition, moreover intraspecific variation can be shown (Linoleic type sunflower, oleic acid type sunflower) (Karaca and Aytaç 2007). Olive, peanut, coconut and sesame are important for human nutrition and these vegetable oils are rich in oleic and linoleic acid (Baydar and Erbaş 2000). Another fatty acid can be found in vegetable oils is erucic acid ($C_{22:1}$). It has been determined that erucic acid has undesirable effects on muscles, heart and animals' growth. The World Health Organization (WHO) determined the rate of erucic acid in edible oils up to 5%. Cocklebur oil does not contain erucic acid (Tosun and Özkal 2000, Zhu et al. 2006). Cocklebur oil fatty acid composition has properties close to edible oils.

On the other hand, non-edible oils can be used in other industrial area, such as biodiesel production, oils, which are containing linolenic acid, dry quickly so they are widely used in the production of polishes, paints and varnishes.

Different studies record the genotoxic and cytotoxic potentials of oils and extracts (Fan et al. 2019, Zor and Aslan 2020). However, lack of a systematic model to determine these potentials makes it difficult to make decisions with reliable results. The cytokinesis-block micronucleus assay, recently approved by the OECD (2016), is consented one of the most reliable methods for evaluating genotoxicity and cytotoxicity with many biomarkers. The biomarkers are mitotic and replication indexes (proliferative capacity in human lymphocytes), apoptosis, necrosis, and cytostasis percentage for cytotoxicity (Fenech et al. 1999) and micronuclei (clastogenic and aneugenic effects), nucleoplasmic bridges, and nuclear buds for genotoxicity (Fenech 2007).

According to the literature, the vegetable oil and fatty acids composition obtained from cocklebur seeds can be evaluated economically. Also, accordingly fatty acid composition of cocklebur oils, are used in different areas such as industry, medicine, pharmacy and herbal medicine (Uskutoglu 2018). In this study, the fatty acid composition and mineral matter of vegetable oil from cocklebur (*X. strumarium* L.) seeds, which was grown under cultural conditions were investigated, different doses of this oil were evaluated to malondialdehyde (MDA) and superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and acetylcholinesterase (AChE) activities in human erythrocytes. Also, its effects on mitotic index (MI), micronucleus (MN) and replication index (RI) parameters in human peripheral lymphocyte cultures were examined.

Materials and Methods

Plant material

The seeds of cocklebur were collected from Muslubelen (1440 m), -Yozgat, Turkey, on 16 September 2014. The seeds of cocklebur were sown (60 cm × 20 cm) in Yozgat (Topçu), Turkey, at the end of March 2016. When the plants had 3 or 4 leaves, N (150 kg ha^{-1}) was applied to the soil. After the soil was irrigated once. When fruits were mature, cocklebur plants were harvested. The plants are stored at room temperature (25°C), in the shade away from direct sunlight. Then, seeds obtained for the cold press analysis and obtained oil stored at refrigerator (+4°C).

Soil data

The physicochemical properties in experimental area's soil surface layer (at 0–30 cm) are presented in Table 1. According to the Table 1, it can be seen that the soil of the experimental area contained a medium level of organic matter (2.49%). The soil of the trial area has a loamy texture.

Table 1. Some soil properties in experiment area

Properties	Values	Class	Properties	Values	Class
Texture class	Loamy	-	P_2O_5 (µg g^{-1})	78	High
pH	7.09	Neutral	K_2O (µg g^{-1})	728	High
Salinity (%)	0.178	Low	Ca (µg g^{-1})	7060	High
$CaCO_3$ (%)	7.15	Medium lime	Cu (ppm)	2.84	Enough
Total N (%)	0.15	Enough	Mg (µg g^{-1})	5604	Very low
Organic matter (%)	2.49	Medium	Fe (ppm)	8.08	High
Mn (µg g-1)	4.07	Low	Zn (ppm)	0.62	Low

Analysis of the fatty acid composition

Pre-treatment: Fatty acids methylation: 0.1 g of oil was taken and put into a 15 mL tube with a cap. Add 1 mL of 2 N methanolic KOH solution and vortex for 2 minutes. After waiting 15 minutes, 10 mL of hexane was added and mixed thoroughly. It was centrifuged at 7000 rpm for 10 minutes. 1 microliter of the upper phase was injected into the GC device. After the fatty acids were methylated, they were analyzed by Shimadzu Gas Chromatography (2025) with Flame Ionizer Detector (FID). Teknokroma brand TR-CN100 column of 60.0 m × 0.25 mm × 0.20 µM is used. The column heat was held initially at 80 °C for 2 min after injection, then increased to 5 °C with 140 °C/min heating ramp for 2 min and increased to 240 °C with 3 °C min^{-1} heating ramp with 5 more minutes, and the injector temperature was set to 240 °C. The total analysis time is 61 minutes. The carrier gas was helium with column flow rate of 30 mL min^{-1}. The gas flows used were determined as H2 = 40 mL min^{-1} and dry air = 400 mL min^{-1} (AOAC 1990).

Determination of nutrient contents

The heavy metals and nutrients in the samples were detected using an iCAP-Qc ICP-MS spectrometer (Thermo Scientific) at the Yozgat Bozok University, Science and Technology Application and Research Center. ICP-MS conditions were listed as follows: The calibration curve was made between 0.1 ppb and 500.0 ppb for the relevant elements, totaling of 7 points. The dilutions of the solutions to be used to draw the calibration curve were made with a 3% nitric acid solution prepared with ultra-distilled water. Plasma power 1450 W, Nebulizer Pressure to 3.01 bars; Spray Chamber Temperature was measured as 2.8 °C. High purity argon gas was used as the gas; plasma gas was set at 0.7 L min^{-1}, Nebulizer gas at 0.9 L min^{-1}. The measurement time was made in 0.01 seconds. The samples were measured 3 times in total (Yetim 2002).

Obtaining of erythrocytes

20 mL blood samples were taken from heparin tubes from 6 healthy male volunteers who did not use cigarettes and alcohol and were not exposed to any chemicals in their workplace. Heparinized whole blood was centrifuged at 2000 rpm for 10 minutes and the supernatant was removed. Then, erythrocytes were prepared by washing three times with phosphate buffer (pH 7.4). The hemoglobin concentration was determined according to the Drabkin (1946) method.

Obtaining of leucocytes

6% dextran prepared in isotonic saline was added to blood samples taken from 6 healthy male volunteers who did not use cigarettes and alcohol and were not exposed to any chemicals in their workplace, and they were subjected to a waiting period of 30 minutes. The upper layer was taken up in another tube containing 2.25 mL EDTA and centrifuged at 1000 rpm. Then leukocytes were prepared by washing with Tris buffer.

Obtaining of lymphocytes

5 mL blood samples were taken from heparin tubes from 6 healthy male volunteers who did not use cigarettes and alcohol and were not exposed to any chemicals in their workplace. The samples were collected into Vacutainer tubes with anticoagulant heparin (Becton Dickinson USA).

Groups and applications

The protocol applied in this study was countenanced by the Yozgat Bozok University Ethics Committee with a protocol number (2017-KAEK-189 2018.12.12 11). For this study, 9 groups (6 samples in each) were composed.

These groups were:
Control group (no oil application was done)
0.01 µL mL^{-1} *X. strumarium* seed oil treated group
0.02 µL mL^{-1} *X. strumarium* seed oil treated group
0.04 µL mL^{-1} *X. strumarium* seed oil treated group
0.08 µL mL^{-1} *X. strumarium* seed oil treated group
0.16 µL mL^{-1} *X. strumarium* seed oil treated group
0.32 µL mL^{-1} *X. strumarium* seed oil treated group
0.50 µL mL^{-1} *X. strumarium* seed oil treated group
1 µL µL mL^{-1} *X. strumarium* seed oil treated group

The oil was added to the cells and left for 30 minutes incubation at 37 °C. Hemolysate was obtained from cells waiting at -20 °C until working time with hypotonic sodium phosphate buffer pH (7.4). The activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), acetylcholinesterase (AChE) and malondialdehyde (MDA) levels from the hemolysate samples compared to the control group (UV-VIS Spectrophotometer (Biotech Engineering/ Spectroscan 60 DV)) were determined.

Measurement of MDA levels

MDA forms a pink colored complex as a result of incubation with TBA at 90 °C under aerobic conditions. The absorbance of this complex is determined on the spectrophotometer at a wavelength of 532 nm. The amount of MDA, the final product of LPO, which reacted with thiobarbituric acid (TBA), was measured using the method of Ohkawa et al. (1979). The absorbance of the mixture with TBA was determined by the spectrophotometer.

Antioxidant enzyme assays

Habig et al. (1974)'s method was used in determining GST activity. The enzyme activity was determined at 340 nm by the GST enzyme conjugated with 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), based on the oxidation of GSH.

In SOD determination, by using the method of Marklund and Marklund (1974), the absorbance increased by autooxidation of pyrogallol at 440 nm in alkaline medium was measured. Firstly, Tris-EDTA buffer and different volumes of supernatant were added to the cuvettes, and then enzyme source was added to them. Then, pyrogallol was added to these mixtures and absorbance was measured at 440 nm in the spectrophotometer.

CAT activity was determined by the method revealed by Aebi (1984). In the first stage, Triton X-100 was added to the supernatant to reveal CAT in peroxisomes, and then dilution was made by adding phosphate buffer. Then H_2O_2 was added for starting the enzymatic reaction and the absorbance measured at 240 nm. The method of Paglia and Valentine (1967) was applied to determine GPx activity. This method is based on the principle of measuring the absorbance created by GR oxidizing nicotinamide-adenine-dinucleotide hydrogen phosphate (NADPH) at 340 nm. The oxidation of NADPH to Nicotinamide-adenine-dinucleotide phosphate (NADP) causes a decrease in absorbance at 340 nm, thus indirectly used to determine the activity of GPx. Enzymatic reaction was started by adding H_2O_2 on this mixture and absorbance values were read at 340 nm for 3 minutes.

Measurement of AChE activities

Cholinesterase catalyzes the breakdown reaction of acetylcholine to thiocholine and acetate. As a product, 5-thio-2- nitrobenzoic acid, which gives yellow color, is formed as a result of the reaction of DTNB with the released thiocholine. The intensity of the resulting color was measured at 412 nm according to Ellman et al. (1961) method. Firstly, Na-K Phosphate Buffer (pH=8.0), sample, etopropazin and DTNB were placed in spectrophotometer cuvettes. At the end of the incubation period, absorbance measurement was made by adding acetylcholine iodide.

Peripheral lymphocytes culture

Culture tubes consisted of 5 mL peripheral blood karyotyping medium (Biological Industries, Israel) and 0.4 mL blood. Lymphocytes were incubated at 37 °C and 5% CO_2 for 72h. The oil was added to the tubes in different

concentrations added at the 24th hour. After 44h incubation times, cytochalasin-B (Sigma-Aldrich, Germany) was added to micronucleus tubes for cytokinesis block. In harvesting, cells were treated by hypotonic solution (0.075 M KCl) and several times fixed in fixative solution (methanol: acetic acid, 3:1, v v-1). In preparation, 3 to 4 drops of cell suspension were dropped onto the slide. Slides were stained with Giemsa (Merck, Germany) (Gümüş et al. 2020).

Microscopic analyses

2000, 500, and 1000 cells were counted in MI, RI and MN evaluation, respectively. The formulae are given below.

MI (%) = (Cells in the metaphase / Total cells) × 100

RI = [(1 × mono-) + (2 × di-) + (3 × tri-) + (4 × polynucleate cells)] / Total cells

MN (%) = (Number of micronucleus / Total cells) × 100 (Gümüş et al. 2020).

Statistical analyses

The data obtained in the study were evaluated by using One Way Analysis of Variance (ANOVA) and Tukey test in Windows SPSS 20.0 computer program. P <0.05 value was considered statistically significant. Data were signified as the means ± standard deviation (S.D).

RESULTS AND DISCUSSION

Fatty acid composition of the cocklebur seeds

In this study, seeds of cocklebur averaged 30% oil content. Other studies showed that cocklebur oil content can reach up to 37-42% (Klimakhin et al. 2015, Rozina et al. 2017). Fatty acid compositions of cocklebur oil are given in Table 2. Fatty acids determined in oil obtained from cocklebur seeds were palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), linolenic acid ($C_{18:3}$), arachidic acid ($C_{20:0}$), and gondoic acid ($C_{20:1}$). The highest fatty acid component was linoleic acid ($C_{18:2}$) with 72.624%, followed by oleic acid ($C_{18:1}$) with 16.87%. According to other studies, the linoleic acid content was determined as 76.97% (Cesur et al. 2018, Coşge Şenkal et al. 2019). Also, cocklebur seed-oil were consisted of saturated palmitic (5.59%) and stearic acids (2.14%); monounsaturated oleic acid (20.07%); and the more valuable polyunsaturated linoleic (68.06%). Our results showed similarities to the other studies (Klimakhin et al. 2015, Coşge Şenkal et al. 2019). The linoleic acid content (72.62%) was close to safflower and sunflower oils, but higher than soybean, olive and peaunt oils (Coşge Şenkal et al. 2019, Gursoy 2019). Chemical and physical properties of oils can change with their fatty acid composition. Saturated and unsaturated fatty acids are found in all vegetable oils and their ratios can be changed (İmer and Taşan 2018, Gursoy 2019). Clinical observations established a 1:5 ratio of polyunsaturated fatty acids omega-3 and omega-6 was most favorable for treating cardiovascular diseases (Klimakhin et al. 2015). Quality in oils is the ratio of unsaturated fatty acids to saturated fatty acids. The high rate of this ratio shows the quality of the oil and its importance in terms of benefiting human health. Fatty acid compositions determined in our study are compared with other oils (Table 3). According to Table 3, the highest ratio was in canola. The other highest unsaturated fatty acids rates are safflower, sunflower and olive, respectively. In this study, cocklebur oil was the second highest oil with unsaturated fatty acid ratio.

Table 2. Fatty acid compositions of cocklebur oil

Fatty acid	Structure	Formula	Amount (%)
Myristic	14:0	$C_{14}H_{28}O_2$	0.034
Palmitic	16:0	$C_{16}H_{32}O_2$	5.902
Palmitoleic	16:1	$C_{16}H_{30}O_2$	0.067
Stearic	18:0	$C_{18}H_{36}O_2$	2.697
Oleic	18:1	$C_{18}H_{34}O_2$	16.874
Linoleic	18:2	$C_{18}H_{32}O_2$	72.624
Linolenic	18:3	$C_{18}H_{30}O_2$	0.255
Arachidic	20:0	$C_{20}H_{40}O_2$	0.036
Gondoic	20:1	$C_{20}H_{38}O_2$	0.16
Behenic	22:0	$C_{22}H_{44}O_2$	0.84
∑SFA			9.509
∑MFA			17.101
∑PFA			72.879
Total			99.489

∑SFA: Saturated fatty acids, ∑MFA: Monounsaturated fatty acids, ∑PFA: Polyunsaturated fatty acids

Table 3. Fatty acid and (P/S) percentages of some crops (Baydar and Erbas 2014; Gursoy 2019)

Species	Unsaturated Fatty Acid (%)	Saturated Fatty Acid (%)	P / S Ratio
Sunflower	89	11	8.1
Soybean	85	15	5.7
Peanut	82	18	4.6
Olive	86	14	6.1
Canola	94	6	15.7
Safflower	90	10	9
Cocklebur*	90	9.5	9.5

*Our results

Macro- and microminerals content

Macro- and microminerals concentrations obtained our study are compared with other oil crops (Table 4). This study showed that the highest macromineral and micromineral contents are K and P and Na, respectively. The basic nutrients needed for the production of plants are also essential for the growth and development of all living things. Minerals, which constitute 4%–6% of the human body, are critical in nutrition (Klimakhin et al. 2015). The most abundant element was measured as Na at 0.15±0.02 ppm. Zn and Fe contents of cocklebur were found to be low compared to K and P levels. Mineral K in sunflower, sesame, flaxseed, soybean, corn and olive oil have detected in the range of 5.93-47.2 ppm. It is an important mineral in plants for metabolic, physiological and biochemical functions (Iskander 1993, İmer and Taşan 2018, Demir and Taşan 2019). Cocklebur seed detected the trace elements Se, Mn, Zn, and Cu, which are valuable for humans (Klimakhin et al. 2015).

Table 4. Macro and micro minerals in the vegetable oils (ppm) (Imer and Taşan 2018).

Vegetable oil	K	P	Fe	Mn	Zn	Cu	Na
Sunflower	7.10±1.43	22.39±4.41	1.03±0.20	**	0.58±0.10	**	4.45±0.32
Safflower	1.99±0.29	5.96±0.57	0.74±1.10	**	0.35±0.05	**	6.97±0.90
Flaxseed	4.40±0.49	17.43±2.35	0.86±0.14	**	0.39±0.06	**	9.73±1.18
Hazelnut	9.69±1.39	36.49±3.08	0.45±0.06	**	0.32±0.07	**	7.93±1.41
Sesame	16.26±3.79	50.10±10.78	2.16±0.57	**	0.24±0.07	**	8.69±1.92
Peanut	51.73±13.92	36.57±7.71	1.46±0.26	**	0.14±0.04	**	8.23±1.09
Cocklebur*	17.53± 2.13	173.00±0.11	0.11±0.02	**	0.02±0.01	**	0.15±0.02

Values are mean±SD in each group. * Our results, **not detected

Oxidative stress parameters and AChE activities of samples

Results of antioxidant enzyme activities of erythrocytes and leucocytes

Antioxidant enzyme activities (GST, SOD, GPx and CAT) of 0.01, 0.02, 0.04 µL mL^{-1} oil which was obtained from the seeds of *X. strumarium*, treated human leucocytes and erythrocytes did not show any differences compared to control group (Table 5 and Table 6). Treatment with higher doses of *X. strumarium* oil significantly decreased cell antioxidant enzyme activities.

Table 5. Enzyme activities of different treatment groups of erythrocytes

Groups (µL mL^{-1})	SOD (U $mgHb^{-1}$)	CAT (U $mgHb^{-1}$)	GPx (U $mgHb^{-1}$)	GST (U $mgHb^{-1}$)	AChE (U $mgHb^{-1}$)
Control	596.94 ±18.21a	372.18±9.94a	69.62±6.76a	41.23±2.09a	29.1±4.57a
0.01	588.76±9.9 a	374.51±11.25a	68.37±7.11a	39.03±3.41a	28.78±2.81a
0.02	579.86±11.53a	351.7±15.02a	65.92±5.78a	39.28±2.67a	26.19±3.22a
0.04	571.64±18.47a	349.76±16.82a	61.45±5.99a	36.42±4.02a	26.66±3.73a
0.08	411.58±7.22b	243.54 ± 8.22b	59.01±8.17a	28.44±3.29b	25.41±2.94a
0.16	342.06±8.15c	238.71±16.11b	35.06±3.92b	20.08±2.24c	19.71±2.62b
0.32	280.71±10.03d	152.02±9.88c	24.04±4.18c	15.19±2.15d	18.09±1.1b
0.50	216.08±6.99e	101.29±12.04d	13.12±5.03d	11.17±3.65d	14.28±2.04c
1.00	127.91±12.56f	62.49±8.67e	5.11±2.12e	2.26±0.69e	10.16±1.88d

Values are mean ± SD in each group. Significance at $P < 0.05$. Within each column, means superscript with different letters are significantly different.

Table 6. Enzyme activities of different treatment groups of leucocytes

Groups (µL mL^{-1})	SOD (U $mgprotein^{-1}$)	CAT (U $mgprotein^{-1}$)	GPx (U $mgprotein^{-1}$)	GST (U $mgprotein^{-1}$)
Control	467.88±23.27a	301.92±17.01a	64.12±7.65a	34.22±5.21a
0.01	455.1±16.24a	292.31±14.47a	63.83±6.17a	33.06±7.21a
0.02	449.53±16.8a	293.05±11.73a	60.23±5.29a	30.48±3.76a
0.04	438.11±14.32a	284.08±16.72a	57.8±3.01a	30.63±4.89a
0.08	359.34±18.05b	216.92 ± 8.83b	48.06±5.14b	26.43±8.91a
0.16	313.7±11.14c	188.27±10.11c	37.77±4.36c	15.52±2.73b
0.32	234.31±13.22d	140.47±7.98d	21.01±7.82d	8.67±3.01c
0.50	173.06±21.74e	98.52±17.3e	18.64±5.24d	6.89±2.72c
1.00	99.52±27.24f	46.15±22.05f	4.47±2.09e	2.13±1.01d

Values are mean±SD in each group. Significance at $P < 0.05$. Within each column, means superscript with different letters are significantly different.

Results of lipid peroxidation levels of erythrocytes and leucocytes

MDA levels (GST, SOD, GPx and CAT) of 0.01, 0.02, 0.04 µL mL^{-1} *X. strumarium* seed oil treated human leucocytes and erythrocytes did not show any differences compared to control group (Figure 1 and Figure 2). Treatment with higher doses of *X. strumarium* oil significantly elevated cell MDA levels.

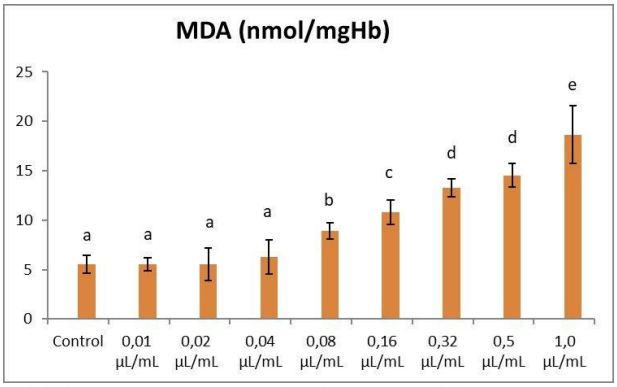

Figure 1. MDA levels of different treatment groups of erythrocytes. Each bar represents mean ± SD in each group. Columns superscripts with different letters are significantly different. Significance at $P < 0.05$.

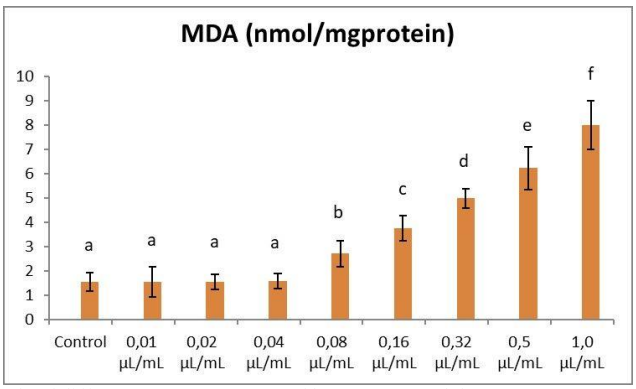

Figure 2. MDA levels of different treatment groups of leucocytes. Each bar represents mean±SD in each group. Columns superscripts with different letters are significantly different. Significance at $P < 0.05$.

Results of AChE activities of erythrocytes

Lower doses of *X. strumarium* seed oil treated erythrocytes did not show significant changes compared to control (Table 5). Treatment with higher doses (above 0.04 µL ml^{-1}) showed decreased AChE activity as compared to control cells.

Results of mitotic index

MI values are summarized in Figure 3. There was a decrease in MI values with increasing doses of *X. strumarium*. Significant changes were observed in 0.16, 0.32 and 0.50 µL mL^{-1} concentrations compared with the control ($P < 0.05$).

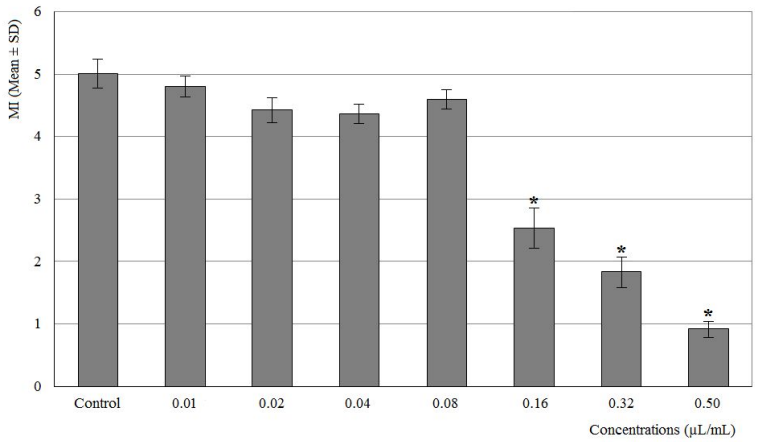

Figure 3. Mitotic index values in peripheral lymphocyte cultures exposed to different concentrations of *X. strumarium*. *Significantly different compared to control ($P < 0.05$).

Results of replication index

RI values are shown in Table 7. There was a decrease in RI values with increasing concentrations. 0.16, 0.32 and 0.50 µL mL^{-1} concentrations compared with the control ($P < 0.05$).

Table 7. Replication index values in peripheral lymphocyte cultures exposed to different concentrations of *X. strumarium.*

Concentrations (µL mL^{-1})	RI (Mean ± SD)
Control	1.282 ± 0.026
0.01	1.212 ± 0.019
0.02	1.174 ± 0.013
0.04	1.170 ± 0.011
0.08	1.184 ± 0.015
0.16	1.080 ± 0.010*
0.32	1.056 ± 0.006*
0.50	1.039 ± 0.008*

*Significantly different compared to the control ($P < 0.05$)

Results of micronucleus

MN rates are summarized in Figure 4. There was an increase in MN values with increasing doses of *X. strumarium*. Significant changes were observed in 0.16, 0.32 and 0.50 µL mL^{-1} concentrations compared with the control ($P < 0.05$).

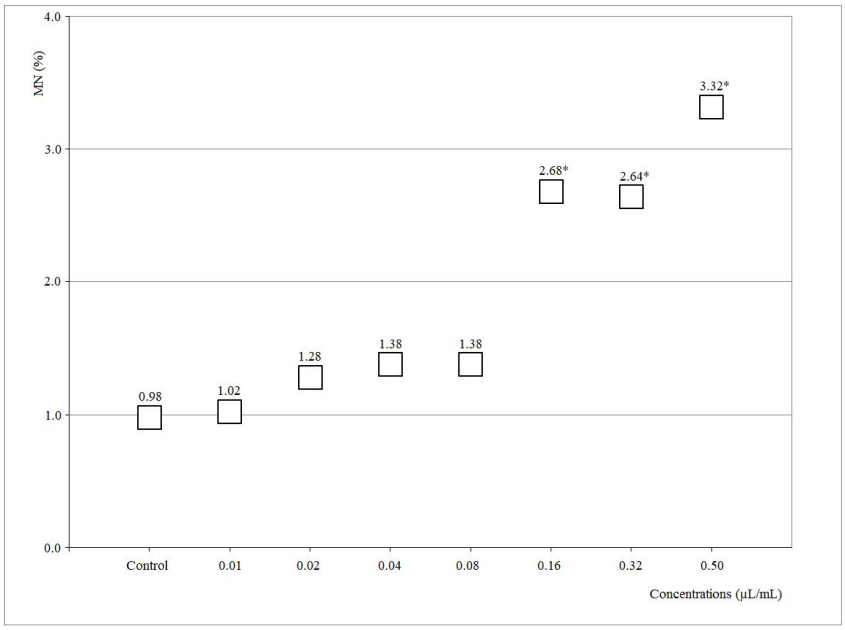

Figure 4. Micronucleus values in peripheral lymphocyte cultures exposed to different concentrations of *X. strumarium*. *Significantly different compared to control ($P < 0.05$).

Cocklebur has been used in many areas from traditional medical treatment to modern pharmacology and, for this reason, it is very important to determine effects on the human genome. The genotoxicity results showed a genotoxic effect to the dose-dependent (0.16, 0.32, and 0.50 µL mL^{-1}). In addition, MI and RI provide a measure of proliferative capacity of the cells and therefore can be used as indicators of the cytotoxic effects of the extracts investigated. The cytotoxicity results showed significant decreases in MI and RI values (0.16, 0.32, and 0.50 µL mL^{-1}). Witte et al. (1990) indicated that *X. strumarium* had medium to strong allergenic effects and was poisonous to mammals, and carboxyatractyloside and atractyloside were considered to be the basic toxic compounds. In literature, some reports have recorded these toxic and allergenic effects of monomers or extracts of *X. strumarium*; however, they are limited with few in vitro and usually in vivo animal experiments. A review summarized the toxicities and side effects of extracts of *X. strumarium*. These effects are death, weight loss, enlarged hepatic cell space, karyolysis, inflammatory cell infiltration, depressing the action of central nervous system, and decreasing hatch rate in mice, swine and zebrafish (Fan et al. 2019). Xue et al. (2014) showed that carbxyatractyloside and atractyloside inhibited cell proliferation and improved LDH activity at high concentration (100 µmol L^{-1}). In addition, Yu et al. (2013) reported that the water extracts of *X. strumarium* fruits could inhibit growth of HK-2 cells at high concentrations (100 µg mL^{-1}). Moreover, hydroalcoholic extracts of aerial parts of *X. strumarium* also caused DNA damage at different concentrations (25-100 µg mL^{-1}) through comet assay, chromosome aberrations, and sister chromatid exchanges (Piloto 2014). Su et al. (2016) indicated that the different components of *X. strumarium* had cytotoxic potentials and the ethyl-acetate fraction of methanol extracts of fruits of *X. strumarium* was the most toxic part. Despite all these reports, there is no study showing the cytotoxic and genotoxic effects of the cocklebur on human peripheral lymphocytes in vitro.

The toxic effect of oils obtained from plants has also been investigated in previous studies (Zaoui 2002). Exposure to xenobiotics such as plant oils may cause lipid peroxidation (LPO) and change the antioxidant enzyme activities such as CAT, SOD, GST and GPx. These enzymes are parts of first line defence against reactive oxygene species (ROS) (Zaoui 2002, Bas and Kalender 2016). These parameters are proven indicators of oxidative stress process (Pathak and Khandelwal 2006). Because they are potential targets for xenobiotic toxicity (Patra et al. 2011). So, we investigated their activities for understanding the effects of *X. strumarium*'s seed oil. In our study, SOD, CAT, GST and GPx activities of human erythrocytes and leucocytes significantly decreased in increasing doses of oil compared with not-treated cells, especially from the 0,08 µL mL^{-1}. As shown in numerous studies, changing of antioxidant enzyme activities correlate with increased concentrations of malondialdehyde (MDA) (Bas and Kalender 2016). MDA is the main oxidation product of LPO. Therefore, increasing in the MDA level is a major indicator of LPO (Comelekoglu et al. 2012). For this reason, we investigated its value for assignation of oxidative stress caused by LPO in leucocytes and erythrocytes. LPO has a main function in xenobiotic toxicity because of causing unwanted effects on cell and organelle membrane rigidity and osmotic fragility (Bas et al. 2014). In this study, increased MDA levels were observed in *X. strumarium*'s seed oil treated blood cells. This increase may explain with harmful effects of *X. strumarium*'s seed oil on cell membranes.

Acetylcholinesterase (AChE) has a main role in neurotransmission mediated by acetylcholine which is a neurotransmitter. The evaluation of AChE activity in cells is an important mark that diagnosis of poisoning induced by reversible and irreversible inhibitors (Santi et al. 2011). Some components of essential oils can cause neurotoxic effects on organisms, especially monoterpenes that found in plant oil can be act as competitive inhibitor of AChE (Kostyukovsky et al. 2002). In a previous study, essential oil of *Hypericum perforatum* caused decreasing of AChE activity (Bas and Ersoy2020). Also, Polatoğlu et al. (2016) indicated that AChE enzyme activity may be decreased by plant oil exposing. According to the results of MDA values, activities of AChE and antioxidant enzymes, it is clear that *X. strumarium*'s seed oil causes adverse effects in human leucocytes and erythrocytes especially from the 0.08 µL mL^{-1}.

Conclusion

The fatty acid component is changeable to a large scale depending on many of factors. Therefore, to know the variation occurred in fatty acid component of an oil-seed under given condition is very important for oil quality. Oil quality depends on food technology and processing manner for crude oil. Knowing the fatty acid component of vegetable oil makes it possible to produce oil for special using purposes. For this purpose, it would be possible to produce suitable oils by cultivating the desired species in suitable conditions. In edible oils, oleic and linoleic acid are the most important fatty acid components that are determine the oil quality. These two fatty acids were found to be significant in cocklebur seed oil. According to the results, it has been determined that it is close to the edible oils. However, in this study, it is thought that the chemical components found in cocklebur oil, which are different from edible oil, have toxic effects. In the genotoxic study, peripheral blood culture was performed in a wide dose range. Although cytotoxic genotoxic effects are detected in high concentrations, cocklebur oil can be evaluated at lower doses. Antioxidant results also support this opinion. Thus, it is thought that cocklebur seed oil, known as weed, cannot be recommended as alternative edible oil, but it can be used in different areas as an industrial crop and will contribute to the economically. It was concluded that more studies should be done on this vegetable oil.

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Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The contribution of the authors is equal

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Correlation among germination and seedling parameters of *Brassica juncea* under PEG 6000 and NaCl treatments

Fatma Kayacetin [1]

[1]Medical and Aromatic Plants Program, Kalecik Vocational School, Ankara University, Ankara/Turkey
Correspondence: fatma.kayacetin@ankara.edu.tr

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Abstract
Brown mustard (*Brassica juncea* L.) has potential for industrial use because of the high erucic acid content of the oil. This study evaluated the association under stress treatments [PEG 6000 (0, –0.2, –0.4, –0.6, –0.8, –1.0 and –1.2 MPa) and NaCl (0, 5, 10, 15, 20, 25, 30 and 35 dSm^{-1})] among germination and early seedling growth of *B. juncea* originated from Izmir, Turkey. Germination and seedling parameters as ISTA method to evaluate eleven seed parameters viz. germination percentage, final germination percentage, mean germination time, germination index, root length, shoot length, root shoot ratio, root length reduction, shoot length reduction, seedling fresh weight, seedling dry weigh. Statistical techniques correlation between different parameters both NaCl and PEG 6000 were analysed. Results exhibied that positive and significant correlation was noted among several germination and seedling parameters subjected to stress excluding root–shoot ratio that showed negative correlation with shoot length under the influence of both stress. Similarly, significant and negative correlation of mean germination time was detected with germination speed and final germination percentage. The objective and aim of this study was to determined correlation coefficients among studies traits of *B. juncea* under PEG 6000 and NaCl treatments. Such information about differential responses over time may be useful for identifying critical parameters for screening this species. The present research can help to plant breeders and agronomist in deciding the selection criteria.

Key words
Brown mustard, Salt and drought stress, Correlation coefficient.

Introduction
Brown mustard (*Brassica juncea* L.) from Brassicaceae family is an annual crop. It is cultivated as food, fodder, oil and as medicinal plants in different cultures and many parts of the World. It is generally grown as alternative feed crops. This species are used as a condiment and vegetable oil (Kayacetin, 2020).

Salinity and drought are major biotic stress detected (Serrano et al. 1999; Mitra, 2001; Blum, 2009). The plant's reaction to stress relies on the genotypes, the severity and duration of water deficit along with level of plant growth (Nezhadahmadi et al., 2013). Brown mustard behave differently under different under stress conditions. Water stress because of NaCl and PEG 6000 acts by declining the percentage germination and seedling growth parameters (Kayacetin, 2021). Evaluation of salt and drought tolerance may be attempted on control conditions and salt and drought stress. The correlation association among various germination traits on different crops have been reported previously (Fuller et al., 2012; Thabet et al., 2018; Ahmed et al., 2019).

Correlation coefficients define the level of relationship among treatments NaCl and PEG 6000. It is significant in plant breeding and give practical information providing evidence of the relationship between several desired traits. It provides a basic concept of the relationship between various germination traits useful for plant breeding in the selection of genotypes with desirable traits (Ghafoor et al., 2013; Ali et al., 2008). Information about correlation coefficients between the studied parameters influence in selection of superior genotypes (Afroz et al., 2004).

Current study targeted to screen germination and seedling parameters relevant to PEG 6000 and NaCl stress responses with an emphasis on the germination measure, investigating salt and drought stress responses in brown mustard. This study will help in understanding the differences between responses and adaptations to PEG 6000 and NaCl stress in *B. juncea* at the seed germination and early seedling growth. It help to correlate the assessed parameters with PEG 6000 and NaCl response of seed germination and seedling growth. The knowledge of association between parameters and their direct and indirect contribution towards expression of seed quality parameters are additional help to plant breeder in deciding the selection criteria.

Materials and Methods
The study was designed to at the Oilseed Crop Unit, Central Field Crops Research Institute Yeni–mahalle, Ankara, Turkey. The experiments were carried out using Completely Randomized Design, with 3 replications. Two experiments were performed. In the first experimental, factor was PEG levels (0.0, –0.2, –0.4, –0.6, –0.8, –1.0, and –1.2 MPa). In the second experiment, factor was NaCl levels [(0, 5 (–0.2 MPa), 10 (–0.5 MPa), 15 (–0.7 MPa), 20 (–1.0 MPa), 25 (–1.3 MPa), 30 (–1.5 MPa) and 35 dSm^{-1} (–1.8 MPa)] (Model WTW Cond. 314i, Germany). Distilled water used as a control in both experiments. Three replications of 20 seeds of each Petri dish® were germinated in two layered Whatman@qualitative filter paper, Grade 1 with 10 ml of respective test solutions in glass Petri dishes (100 × 10 mm) with respective concentrations of PEG and NaCl in separate experiments to induce drought and salt stress. The seeds were germinated at 22 ± 1 °C (Fallah–Toosi and Baki, 2013) in the dark for 10 days. Rate of germination was measured on daily basis. The filter papers and the Petri dishes in the experiment were replaced after every 2 days to prevent error and over accumulation of both PEG and NaCl. The percentage of germination was measured every day until no increase in count was was noted (ISTA, 1996). The seeds were counted germinated when ~2mm radicle emerged (Huang and Redmann, 1995). These seedlings from each replicate were measured at the end of tenth day of standard germination test and were evaluated for germination speed, mean germination time, final germination percentage, germination index, root length, shoot length, root/shoot ratio, root length reduction, shoot length reduction, and seedling fresh weight. The seedling dry weights were measured by drying the samples in an oven at at 70 °C for 48 h (Böhm, 1979) (Table 1).

Table 1. The names, and descriptions of measurement for *Brassica juncea*

Name	Abbreviation	Description of Measurement
Germination speed	GS	$GS = n_1/d_1+n_2/d_2+n_3/d_3+ \ldots\ldots nn/dn$ (Panuccio et al., 2014) n= count of the germinated seed; d= count of the days
Final germination percentage	FGP	FGP–%: (number of normal seedling/number of seedling)×100 (Kandil et al., 2012)
Mean germination time	MGT	MGT–day= $n_1 \times d_1 + n_2 \times d_2 + n_3 \times d_3 + \ldots\ldots$ /Total number of days (Ellis et al., 1980) n= count of the germinated seed; d= count of the days
Germination index	GI	GI–%= (germination percentage in each treatment/germination percentage in the control) × 100 (Panuccio et al., 2014)
Root length	RL	was measured with a scale (in cm)
Shoot length	SL	was measured with a scale (in cm)
Root shoot ratio	R/S	was expressed as the percentage of the shoot length to root length
Root length reduction	RLR	root length in control treatment − root length under the influence of stress
Shoot length reduction	SLR	shoot length in control treatment − shoot length under the the influence of stress
Seedling fresh weight	SFW	was recorded in g using a sensitive balance
Seedling dry weight	SDW	was measured in mg with a sensitive balance soon after drying

Statistical analysis

Completely Randomized Design with three replications was used. Correlation analysis among treatments was done for both seed germination and growth parameters separately for PEG 6000 and NaCl treatments ($p<0.05$; $p<0.01$) using MINITAB.

Result and Discussion

PEG Treatments

Correlation coefficients among germination and seedling parameters of brown mustard under PEG 6000 treatments is detected in Table 2. Significant and positive correlation of final germination percentage was determined with germination speed (0.995). Significant and negative correlation of mean germination time was noted with germination speed (–0.981) and final germination time (–0.993). Similarly, germination index expressed positive and significant correlation with germination speed (r = 0.995) and final germination percentage (r = 1) and significant and negative correlation with mean germination time (–0.992). Root length expressed positive and significant correlation with germination speed (r = 0.973), final germination percentage (r = 0.984) and germination index (0.985) and significant and negative correlation with mean germination time (–0.966). Taking into account shoot length expressed significant and positive correlation with germination speed (r = 0.977), final germination percentage (r = 0.988), germination index (0.989) and root length (0.999) whereas parameter mean germination time (–0.972) was showed significant and negative correlation. Root shoot ratio expressed significant and positive correlation with germination speed (r = 0.879), final germination percentage (r = 0.92), germination index (0.92), root length (0.944) and shoot length (0.946); significant and negative correlation with mean germination time (0.932). Root length reduction expressed significant and negative correlation with germination speed (r = –0.973), final germination percentage (r = –0.984), germination index (–0.984), root length (–1), shoot length (–0.999) and root shoot ratio (r = –0.942) while root length reduction demonstrated significant and positive correlation with mean germination time (0.964). Shoot length reduction indicated significant and negative correlation with germination speed (r = –0.978), final germination percentage (r = –0.989), germination index (–0.989), root length (–1), shoot length (–1) and root shoot ratio (r = –0.944) whereas shoot length reduction showed significant and positive correlation with mean germination time (0.973) and root length reduction (0.999). Seedling fresh weight expressed significant and positive correlation with root length (0.919) and shoot length (0.906); significant and negative correlation with root length reduction (–0.921) and shoot length reduction (–0.907). Rest of the parameters showed non–significant correlation. Seedling dry weight possessed significant and positive correlation with germination speed (r = 0.957), final germination percentage (r = 0.978), germination index (0.978), root length (0.991), shoot length (0.993), root shoot ratio (r = 0.972) and seedling fresh weight (0.879). In other respects positive and significant correlation with mean germination time (–0.97), root length reduction (–0.99) and shoot length reduction (–0.992). These obtained results agreed with those obtained by Rauf et al., (2007) who finded a positive and significant correlation between germination rate, root length and shoot length but a negative and non significant and correlation between germination rate and root/shoot length ratio in 16 wheat varieties under PEG 6000 stress. And similar conclusions were displayed by Hellal et al. (2018) for barley genotypes under PEG 6000 stress, the correlation analysis expressed that root/shoot length were the correlated parameters. Wheat, root length, fresh weight, dry weight had positive and significant correlation among themselves under the influence of stress and under controlled conditions long–lived and not comprehensively possible with all the parameters studied while shoot length showed a non–significant correlation (Ahmed et al., 2019). In barley, root control options, but drought fresh weight and long and short time and germination percentage and fresh varieties are mandatory (Thabet et al., 2018).

NaCl Treatments

It is evident from Table 3 all the 11 parameters were significantly associated with germination and seedling parameters of brown mustard under NaCl treatments. Significant and positive correlation of final germination percentage was determined with germination speed (0.959). Significant and negative correlation of mean germination time was noted with germination speed (–0.967) and final germination time (–0.897). Similarly, germination index showed positive correlation with germination speed (r = 0.959) and final germination percentage (r = 1); significant and negative correlation with mean germination time (–0.898). Root length indicated significant and positive correlation with germination speed (r = 0.966), final germination percentage (r = 0.911) and germination index (0.912) whereas root length showed significant and negative correlation with mean germination time (–0.921). Shoot length expressed significant and positive correlation with germination speed (r = 0.976), final germination percentage (r = 0.956), germination index (0.956) and root length (0.988) while shoot length demonstrated significant and negative correlation with mean germination time (–0.914). Root shoot ratio expressed significant and positive correlation with germination speed (r = 0.892), root length (0.936) and shoot length (0.877); significant and negative correlation with mean germination time (0.903). Final germination percentage and germination index showed non–significant correlation. Root length reduction possessed significant and negative correlation with germination speed (r = –0.968), final germination percentage (r = –0.912), germination index (–0.912), root length (–1), shoot length (–0.988) and root shoot ratio (r = –0.938); significant and positive correlation with mean germination time (0.923). Shoot length reduction expressed significant and negative correlation with germination speed (r = –0.975), final germination percentage (r = –0.964), germination index (–0.964), root length (–0.981), shoot length (–0.999) and root shoot ratio (r = –0.859); significant and positive correlation with mean germination time (0.913) and root length reduction (0.981). Seedling fresh weight noted significant and positive correlation with germination speed (r = 0.983), final germination percentage (r = 0.944), germination index (0.944), root length (0.995), shoot length (0.997), root shoot ratio (r = 0.912); significant and negative correlation with mean germination

time (–0.933), root length reduction (–0.996) and shoot length reduction (–0.993). Seedling dry weight showed significant and positive correlation with germination speed (r = 0.986), final germination percentage (r = 0.96), germination index (0.961), root length (0.987), shoot length (0.998), root shoot ratio (r = 0.885) and seedling fresh weight (0.997). Contrarily, negative and positive correlations with parameters mean germination time (–0.935), root length reduction (–0.987) and shoot length reduction (–0.998). Bae et al. (2006) detected a negative correlation between germination and salinity, root and shoot length and also significant positive correlation to germination percentage and root and shoot length germination and early pak–choi, amaranth, cabbage, and sugar beet seedling (p=0.01).

Under both salinity and drought stress, the significant positive association among germination and seedling growth parameters may lead to rapid and high improvement during selection because of correlated response as improvement in on characters may bring improvement in other character. These characters have also been identified as major direct contributors in germination and seedling growth parameters (Maurya et al., 2019).

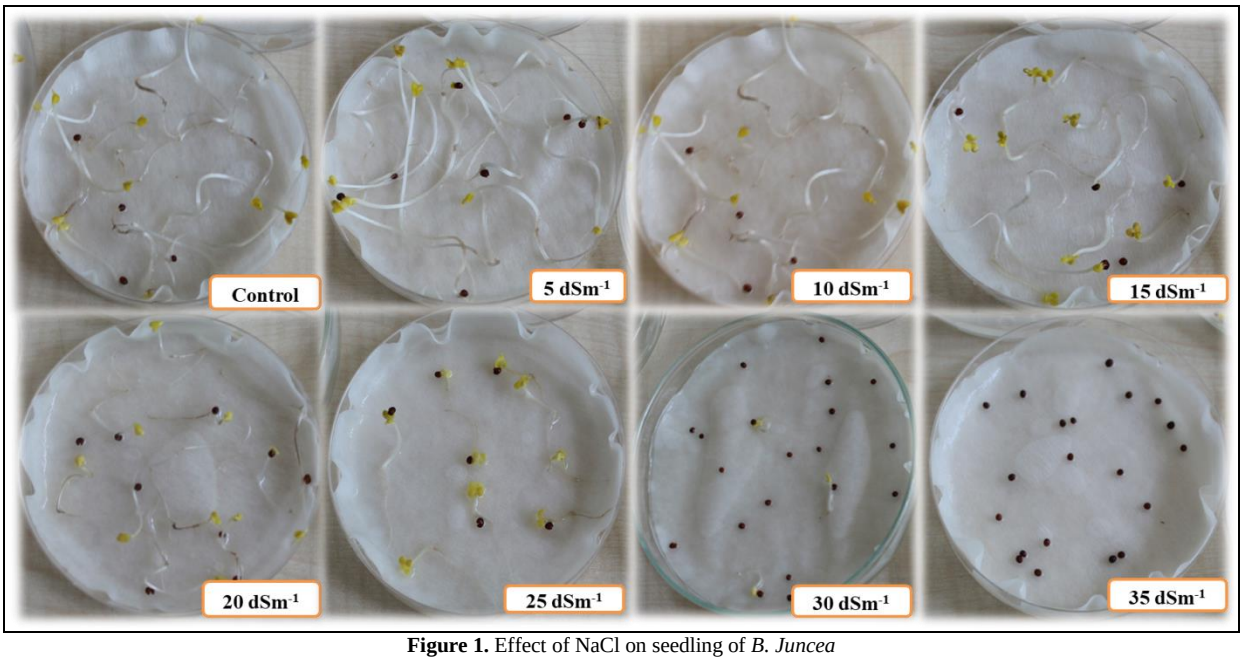

Figure 1. Effect of NaCl on seedling of *B. Juncea*

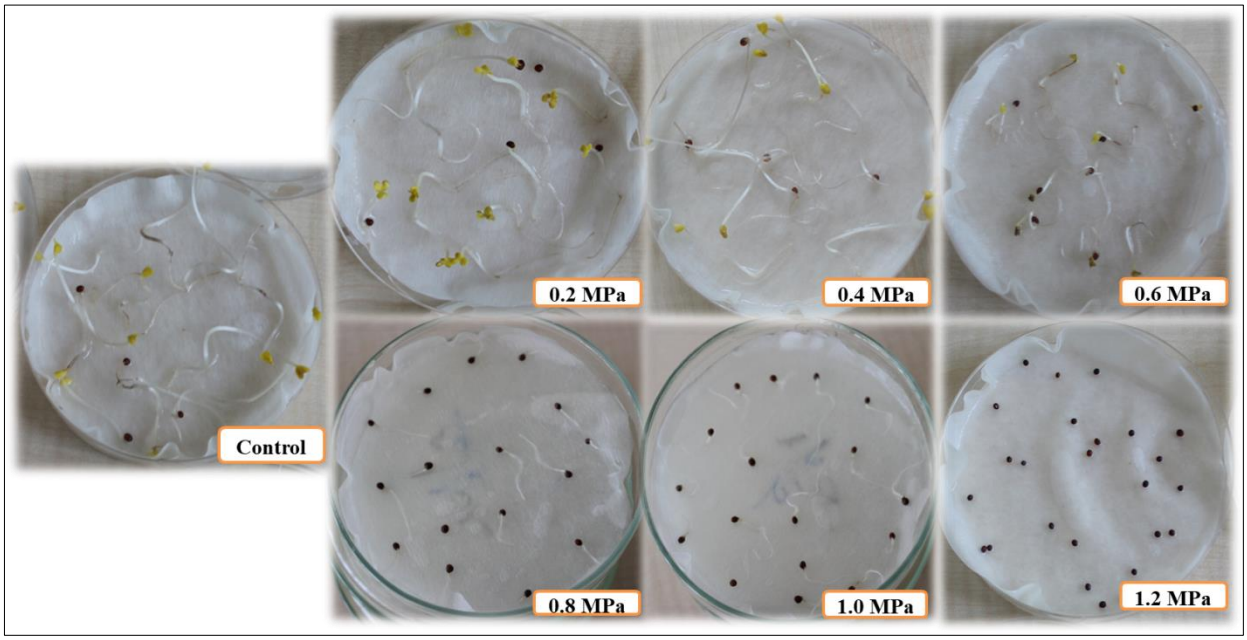

Figure 2. Effect of PEG 6000 on seedling of *B. juncea*

Table 2. Correlation coefficients among germination and seedling growth parameters observed in PEG 6000 treatments of *B. juncea*

	GS	FGP (%)	MGT (day)	GI (%)	RL (cm)	SL (cm)	R/S	RLR (%)	SLR (%)	SFW (mg)	SDW (mg)
GS	1	0.995**	–0.981**	0.995**	0.973**	0.977**	0.879*	–0.973**	–0.978**	0.838	0.957*
FGP (%)		1	–0.993**	1**	0.984**	0.988**	0.92*	–0.984**	–0.989**	0.843	0.978**
MGT (day)			1	–0.992**	–0.966**	–0.972**	–0.932*	0.964**	0.973**	–0.788	–0.97**
GI (%)				1	0.985**	0.989**	0.92*	–0.984**	–0.989**	0.845	0.978**
RL (cm)					1	0.999**	0.944*	–1**	–1**	0.919*	0.991**
SL (cm)						1	0.946*	–0.999**	–1**	0.906*	0.993**
R/S							1	–0.942*	–0.944*	0.825	0.972**
RLR (%)								1	0.999**	–0.921*	–0.99**
SLR (%)									1	–0.907*	–0.992**
SFW (mg)										1	0.879*
SDW (mg)											1

** $p<0.01$; * $p<0.05$

Table 3. Correlation coefficients among germination and seedling growth parameters observed in NaCl treatments of *B. juncea*

	GS	FGP (%)	MGT (day)	GI (%)	RL (cm)	SL (cm)	R/S	RLR (%)	SLR (%)	SFW (mg)	SDW (mg)
GS	1	0.959**	–0.967**	0.959**	0.966**	0.976**	0.892**	–0.968**	–0.975**	0.983**	0.986**
FGP (%)		1	–0.897**	1**	0.911**	0.956**	0.747	–0.912**	–0.964**	0.944**	0.96**
MGT (day)			1	–0.898**	–0.921**	–0.914**	–0.903**	0.923**	0.913**	–0.933**	–0.935**
GI (%)				1	0.912**	0.956**	0.747	–0.912**	–0.964**	0.944**	0.961**
RL (cm)					1	0.988**	0.936**	–1**	–0.981**	0.995**	0.987**
SL (cm)						1	0.877**	–0.988**	–0.999**	0.997**	0.998**
R/S							1	–0.938**	–0.859*	0.912**	0.885**
RLR (%)								1	0.981**	–0.996**	–0.987**
SLR (%)									1	–0.993**	–0.998**
SFW (mg)										1	0.997**
SDW (mg)											1

** $p<0.01$; * $p<0.05$

Conclusion

Correlation analyses showed that most of germination and seedling characters of *B. juncea* under salinity and drought stress and non stress conditions were significant and negatively or positively associated among themselves screening for these desired characters could be beneficial in breeding studies. Higher of values of above parameters, better is the seed quality. Generally mean germination time showed significant and negative association with studied characteristics. Negative and positive strong relationship among the parameters under the influence of drought and salinity condition showing the importance of these parameters for use in future breeding and plant improvement studies.

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Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The contribution of the authors is equal

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Effects of donor plant age and explant types on Asparagus (*Asparagus officinalis* L.) micropropagation

Hatice Büşra YAVUZ[1] **Nuray ÇÖMLEKÇİOĞLU**[2*]
[1]Eskişehir Osmangazi University, Institute of Science, Eskişehir, Türkiye
[2]Eskişehir Osmangazi University, Faculty of Agriculture, Department of Horticulture, Eskişehir,Türkiye
* **Correspondence:** ncomlekcioglu@ogu.edu.tr

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Abstract
Asparagus (*Asparagus officinalis* L.) is a dioecious species in the Asparagaceae family. Male plants are more productive than female plants. More efficient plant propagation can be followed by focusing on the production of male plants with tissue culture techniques. The aim of this study is to determine the micro-propagation effects of shoot tip and lateral bud explants taken from donor plants of different ages (3 and 5 years). Jersey Knight F1 was used in the experiment. To obtain shoots from explants, 0.2 mg l^{-1} NAA (Naphtalen acetic acid), 0.2 mg l^{-1} BAP (benzyl amino purine), 0.2 mg l^{-1} kinetin, 30 g l^{-1} sucrose and 7 g l^{-1} agar containing MS (Murashige & Skoog, 1962) medium was used. The shoots were transferred to proliferation MS medium containing 0.5 mg l^{-1} BAP and 0.2 mg l^{-1} IBA (indole butyric acid), and two subcultures were made. MS medium containing 0.5 mg l^{-1} IBA was used for rooting of the shoots. Shoot development was obtained from all cultured explants. In the first subculture, the average shoot numbers per explant obtained from 3 and 5 years old plants were determined as 7.0 and 7.18, respectively. While an average 9.43 shoots were obtained from shoot tip explant, 4.75 shoots from the lateral bud explant. In the second subculture, an average of 9.13 shoots from 3-year-old plants and 9.55 shoots from 5-year-old plants were obtained. While 10.53 shoots were obtained from the shoot tip, 8.15 shoots were detected from the lateral bud. The proliferation coefficient of the shoot tip explant in the first subculture differed significantly compared to the lateral bud explant. In contrast, the difference in proliferation coefficients decreased in the second subculture. 48.0% and 58.0% rooting rate were obtained from the explants of the 3 and 5 years old plants respectively. The average rooting were 65.0% in the shoot tip explant and 41.0% in the lateral bud explant.

Key words
Asparagus officinalis, micropropagation, donor age, shoot tip, axillary bud

Introduction
Asparagus (*Asparagus officinalis* L.) is an herbaceous and perennial monocot plant species. It's economic and nutritional value is quite high. It is a product that is harvested in the early season especially in the spring (Rasad et al., 2019). It can be grown worldwide and there are approximately 300 species (Kubota et al., 2012). While nearly 100 species grow in Anatolia and Europe, it is known that 12 species naturally grow in Turkey. These wild species are *Asparagus acutifolius* and *Asparagus verticillatus* (Altunel, 2021).
Asparagus (2n=20) is a dioecious plant. Male plants are more productive than female plants. Many breeding programs around the world focus on the production of such male hybrids (Desjardins, 1992). When a 100% male hybrid with superior characteristics is obtained, tissue culture propagation of the parents is required for large-scale seed production. Propagation of asparagus from seed is not efficient due to the low germination rate of this plant. Propagation by dividing the rhizome of the plant is a time-consuming process. 2-4 new plants are obtained from a plant under optimum conditions and within a year (Sarabi & Almasi, 2010). At the same time, production depends on the season. Tissue culture techniques allow vegetative propagation of male plants. Since microclonal reproduction is not dependent on the season, production can be made throughout the year. It allows the reproduction of healthy, high-yielding and high-quality male plants preferred in production with the selection of donor plants.
Although micropropagation has been practiced for a long time in asparagus, low propagation coefficient, weak roots or lack of root formation are important problems. The success of tissue culture is influenced by many factors that originate from the donor plant from which the explant is taken or are related to the conditions during the application of the culture technique. To improve asparagus micropropagation, genotype (Conner et al., 1992; Fortes et al., 1997), explant type (Inagaki et al., 1981; Harada & Yakuwa 1983; Maung et al. 2019), culture system, type and concentration of basal medium, carbohydrate sources (Harada and Yakuwa 1983; Levi and Sink 1991; Bojnauth et al. 2003; Pontaroli and Camadro 2005), growth regulators (Azad and Amin 2017), and growth retardants (Khunachak et al. 1987) should be considered.
The aim of this study is to determine the micropropagation effects of shoot tip and lateral bud explants taken from donor plants of different ages (3 and 5 years) in asparagus.

Material and Methods
This research was carried out in the tissue culture laboratory of Eskişehir Osmangazi University, Faculty of Agriculture, Department of Horticulture (Eskişehir, Turkey). 3 and 5 years old plants of Jersey Knight F1 asparagus variety obtained from Nomad Tarım A.Ş. were used as donors. Shoot tip and lateral (axillary) buds were used as explants. All tissue culture applications were made under aseptic conditions and a laminar air flow sterile cabinet was used.

Disinfection of explants
For disinfection of plant material, the shoots were cut into 3-4 cm lengths containing the apical meristem and lateral buds (Figure 1). After shaking for 20-30 seconds in 70% ethyl alcohol in separate groups, they were kept in 10% commercial bleach solution containing sodium hypochlorite (5%) for 15 minutes. Then rinsed 3-4 times with sterile distilled water.

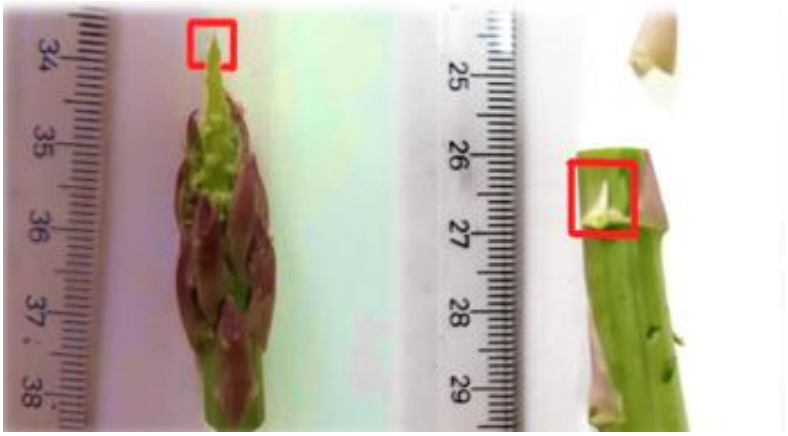


Figure 1. Cutting shoot tip and lateral bud explants

Nutrient Media

MS (Murashige & Skoog 1962) nutrient medium containing 30 g l^{-1} sucrose and 7 g l^{-1} agar was used as the basic medium. The pH of the nutrient media was adjusted to 5.9. To obtain shoots from explants, 0.2 mg l^{-1} NAA, 0.2 mg l^{-1} BAP and 0.2 mg l^{-1} kinetin were added. In order to proliferate the developed shoots, they were transferred to the multiplication (propagation) medium containing 0.5 mg l^{-1} BAP and 0.2 mg l^{-1} IBA. The shoots obtained after the second subculture were transferred to a rooting medium containing 0.5 mg l^{-1} IBA (Table 1).

Sterilization of the nutrient media was done by autoclaving at 121 °C for 15 minutes. Shoot tips and lateral buds from sterilized shoots were cut aseptically in a laminar air flow sterile cabinet and used as explants. The explants, which were cut with the help of scalpel and forceps, were placed on the nutrient medium in contact with the medium and without being immersed in the medium. 100 ml glass jars were used for planting. 5 explants were planted in each jar.

Table 1. Nutrient media and contents

	Nutrient media and contents
Shoot Induction Medium	MS + 0.2 mg l^{-1} NAA + 0.2 mg l^{-1} BAP + 0.2 mg l^{-1} kinetin + 30 g l^{-1} sucrose + 7 g l^{-1} agar
Shoot Multiplication Medium	MS + 0.5 mg l^{-1} BAP + 0.2 mg l^{-1} IBA + 30 g l^{-1} sucrose + 7 g l^{-1} agar
Root Induction Medium	MS + 0.5 mg l^{-1} IBA + 30 g l^{-1} sucrose + 7 g l^{-1} agar

Incubation

The glass jars, whose explant planting was completed, were taken to the climate room adjusted to 25 ºC temperature and 16/8 hour photoperiodic arrangement.

Experiment Design and Statistical Analyses

The study was carried out in four replications, with 25 explants in each replication (5 jars and 5 explants in each jar), using a total of 100 explants for each application. The results were analyzed by analysis of variance (ANOVA) using the Tarist Statistics Program (Açıkgöz et al., 2004). Biplot (principal component method) was made with Minitab 17 statistical program (Anderson, 1984). Means were compared with Least Significant Different (LSD).

Result and Discussion

In the experiment, shoot tip and lateral bud explant types were used from 3 and 5 years old donor asparagus plants. Shoot induction rate, the number of shoots per explant in the first and second subcultures (proliferation coefficient), and rooting rates were determined.

Shoot Induction

Shoot development started within 2 - 3 weeks after the shoot tip and lateral bud explants were planted (Figure 2). All explants taken in all of the studied donor age and explant types formed 100% shoots.

Figure 2. Shoots obtained in the shoot induction medium

In the experiment, the shoot formation of all explants is in agreement with the study of Paudel et al. (2018) that the combination of auxin and kinetin used in MS medium had a positive effect on shoot growth and development *in vitro* propagation of *Asparagus racemosus* Wild. Likewise, Sallam (2019) stated that 100% shoot was obtained from shoot tip and single node (axillary) explants.

Shoot Multiplication

The shoots that developed to 5-6 cm long in the shoot induction medium were transferred to the multiplication medium. Two subcultures were made in the study. Shoot multiplication coefficient data were obtained after 5 weeks of culture (Figure 3). In Table 1, the variance analysis table of the shoot numbers obtained per explant in the first subculture is presented. In the first subculture, it was determined that the donor plant age was not important in the number of shoots obtained, but showed significant differences ($P \leq 0.01$) according to the explant type. The interaction of donor plant age and explant type was found to be not significant.

Table 1. Table of variance analysis of donor plant age (A) and explant types (B) on average shoot numbers per explant in the first subculture

Variation source	Degrees of freedom	Sum of Squares	Mean Square	F Value	Table Value %5	Table Value %1
Repeat	3	2.273	0.758	1.949ns	3.860	6.990
Donor Plant Age (A)	1	0.123	0.123	0.315ns	5.120	10.560
Explant Type (B)	1	87.422	87.422	224.961**	5.120	10.560
A*B	1	0.063	0.063	0.161ns	5.120	10.560
Error	9	3.498	0.389			
Total	15	93.378	6.225			

ns = not significant, * = significant at alfa level %5, ** significant at alfa level %1

In the first subculture, the average number of shoots obtained from the explants of 3 and 5 years old donor plants were determined as 7.0 and 7.18 shoots, respectively. The difference between the means was found to be not significant. An average of 9.43 shoots were obtained from the shoot tip explant, while an average of 4.75 shoots were obtained from the lateral bud explant. The explant type was found to be significant ($P \leq 0.01$) in terms of the number of shoots obtained.

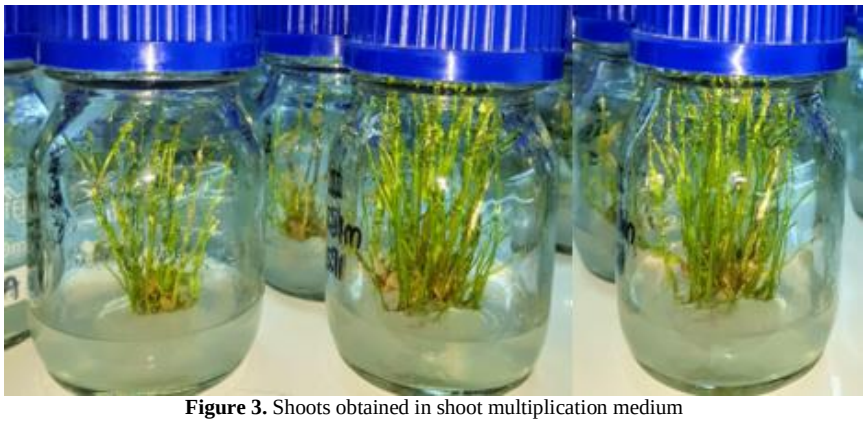


Figure 3. Shoots obtained in shoot multiplication medium

In the second subculture, effects of donor plant age ($P \leq 0.05$) and explant type ($P \leq 0.01$) were significant on the number of shoots obtained per explant. It was determined that the interaction of plant age and explant type was insignificant in the second subculture (Table 2).

Table 2. Table of variance analysis of donor plant age (A) and explant types (B) on average shoot numbers per explant in the second subculture

Variation source	Degrees of freedom	Sum of Squares	Mean Square	F Value	Table Value %5	Table Value %1
Repeat	3	0.613	0.204	2.882ns	3.860	6.990
Donor Plant Age (A)	1	0.723	0.723	10.200*	5.120	10.560
Explant Type (B)	1	22.563	22.563	318.529**	5.120	10.560
A*B	1	0.062	0.062	0.882ns	5.120	10.560
Error	9	0.637	0.071			
Total	15	24.597	1.640			

ns = not significant, * = significant at alfa level %5, ** significant at alfa level %1

In the second subculture, an average of 9.13 shoots were obtained from 3-year-old donor plants, while an average of 9.55 shoots were obtained from 5-year-old donor plants. Compared to the first subculture, 3-year-old donor plants produced more shoots than 5-year-old donors. When the effect of explant type in the second subculture is examined; 10.53 shoots were obtained from the shoot tip explant, while 8.15 shoots were detected from the lateral bud explant (Table 3). In the second subculture the shoot numbers per explant were increased for both explant types compared to the first subculture (Figure 4).

Generally, it was determined that the shoot tip explant was more successful than the lateral bud explant. According to the results obtained, the shoot tip explant gave the highest shoot regeneration rate at the same duration and medium. According to the first subculture data, the shoot tip explant showed a significant difference when compared to the lateral bud explant. On the other hand, as a result of the second subculture, the difference between the proliferation coefficients decreased according to explant types.

Table 3. Average number of shoots per explant according to donor plant age and explant type in the second subculture

Donor plant age	Number of shoot	Explant type	Number of shoot
3-year-old	9.13 **b**	Shoot tip	10.53 **a**
5-year-old	9.55 **a**	Lateral bud	8.15 **b**
LSD (%5)	0.301	**LSD(%1)**	0.432

The column having a different letter(s) are statistically significant

It has been reported by many researchers that the explant type and the optimum hormone concentrations are important in the number of shoots obtained. Rasad et al. (2019) obtained shoots from shoot piece and root explants in different culture media within 2 weeks. They reported that the highest shoot regeneration rate was obtained in the shoot piece explant (6.2 shoot explant^{-1}). Maung et al. (2019) found that apical bud (8.9 shoot explant^{-1}) and lateral bud

explants have micropropagation potential compared to shoot piece explant in micro-propagation. According to the studies, it was determined that the type of explant affects the regeneration capacity.

Figure 4. Shoots proliferation in the first subculture (A) and second subculture (B) medium

In this study, it was determined that the explant type effects on shoot regeneration was more significant than the effects of donor plant age. Shoot tip explants produced more shoots per explant than lateral bud explants. This may be due to different cell division rates at different explant. The shoot tips used as explants are located in the apical meristem and sub-apical meristem region. It has been reported that most of the cell division in asparagus occurs in the apical meristem of the shoot tip (Culpupper & Moon, 1939).

Root Induction

The shoots obtained in the shoot multiplication medium were separated as cluster shoots (containing 4-7 shoots) after 5 weeks. The shoot clusters were transferred to root induction medium containing 0.5 mg l^{-1} IBA for root initiation. Due to the low rooting rate after 8 weeks, these cluster of shoot were retransferred to MS medium containing 1.0 mg l^{-1} IBA (Figure 5). Rooting data were determined after 4 weeks (12 weeks in total)

The variance analysis table of rooting rates is presented in Table 4. It was determined that donor plant age ($P \leq 0.05$) and explant type ($P \leq 0.01$) had significant effects on rooting rates of shoots.

Table 4. Analysis of variance of rooting rates of donor plant age (A) and explant types (B)

Variation source	Degrees of freedom	Sum of Squares	Mean Square	F Value	Table Value %5	Table Value %1
Repeat	3	18.500	6.167	1.762**ns**	3.860	6.990
Donor Plant Age (A)	1	25.000	25.000	7.143*****	5.120	10.560
Explant Type (B)	1	144.000	144.000	41.143******	5.120	10.560
A*B	1	4.000	4.000	1.143**ns**	5.120	10.560
Error	9	31.500	3.500			
Total	15	223.000	14.867			

ns = not significant, * = significant at alfa level %5, ** significant at alfa level %1

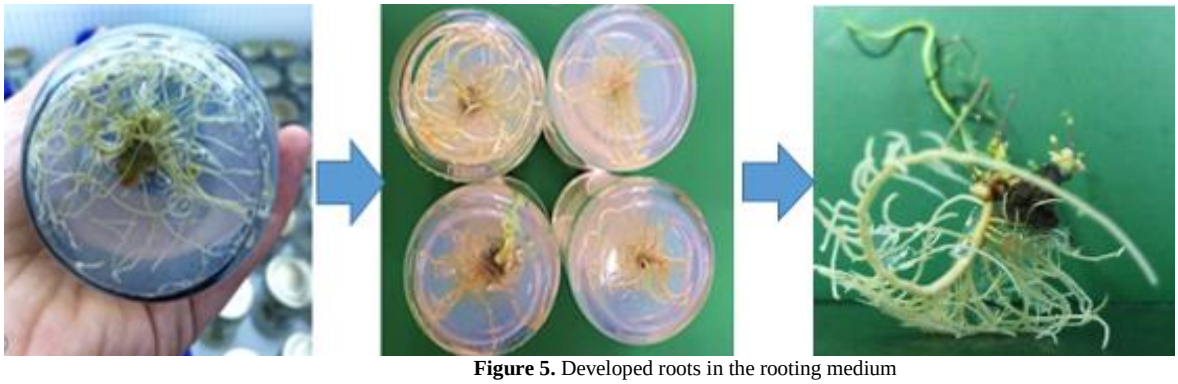


Figure 5. Developed roots in the rooting medium

While a rooting rate 48.0% was obtained in 3-year-old donor plant explants, a rooting rate of 58.0% was obtained from 5-year-old donor plant explants. It was determined that the explant type had a more significant effect on the rooting rate compared to the age of the donor plant. As an average value, 65.0% rooting was determined in the shoot tip explant, while 41.0% rooting was determined from the lateral bud explant. The shoot tip explant formed more roots than the lateral bud explant (Table 5).

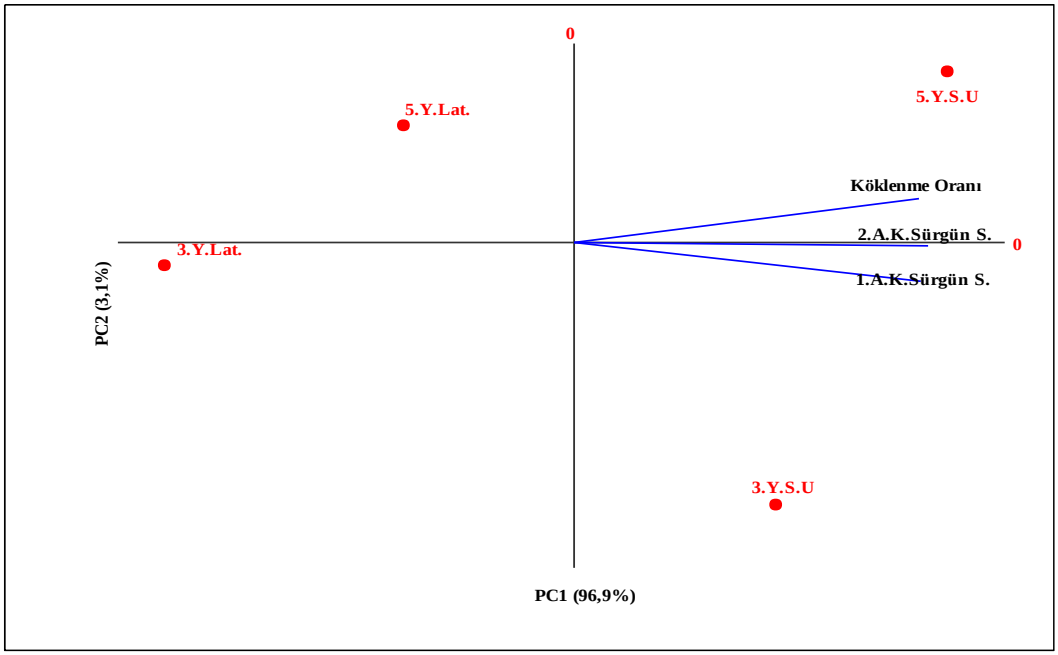
Table 5. Rooting rates (%) by donor plant age and explant type

Donor plant age	Rooting rate	Explant type	Rooting rate
3 year-old	48.0**b**	Shoot tip	65.0**a**
5 year-old	58.0**a**	Lateral bud	41.0**b**
LSD(%5)	2.117	**LSD(%1)**	3.040

The column having a different letter(s) are statistically significant

The rooting rates presented in Table 5, are belong to cluster shoots. The rooting rate obtained was found to be compatible with the study of Maung et al. (2019) in which cluster shoots achieved higher root rate than single shoots. Hormone type, concentration and rooting time effect are also very important in *in vitro* rooting of asparagus. In previous studies, the effects of different auxin hormones have resulted in different results. The reason for this difference may be the effect of many factors, but the most important factor may be the response of the genotype. It has been stated that the most suitable auxin hormone for *in vitro* rooting of asparagus is IBA (Azad & Amin 2017; Maung et al., 2019; Sallam, 2019). Certain metabolic products can accumulate in the nutrient medium during shoot development in *in vitro* cultures. At the same time, some components in the medium may be consumed. Even if the plants are capable of rooting, one or both of the conditions may inhibit root formation. It has been stated that reculturing the plantlets in the culture medium, can remove root inhibitors or it is possible to use a new root promoter (Slabbert et al., 1990).

Figure 5 shows the biplot plot of shoot numbers and rooting rate obtained in the first and second subcultures of the donor plant age and explant type. The principal components biplot analyzes of the examined traits and the variance of donor plant age and explant type were explained at a rate of 100%. The shoot numbers obtained per explant in the first and second subcultures showed higher performance in the shoot tip explant. Likewise, it was determined that the rooting rate showed high performance and high stability. Shoot tip explants were more successful than the lateral explant type in both subcultures.



3.Y.Lat= 3 years lateral, 3.Y.S.U=3 years shoot tips, 5.Y.Lat= 5 years lateral, 5.Y.S.U=5 years shoot tips

Figure 5. Biplot graph of the relationship between shoot numbers and rooting rates per explant in first and second subcultures.

In general, the advantages of micropropagation in artificial nutrient media and in obtaining a new plant under aseptic conditions are at the forefront. By associating *in vitro* culture techniques with asparagus breeding; it is important for many reasons such as the difficulties in creation of homozygous parent lines due to the dioecious nature of asparagus, the preference of male plants due to their higher performance, the difficulties of propagation by seed and the low vegetative (rhizome root) reproduction coefficient. Micropropagation offers the easiest, the fastest and the most reliable method in asparagus breeding and propagation.

As a result, it was determined that the effects of explant type on micropropagation was more important than the plant age of donor plant. It was concluded that harvesting the donor plant in the season could affect the success of the study positively due to active cell growth and development in the explant used. It has been determined that efficient mass micropropagation is possible with both explant types.

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Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The contribution of the authors is equal

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Technical efficiency analysis of melon (*Coloncynthis citrullus l*) production among smallscale farmers in federal capital territory, Nigeria

Elizabeth EBUKIBA[1] **Glory AKPEJI**[1] **Luka ANTHONY**[1*]
[1]Department of Agricultural Economics, University of Abuja, PMB 117, Gwagwalada, Federal Capital Territory, Nigeria
***Correspondence:** gqluka11@gmail.com

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Abstract
This study evaluated technical analysis of melon production in Federal Capital Territory, Nigeria. The specific objectives were to**:** evaluate the technical efficiency in melon production among smallholder farmers, determine the factors influencing the technical efficiency in melon production among smallholder farmers, and identify the constraints of melon production in the study area. Primary data were used for this study. Multi-stage sampling technique was adopted and used for the study. Data were obtained using structured questionnaire. The questionnaires were administered to two hundred and sixteen (216) smallholder melon farmers in the study area. Data were analyzed using descriptive statistics, and Stochastic frontier production function. The results showed that majority (80.65%) of the sampled smallholder melon farmers had mean age of 44 years. Majority (76.9%) were married, (44.9%) had no formal education. The average member per each household was 5 persons in the study area. The average years of experience in melon production by the smallholder farmers was 7.4years. The average farm size cultivated by melon farmers was 2.15ha. The results of the stochastic frontier of the production function revealed that the statistically significant factors influencing the total output of melon were; seed input ($P<0.01$), family labour ($P<0.01$), and Chemical input ($P<0.01$). The findings of this study also revealed that, the average technical efficiency score level of the smallholder melon farmers was 50% this implies that farmers were able to attain 50% level of resource use but have a gap of 50% to reach the optimum level of melon production while the factors influencing technical inefficiency were age of the farmer ($P<0.10$) and farming experience ($P<0.05$). The Smallholder melon farmers were faced with the following constraints in melon production: unavailability of improved seed varieties, lack of extension agent, inadequate capital, inadequate transportation and bad roads. The study recommended that farmers should be encouraged to engage in melon production entrepreneurial activities should also be introduced to farmers in order to earn income from non-farm business enterprise. Inputs like improved seeds, and chemicals, should be made available by governments and non-governmental organizations to melon farmers at subsidized rate to increase profit among melon farmers in the study area. Extension agents should teach farmers and provide necessary supports that can boost melon production. Credit facilities should also be made available to farmers at affordable interest rate. Farmers should be encouraged to join farm organizations for easy accessibility of credit and other farm inputs. Melon farmers should be encouraged by making a provision of capital to enhance their productivity.

Key words
Melon, Production, Technical Efficiency, Profitability, Nigeria

Introduction
Agriculture still remains the largest sector of Nigerian economy and employs two thirds of the entire labour force; the production hurdles have significant stifled the performance of the sector (Food and Agriculture Organization- FAO, 2013). The sector is almost entirely dominated by small scale resource poor farmers living in rural areas, with farm holdings of 1- 2 hectares, which are usually scattered over a wide area (Idisi, Ebukiba & Anthony, 2019). Agricultural sector plays a significant role in food security and poverty alleviation. It employs more than 70% of the labour force, accounts for over 70% of non-oil export and most importantly provides over 80% of the food needs of the country (Adegboye, 2004). However, over the years there has been a marked decline in the performance of the sector. Nigeria, consequently, had to resort to large importation of food produce to cope with increasing demand for food (International Institute of Tropical Agriculture- IITA, 2015). The small-scale famers who constitute about 85% of the farmers in Nigeria occupy the vertex in the hierarchy of players in food production and produced about 90% of food consumed in the country (Food and Agricultural Organization, 2017).
Egusi (Colocynthiscitrillus L) generally known as honey dew, is a vegetable crop of West Africa. Melon seed (*Egusi)* plays a vital role in the farming system of West African rural dwellers as cover crop, weed suppressant and soil fertilization through the formation of root nodules that improves the nitrogen status of the soil (Abiola & Daniel,2012). The origin of the crop is Africa. It can be cultivated as mixed cropping system with other crops like yams and cassava in peasant farms and traditional farming systems (Sadiq, Muhammed & Yusuf, 2013). The mainly harvested melon seed is commonly consumed in Nigeria as thickening for sauces and soups, also fried and eaten as snack. Melon seed (*egusi*) is a good source of oil, protein, minerals, vitamins, and energy in form of carbohydrates. The seed contained 4.6g carbohydrates, 0.6g proteins, 0.6 crude fibre, 33mg vitamin C, 17g per100g of edible seeds (Olayniyi, 2008). Valuable vegetable oil is extracted from the seed while the ground seed is used to prepare various delicacies (Yusuf, Sanni, Ojuekaiye &Ugbabe, 2008). Melon is well known and widely cultivated in West Africa (Nigeria, Ghana, Togo and Benin) and many other African Countries for the food in the seeds (Van der Vossen, Denton & Eitahir, 2004). Among the Yorubas in Southwestern Nigeria, it is known and popularly referred to as *Egusi*. Melon plays vital roles in the farming system and in the well-being of West African rural farmers as a good source of energy, weed suppressants and for soil fertilization (Achigan-Dako, Vodouche & Sangare, 2008). It is also used as mulch, leaving high residual nitrogen in the soil after harvesting. Melon is one of the most economically important vegetable crops worldwide and is grown in both temperate and tropical regions (Bisognin, 2002). A high-energy, high-protein concentrate, melon seed ideally complement Africa's prevalent diets based on starch-rich grains (rice, sorghum and maize, for instance) and roots (notably cassava, yam and potato). Although *egusi* is consumed in Nigeria, the cultivation hardly attracts any significant attention and even government has not developed any programme to promote its inherent potentials to hugely improve the household income in poor communities. At the moment only sesame can

compete with the product in the market ten 170kg bags of *egusi* will give a farmer between N900, 000 Naira and N1.1 million under current market price depending on market location, a bag of melon now is N100, 000. Melon is a vital tool against marasmus (lack of calories), kwashiorkor (lack of protein), and other debilitations (Gurudeeban, Satyavani & Ramanathan, 2010). A traditional food plant in Africa, this vegetable has potential to improve nutrition, boost food security, foster rural development and support sustainable land care (National Research Council, 2006). Melon has been recognized as an affordable source of vitamins and micronutrients especially in the rural areas. There is also a prospect for use of the melon seed in the improvement of infant nutrition in view of its high protein and fat content (Van der vossen *et al*. 2004). Almost all the big markets in Nigeria, Benin, Cameroon, Ghana, Togo, and other nearby nations sell the seed. Melon is in high demand in tropical markets, especially in the peri-urban and urban markets. It is also exported to Ethiopia and Sudan where the consumption is high and the extracted yellow oil is in high demand (Schippers, 2000). The World Bank's Rural Development Strategy defines smallholder farmers as those with a low asset base, operating less than 2 hectares of cropland and depending on household members for most of the labour (World Bank, 2003). The crucial role of efficiency in increasing agricultural output has been widely recognized by researchers and policy makers alike. The efficiency of a farm/firm refers to its success in producing as large amount of output as possible given a set of inputs. To determine the efficiency of a particular firm, there is need for efficiency measurement through the production factor inputs and processes. Efficiency measurement has received considerable attention from both theoretical and applied economists. From a theoretical point of view, there has been a spirited exchange about their relative importance of the various components of firm efficiency. From an applied perspective, measuring efficiency is important because this is the first step in a process that might lead to substantial resource savings these resource savings have important implications for both policy formulations and firm management (Omonona *et al.* (2010). Production of melon in Nigeria amounted to 370,000.00ton. Cameroon produced 57,000.00ton; Sudan 45,000.00ton; DR Congo 40,000ton; Central African Republic 23,000.00ton; and Chad 20,000.00ton. Outside Africa, China is important with a production of 25,000.00ton (Van der vossen et al., 2004). This reflected that Nigeria is leading in melon production in Africa by 64.24% of total production as against the China production. To date, very few studies focused on the importance of traditional practices related to African vegetables such as melon, its nutritional value and contributions to rural livelihood. Melon farmers depend on the income generated from the crop to send their children to school, provide shelter and improve their lives. As a household food, it is the most affordable and suitable dietary sources of vitamins and minerals. It includes other bioactive compounds, that are important protective food and highly beneficial for the improvement of diets, provision of vitality of health and prevention of diseases (De Mello, 2000). Despite the socioeconomic, cultural, agronomic and culinary importance of melon, information is lacking on the cultural background that contributes to the traditional farming system (Schippers, 2004; Achigan-Dako *et al*. 2006). It is therefore essential to assess these challenges as it will have bearing on the contributions of melon production to sustainable rural livelihood in the study area. In Nigeria, the problems with smallholder agriculture dwell on the use of traditional technology which is associated with low productivity, the extension services which are not properly funded, and lack of farmers' access to agricultural inputs due to lack of credit facilities. There is dearth of studies on the use of farm plot size, agrochemicals, machinery, labour, improved seeds (which are the inputs involved in melon production) as well as resource use efficiency of smallholder melon farmers in Federal Capital Territory, Nigeria. Nwaru (2011); Nsikak-Abasi, Etim & Onyenweaku, (2013); and Onumadu (2014) observed that the acute shortage of agricultural resources has been complicated by gross inefficiency in resource use. Therefore, issues relating to how these resources are utilized to enhance income of farmers thus impacting on the growth of the economy of the FCT need to be addressed. Many researchers (Udoh, 2005; Mbanasor &Kalu 2008; Eze, 2010; Oluwatusin, 2011; Simonyan, Olukosi, Omolehin &Atala, 2012; Masakure &Henson, 2012; Onubuogu, Chidebelu & Eboh, (2013) have identified resource use inefficiency to be responsible for the poor performance of the agricultural sector in Nigeria. Some critical resources which have been identified with low levels of productivity are capital, labour and land use (Oladeebo & Oyetunde, 2013; Girei & Dire, 2013; Girei, Yuguda & Salihu, 2014; Ohen, Ene & Umeze, 2014).

Despite the nutritional and commercial value of melon, its production remains low in Nigeria (Dauda, Ajayi & Ndor, 2008). To date, very few studies focused on the importance of traditional practices related to African vegetables such as melon, its nutritional value and contributions to rural livelihood. The productivity of farmers can be raised by adoption of improved production technologies or improvement in efficiency or both. But with the low rate of adoption of improved technologies by farmers in Nigeria, improvement in efficiency becomes the best option in productivity enhancement in the short run (Idiong, 2007). Problems of melon production also include inappropriate decision on how best to allocate resources, inadequate use of corresponding production inputs and inadequate adoption of improved technologies by farmers, also farmers might use resources rationally but not at economic optimal level, all these contribute to inefficiency (Idisi, *et al.*,2019). Melon is consumed in many parts of Nigeria, but despite the nutritional and commercial value, its production remains low. Even with its good market price, melon is still produced by farmers on a small scale. Despite the socio-economic importance of melon, production output has been on the decline in recent time. The reason for this decline could be attributed to the problem of scarcity of land resulting from land fragmentation, high cost of inputs, use of traditional techniques, and inefficient allocation of resources or what the factors responsible for inefficiency among melon farmers are. To achieve economic optimum output and thus profitability, resources have to be optimally and efficiently utilized (Abiola and Daniel, 2012). It has been observed that Nigeria has the potential particularly in terms of land and human resources needed to produce enough food for the country. Melon consumption in the Federal Capital Territory is on the increase due to the increasing awareness of its nutritional value and the diversity of its inhabitants, while its production is on the decrease due to inefficiency in the resources use, poor access to modern inputs and credit, poor infrastructure, inadequate access to market, land and environmental degradation, and inadequate research and extension services. Despite the nutritional value of melon, its production remained low in the Federal Capital Territory (Dauda *et al.*,2008). The poor output of melon realized by farmers may be an indication that resources needed in the production of the crop are not being used at their optimal level and this raises the question as to whether it is profitable to grow the crop or not? This situation calls for an examination of the profitability of growing the crop, an assessment of the resources needed for its production and how their resources are managed by its cultivators. Since there is no documented study on cost efficiency of melon production in the area, this study therefore became imperative. There is therefore, the need to provide empirical information on farm level production efficiency in small-scale melon production in the study area. For this to be fully realized there is need to address the problems of rural farmers who produce the bulk of Nigerian agricultural product. This study is significant as it will contribute to research by bridging the information gap in efficiency studies as most of the previous work focused on agronomic issues (Adekunle *et al*., 2003; Dauda *et al*. 2008; Gambo *et al*, 2008). The production of melon is declining in the study area, even though the crop plays many vital socio-economic and cultural roles in the wellbeing of the farmers' and communities in its entirety. Even then, smallholder and traditional melon farmers who use rudimentary production techniques, with resultant low yields, cultivate most of the degraded lands. Empirical evidence remains largely scanty, hence, to fill this dearth in empirical research, this study becomes imperative and essential to assess the efficiency of melon production so as to enhance its productivity, profitability, and sustainability among smallholder farmers in the Federal Capital Territory, Nigeria.

Research Questions

The study is designed to answer the following research questions;

i. What is the level of technical efficiency in melon production among smallholder melon farmers in the study area?
ii. What are the factor influencing technical efficiency among Smallholder melon farmers in the study area?
iii. What are the constraints of melon production in the study area?

Objective of the Study

The broad objective of this study is to analyze the technical efficiency analysis of melon production among smallholder farmers in Federal Capital Territory, Nigeria.

The specific objectives were to:

(i) evaluate the technical efficiency in melon production among smallholder farmers,
(ii) determine the factors influencing the technical efficiency in melon production among smallholder farmers,
(iii) identify the constraints of melon production in the study area.

Methodology

Area of Study

This study was conducted in Federal Capital Territory. Federal Capital Territory is the capital city of Nigeria located in the center of the country. FCT has latitudes $9^{o}\,4^{'}\,60^{o}$ N and longitude $7^{o}\,3^{'}\,60^{o}$ E of the equator. FCT is bounded on the north by Kaduna state, the west by Niger state, at the south east by Nassarawa State and at the North West by Kogi state as shown in fig. (3.1). It is predominantly a

grassy savannah region, thus has potentials to produce both root crops and tubers such yam, and cassava. It also sustains legumes (groundnut & cowpea); grains (maize, sorghum & rice); seeds and nuts (melon seeds & benniseed); animal products (goats, cattle& sheep); fruits and vegetable, the people are predominantly farmers. It has a total land area of about 8000km^2 with a total population of about1,405,201 people according to 2006 population census but has grown to 2,245,000 in 2010 (NPC, 2006). Abuja is characterized with two main seasons; rainy season within April to November and dry seasons within December to march, with a temperature of 30^{oc} -37^{oc}, which drops to about 25^{oc} - 27^{oc} during rainy seasons.

Sampling Technique and Sample Size

Multistage sampling technique was adopted for this study, in the first stage purposive sampling procedure was adopted for selecting FCT because of the dominance and, the number of the smallholder melon farmers in the study area. In the second stage, two (2) area councils Gwagwalada and Kwali were also purposely selected because of the concentration of the small holder melon producers in the Area Councils. In the third stage simple random sampling was employed to select three (3) districts from each of the ten (10) wards in the two Area council selected. The required sample size was determined by Cochran's proportionate probability to sample size sampling methodology (Cochran, 1977). A total sample frame of 4,300 farmers is available, and a total sample size of 216 respondents was selected as shown in Table 1. The required sample size (216) was determined as used by Cochran (1977), as shown in equation 1

$$n = \frac{pqZ^2}{e^2} = 216 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (1)$$

Where,
n= Sample Size
p=0.17
q=0.83
Z=1.96($\alpha = 0.05$)
e = 0.05 Allowable Error

Table 1. Sample size and sample frame of the melon farmers in the study area

Area Councils	Ward No of Farmers	Proportion	Sample Size	Area Councils
Gwagwalada	Paiko	400	0.093	20
	Tunga-maje	800	0.186	40
	Gwako	700	0.163	35
Kwali	Ashara	900	0.209	45
	Kilankwa	906	0.210	46
	Kwali ward	600	0.139	30

(Author's Computation, (2021)

Methods of Data Collection

Data were collected through primary source. Primary data were obtained through the use of a well-structured questionnaire. Data were collected based on the socio-economic variables such as gender, age, farming experiences, educational status, household size and income level of the respondents, as well as costs, returns, profitability variables and factors influencing melon production in the study area. The structured questionnaire was administered directly to the sampled respondents by the researcher with the help of trained research assistant who also interpret the questions in local language where necessary. The instrument for data collection for this study were checked for its validity and reliability by the team of researchers and the other scholars.

Method of Data Analysis

The data collected were scrutinized, collated and coded for analysis using both descriptive and inferential statistics in order to achieve the stated objectives. The following tools of analysis were employed to achieve the stated objectives of the study:

(i) Stochastic Frontier Model (ii) Test Statistics

Stochastic frontier Cobb-Douglas production function

This tool was used to achieve objective (i), which was to analyze the technical efficiency in melon production**.**

The implicit form of the model is specified as:

$$Y_i = f(X, \beta) + \varepsilon_i(V_i - U_i) \dots\dots\dots\dots (2)$$

Where,

$$\varepsilon_i = (V_i - U_i) \dots\dots\dots (3)$$

Y_i= Quantity of Melon Output of the ith farm
X_{ii} = Vector of the Inputs used by the ith farm
β = Vector of the Parameters to be Estimated
ε_i= Two sided Error term
V_i= Error Term due to measurement or faulty data
U_i = Random Error Outside Farmers Control

The explicit model is specified as follows:

$$LnY_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + (V_i - U_i) \dots\dots (4)$$

Where:
Ln = The Natural Logarithm
Y = Output of Melon (kg)
β_0 = Constant term
$\beta_0 - \beta_1$ = Regression Coefficients
X_1 = Quantity of Melon Seed (kg)
X_2 =Quantity of Fertilizer (kg)
X_3 = Family Labour used (Man days)
X_4 = Hired Labour used (Man days)
X_5 = Farm Size
X_6 = Chemical
V_i = Random variability in the production that cannot be influenced by the farmer.
U_i = Technical inefficiency effects predicted by the model.

The Inefficiency model is stated as follows:

U_i= $\alpha_0 + \alpha_1 Z_1 + \alpha_2 Z_2 + \alpha_3 Z_3 + \alpha_4 Z_4 + \alpha_5 Z_5 + \alpha_6 Z_6 + \alpha_7 Z_7 + \alpha_8 Z_8 \dots\dots (5)$

Where,
U_{ii}= Inefficiency Component
Z_1=Gender (Dummy Variable (1= Male and 0= Female)
Z_2 =Age of Farmers (years)
Z_3=Household size (Number of Persons)
Z_4 =Educational level (Number of Years of Formal Education)
Z_5 = Marital status (dummy variable: 1, Married and 0 Otherwise)
Z_6 = Membership of Cooperative Society (Years of Participation)
Z_7= Access to Credit (Amount borrowed in Naira\)
Z_8= Extension visit (Number of Visit per year)
α_0= Constant Term
α_1 –α_8 = Parameters to be estimated

This were included in the model to indicate their influence on the technical efficiency and it was used to achieve objective (ii).

Results and Discussion

Estimates and Distribution of Technical Efficiency Level in Melon Production Among Smallholder Farmers

Table 3 presents the distribution of technical efficiency score of melon production among the small holder farmers. The results showed that 5.09% of the sampled farmers had a technical efficiency score of 0-0.20 while 40.28% of the melon farmers score 0.21-0.4.0 level of technical efficiency 22.69% had a technical efficiency score 0.41-0.60, also about 24.07% of the sampled respondent attained 0.61-0.80 level of technical efficiency and only 7.87% were able to reach 0.81-1.0 level of technical efficiency. The maximum value of technical efficiency attained by individual farmer was 0.9990 while the minimum value was 0.01 with mean technical efficiency of 0.445 level of technical efficiency on individual basis. This implies that melon farmers were technically efficient to some extent but having a gap of 50% of inefficiency that need to be filled up to attain the level of perfection in technical efficiency by adopting innovation, new technology and the use of modern method of agricultural practices.

Table 2. Distribution of technical efficiency score level in melon production among smallholder farmers

Technical Efficiency	Frequency	Percentage
0-0.20	11	5.09
0.21-0.40	87	40.28
0.41-0.60	49	22.69
0.61-0.80	52	24.07
0.81-10	17	7.87
Total	216	100
Minimum Value	0.01	
Maximum Value	0.9990484	
Mean Value	0.4450571	
Standard Deviation	0.230582	

Source; Field Survey Data, (2021)

Maximum Likelihood *Estimates and Factors Influencing Technical Efficiency of Melon Production Among Smallholder Farmers in the Study area*

Table 2 presents the results of the maximum Likelihood (MLE) of the parameters of the Stochastic production frontier model and the inefficiency component model was also estimated for smallholder melon farmers. The MLEs stochastic production frontier model with half-normal distributional assumptions on the value of efficiency error term were estimated. The value of gamma estimated showed the measure of the level of the inefficiency in the various parameters included in the model and the values ranges from 0 to 1. Gamma estimate was 0.0092024. This indicates the total output of inefficiency of the melon farmers in the study area. The parameter of sigma square was 0.0522657 and the value of Log likelihood estimated was 12.211062 and was significant at (P<0.01) probability level. The computed average value of technical efficiencies for smallholder melon farmers was 0.45. This implies that, averagely the sampled smallholder farmers were able to obtain 50% of potential output of melon from a given combination of farm inputs, in a short run, there is a shortfall scope of (50%) of increasing the efficiency of melon production among smallholder melon farmers, through adoption of innovation, new technology and techniques used by best smallholder farmers in the study area. This result shows that small-holder melon farmers were technically efficient to some extent in melon production in the study area. The coefficient of seed input was statistically significant at (P<0.01), seed input influence the total output of melon positively. The coefficient of seed input (1.7270) signifies that a unit/percentage increase in the quantity of seed planted by farmers results in the increase of the total output of melon by (1.727%) among smallholder farmers in the study area. This result is in line with (Ibrahim et al, 2014) who reported that the quantity of seed determines to a large extent, the output obtained. If correct seed rates and quality of seeds are not used, output will be low even if other inputs are in abundance. Quality of seed planted is a determinant factor of the quality and quantity of total output of produce among farmers in the study area. Family labour influence the total output of melon positively the magnitude of the coefficient of family labour was (0.000147) and was statistically significant at P<0.01). This indicates that a unit increase in the family labour by one person will lead to increase in the total output of melon seed by (0.000147%) in the study area. Chemical input was statistically significant at (P<0.01) and it influence the total output of melon positively. The magnitude of the coefficient of chemical input (0.04669) implies that a unit change in the quantity of chemical input applied by the farmers in order to protect the melon seed from damage from pests and diseases will results in the increase in yield of melon total output by (0.0467%) among smallholder farmers in the study are. The inefficiency component of the model was also estimated and presented in table 2. The negative sign of the estimated parameters indicates that the variable reduces total output inefficiency (increases efficiency). The positive signs of the estimated coefficients increase inefficiency (decreases technical efficiency). The results revealed that the age of the smallholder melon farmers and farming experience were the only significant variables, and therefore reduces technical inefficiency (or increase technical efficiency). The coefficient of the age of the smallholder farmer was positive and statistically significant at (P<0.01) the positive sign of the age of farmer increases technical inefficiency (decreases efficiency). The coefficient of the age of farmer (0.103) implies that a unit increase in the age of farmers will result in the decrease in technical efficiency in melon production by (0.103%) in the study area this could be as the result of the fact that as age increases energy for farm operations decreases and productivity slows down with increase in age also older farmers are risk averse and they don't embrace or adopt new innovations and technology easily. This is in line with Ebukiba et al, (2020) who opined that as a result of old age, farmers could become unproductive as they advance in age. Farming experience influence the technical inefficiency negatively and it was statistically significant at (P<0.01). The coefficient of farming experience was (-0.0073) this implies that a unit increase in farming experience by one year will results in the decrease in technical inefficiency (by 0.00735%) this result show that as farmers experience increases technical inefficiency decreases while technical efficiency increases with increase in experience of a farmer in the study area. This is in agreement with Ebukiba *et al* (2020) who reported that Farming experience increases the level of efficiency as the farmers accumulated experience results in increase in farm productivity. This result is also consistent with the findings of (Ibrahim *et al*., 2014) which indicates that the negative sign on the years of farming experience variable indicates that an increase in the number of years in melon production, decreases farmers experience enhances technical efficiencies.

Table 3. Results of the maximum likelihood estimates of the stochastic frontier model of mellon production among smallholder farmers

Variables	Parameter	Coefficients	Standard Error	Z-score
Total Output (Y_i)				
Constant	β_0	1.726899	0.1976439	8.74
Seed Input	β_1	0.1030078	0.01638	6.29
Fertilizer Input	β_2	-0.0002228	0 .0002184	-1.02
Family Labour Input	β_3	0.0001478	0 .0000256	5.77
Hired Labour	β_4	-6.78e-06	0 .0000121	-0.56
Farm Size	β_5	0.0197973	0.0194992	1.02
Chemical Input	β_6	0 .0466952	0 .0070991	6.58
Inefficiency Component				
Age	Z_1	0.0034965	0.001988	1.76
Education Level	Z_2	-0.0116905	0.0175816	-0.66
Cooperative Association	Z_3	-0.02008	0.0458424	-0.44
Household Size	Z_4	-0.0031938	0.0060213	-0.53
Farming Experience	Z_5	-0.0073553	0.0042424	-1.73
Access to Credit	Z_6	-7.77e-10	4.76e-08	-0.02
Extension Visit	Z_7	0.0107203	0.0153138	0.70
Farm Size	Z_8	-0.0184086	0.014473	-1.27
Diagnostic Statistics				
Sigma^2	σ^2	0.0522657	0.0050834	
Gamma	γ	0.0092024	0.2450031	
Log likelihood		12.211062		

Source: Field Survey (2021) Computed from STATA Version 12
***, **, ***. Significant at 1%, 5% and 10% Respectively**

Constraints Faced by Smallholder Farmers in Melon Production in the Study area

Table 4 shows that 31.9% among the sampled respondents were faced with the constraint of unavailability of improved seed varieties and it was ranked first out of the various challenges based on the opinion of the smallholder farmers in the study area. Also 23.6% of the melon farmers were faced with lack of extension agents to explain to them the new innovations and use of technology involved in melon production and it was ranked 2nd table 4.5 depicts that 19.0% of the sampled respondents were faced with the constraint of inadequate transportation and it was ranked third 3rd 17.6 faced challenge of capital to in large melon production while other constraints militating against melon production in the study area according to the farmers include bad roads, outbreak of pest and diseases and high costs of farm inputs and availability. This result finding is in consonant with the findings of Sodiya et al., (2011); Ibrahim et al., (2014) von Braun and Torero, (2008)

Table 4. Results of the analysis of constraints face by smallholder melon farmers in the study area

Constraint	Frequency	Percentage	Rank/ Remarks
Unavailability of improved seed	69	31.9	1st
Lack of Extension agent	51	23.6	2nd
Inadequate transportation	41	19.0	3rd
Inadequate capital	38	17.6	4th
Bad roads	26	12.0	5th
Outbreak of pest and Diseases	24	11.1	6th
Lack of land availability	22	10.2	7th
High cost of farm inputs and affordability	**22**	10.2	7th
Inadequate marketing system	20	9.3	8th
Unattractive Price i.e fluctuations	19	8.8	9th
Poor information network	14	6.5	10th
Government policies	14	6.5	10th
Limited scale and uneven distribution	11	5.1	11th
Unavailability of hired labour	9	4.2	12th

Source: Field Survey (2021)

Conclusion

Based on the results of the findings emanating from this research work the study concludes that melon production were not technically efficient in resource use in the study area. That is farmers were making profits even though the level of profit was low. The determinant factors influencing the total output of melon among the smallholder farmers were: seed input, household size, access to credit, family labour, chemical input and extension visit. The mean value of the technical efficiency attained by individual smallholder melon farmer was 50% with a gap of 50% that need to be filled in the short run by adopting farm practices that must be efficient. The factors influencing technical inefficiency was age of the farmer and farming experience accumulated by the farmers over the years in melon production. Smallholder melon farmers were faced with the following constraints in melon production in the study area: unavailability of improved seed varieties, lack of extension agent, inadequate capital, inadequate transportation and bad roads.

Recommendations

The following recommendations were made based on the findings emanating from the study.

1. Inputs like improved melon seed varieties and chemicals should be made available to melon farmers at subsidized rate by the government to increase profit among melon farmers in the study area. Credit facilities should also be made available to farmers at affordable interest rate in the study area.
2. Extension agents should teach farmers and provide necessary supports that can boost melon production in the study area. Credit facilities should also be made available to farmers at affordable interest rate in the study area.
3. Farmers should also be encouraged to by non-governmental agricultural agencies to join farm organizations for easy accessibility of credit and other farm inputs.
4. Agricultural development projects (ADP) in the F.C.T should intensify *egusi* melon production awareness and mobilize the local industries for *egusi* melon processing and extraction of oil for better utilization in the study area thereby boost more interest in production which will in turn increase profit and reduce the labour cost involved in its production.

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Some fruit quality characteristics of 'Grand Naine' banana fruits during various ripening stages

Ömer Faruk BİLGİN*[1] Gözde NOGAY[1] Nesibe Ebru KAFKAS[1] Salih KAFKAS[1]

[1]University of Çukurova, Faculty of Agriculture, Department of Horticulture, Adana, Turkey.
***Correspondence:** ofarukbilgin35@gmail.com

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Abstract

During ripening and ageing, the chemical components and antioxidant capabilities of bananas alter dramatically. The effects of different ripening stages (green, ripen and over ripe) of the flesh of banana cultivar 'Grand Naine' on the total phenolic substance, antioxidant capacity of banana flesh, total sugar, sucrose , fructose , and glucose content were investigated. The total phenolic content was found to be 10.54 mg GAE /100g in the green (first stage) banana, 9.03 mg GAE /100g in the medium ripe banana (fourth stage) and 13.46 mg GAE /100g in the over ripe (seventh stage) banana. When comparing the antioxidant contents, the highest DPPH (%) radical scavenging value was obtained from the fruits of the fully ripe (seventh stage) banana (51.64%), while the lowest %DPPH radical scavenging value was obtained from the fruits of the fully ripe (seventh stage) banana (17.06%). In the Frap assey, the overripe banana had the highest content of Trolox equivalents (TE), 1.24 mg TE / g FW, followed by the medium ripe (0.63 mg TE / g FW) and the green banana (0.56 mg TE / g FW). The HPLC sugar profiles displayed that sucrose is the most important sugar, followed by fructose and glucose in all ripening stages of banana fruit pulp samples. The content of soluble sugars (sucrose, glucose and fructose) in the 'Grand Naine' banana increased during the fruit ripening stages. 'Grand Naine' showed increasing sugar content as ripening progressed and was highest when the fruit was fully ripe.

Key words

Total Phenol Content, Dpph Scavenging Activity, Solubla Sugar Content, Banana, sugars, HPLC.

Introduction

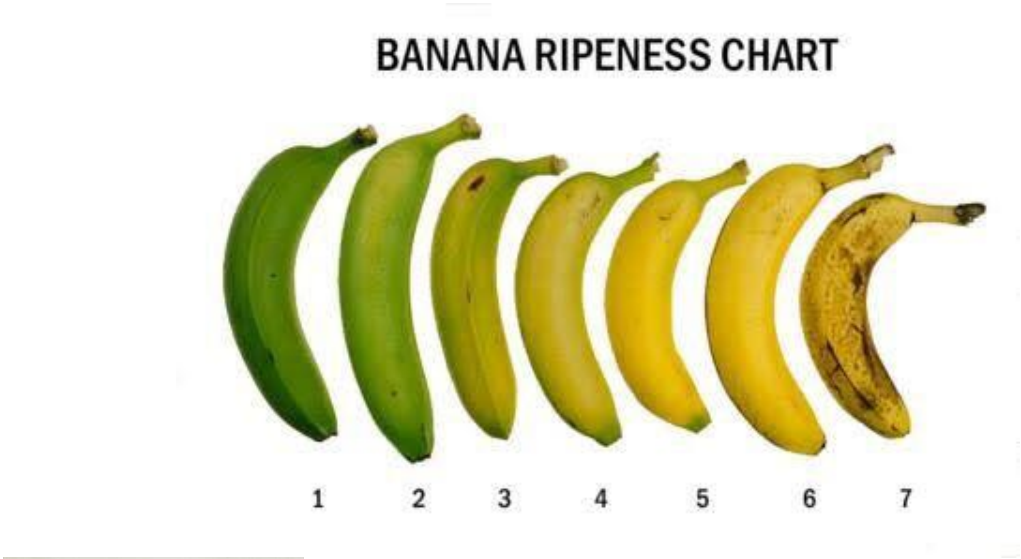
The banana (*Musa cavendishii* L.) cultivated as a fruit, belongs to the Musaceae family of the order Scitamineae. The Musaceae family has two major genera, Musa and Ensete. The genus Musa includes the edible cultivated species and Esnete, the wild species found in the forests of East Africa. There are 4 subgenera within the genus Musa. These are Australimusa, Eumusa, Callimusa, and Rhodochlamys. Among these subgenera, Australimusa and Eumusa are important species. The species of Callimusa and Rhodochlamys are used as ornamentals. The most important Australimusa species is *Musa textilis*, from which fibers called Manila hemp are obtained and used in the textile industry world trade today (Kuchi et al., 2017) . It is native to southern China, India, and the islands between India and Australia. In our country, it was first introduced in the middle of the 18th century. It was brought to Alanya from Egypt as an ornamental plant, and when it was realised that its fruits could also be eaten, it was brought to Anamur and cultivated in 1935 (Akova, 1997) . Banana cultivation is carried out worldwide under tropical conditions in countries such as India, Ecuador, Brazil, the Philippines, Indonesia and Costa Rica, and under subtropical climatic conditions in countries such as Egypt, Spain, South Africa, Lebanon, Portugal, Jordan, Israel and Turkey. In our country, banana cultivation was carried out under greenhouse conditions in Anamur and Bozyazı locations in Mersin province and in Alanya and Gazipaşa locations in Antalya provinces of the Mediterranean region in limited areas called microclimate areas until a few years ago, and in Anamur and Bozyazı, which belong to these areas. On the other hand, it is mostly grown in the open-field conditionss in Alanya and Gazipaşa (Gubbuk et al., 2017). The latitude and longitude of our country are far outside the banana growing areas of the world. Nevertheless, banana is the most important economically cultivated species among tropical fruits in our country. In recent years, in terms of cultivated areas, especially in the Mediterranean region, outside the microclimate areas, it has been noted that greenhouse cultivation has greatly increased and this expansion is moving towards the Aegean region. Banana is one of the most favorite fruits in the global market (Meechaona et al., 2007). This tropical fruit can fight oxidative stress caused by harsh sunshine and high temperatures by increasing its antioxidant content. Several studies have shown that both the banana pulp and peel contain antioxidants such as vitamins, B-carotene, phenolic compounds (catechin and epicatechin), lignin, and tannins, as well as anthocyanins (Lim et al., 2007; Someya et al., 2002; Wall, 2006). Bananas are also rich in potassium and phosphorus (Hardisson et al., 2001; Leterme et al., 2006; Wall, 2006). There is augmenting evidence that banana peels have higher groups of phenolic constituents and antioxidant attributes than fruit. (Kondo et al., 2005; Someya et al., 2002) and minerals than banana pulp (Emaga et al., 2007; Forster et al., 2002). However, most of the aforementioned studies have focused on a single variety, which is the best known banana variety, namely Musa acuminata AAA (Cavendish subgroup) (González-Montelongo, Lobo, et al., 2010; Someya et al., 2002; Vijayakumar et al., 2008).

The aim of this study was (i) to compare the antioxidant activity, total phenolic content, and soluble sugar content of banana cultivar 'Grand Naine' at different stages of ripening under greenhouse conditions in the Mersin region.

MATERIALS AND METHODS

Materials

The plants of 'Grand Naine' banana variety grown under greenhouse condition in Kazanlı region of Mersin provinces in Turkey was used as material. The fruits harvested in the three stages as mentioned in Figure 1 and were analyzed first stage, fourth stage, seventh stage



1) Firstly Stage

2) Fourth Stage

3)Seventh Stage

Method

The fruits harvested from the greenhouse were immediately taken to the Enstrumental Analysis Laboratory at Çukurova University, Agriculture Faculty, Horticulture Department. The fruits peeled and fruit pulp were homogenized and stored at -20 ºC until analysis. The analyzes of total phenolics and antioxidant activity and individual sugars were performed in 3 replicates using a spectrophotometer and HPLC (High Performance Liquid Chromatography), respectively.

Total phenol

After the fruit samples were homogenized and weighed, the determination of total phenols of banana samples of different ripening stages was carried out by modifying the spectrophotometric method of Spanos & Wrolstad, (1990) . The metrical values were computed from the absorbance value at a wavelength of 760 nm in the spectrophotometer (MultiskanTM GO microplate spectrophotometer) and the calibration curve developed with gallic acid. Results were represented as mg gallic acid equivalent/100 g weight (mg/ GAE 100 g).

DPPH Scavenging Activity(%)

Total antioxidant capacity was determined from the reducing antioxidant power of iron (FRAP) in according to Benzie & Strain, (1996) method and DPPH described by (Okatan et al.,(2021) . The antioxidant capacity of banana pulp samples at different ripening stages was investigated by evaluating the radical scavenging effect on the DPPH (1,1-diphenyl-2-picrylhydrazyl radical). The result was determined according to a procedure described by Sultana et al. (2008) with slight modifications. Briefly, 5.0 ml of a freshly prepared DPPH (methanolic 1,1-diphenyl-2-picrylhydrazyl) solution at a concentration of 0.025 g/l added to 1.0 ml of an extract having 25 µg/ml dry weight in methanol. The mix was vibrated, held in the dark, and let to stand at room temperature for 2 hours. The absorbance of the consequent solution was estimated at 515 nm using a UV spectrophotometer (Shimadzu UV-1601PC, Tokyo, Japan) and likened to a blank sample of methanol without DPPH. The outcomes were represented as the ratio of inhibition of the DPPH radical and computed according to the subsequent equation:

DPPH -Percentage % of reduction power =($\frac{Ac-As}{Ac}$**)**x100

Ac : control

As : sample

Ab : Blank

For the extraction of the reducing antioxidant power of iron (FRAP), 1 g of the frozen banana pieces was put in an aluminum foil-wrapped flask including 50 mL of an 80% methanol solution. The flasks were shaken in an incubator at 30°C and 150 rpm for 24 hours. The samples were centrifuged at 3200 rpm for 20 minutes and the supernatant was gathered. For the FRAP method, 100 uL of the extract was blended with 2.9 mL of FRAP reagent including 30 mM acetate buffer, pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 µM HCl and 20 mM FeCl3 at a ratio of 10:1:1 (v/v/v)) and vortexed. Samplings were set in a water bath (ST30, Nueve, Turkey) for half of an hour at 37°C, and absorbance was determined at 593 nm. Values were represented as millimoles TE mg /g-1 iron equivalent Fe (II) per gram of banana samples.

Sugar Analysis

Using Valero et al., (2007) method modifieded by Kilic et al.,(2021) and Urün et al., (2021), the total amount of sugar, fructose composition, and sucrose composition (mg per 100 g fresh weight) in banana fruit at various stages of maturity were examined. Prior to analysis, the peeled banana samples were ground. 1 g of the banana samples was put to 4 mL of ultrapure water (Millipore Corp., Bedford, MA, USA). The reaction mixture was set in an ultrasonic bath and sonicated at 75-80 C for 15 min. It was then centrifuged at 5500 rpm for 15 minutes and purified (Whatman nylon syringe filter, 0.45 µm, 13 mm diameter) before HPLC analysis. The HPLC instrument (Shimadzu LC 20A high performance liquid chromatography instrument VP, Kyoto, Japan) includes an inline degasser, pump, and controller, as well as a refractive index detector (Shimadzu RID 20A VP, Kyoto, Japan) with an automatic injector (20 L injection volume) that is connected to a PC running Class VP Chromatography Manager software (Shimadzu, Kyoto, Japan). Splits were performed at 70 °C with a flow rate of 0.6 ml min-1 on a 300 mm 7.8 mm i.d., 5 m reversed-phase Ultrasphere Coregel-87 C analytical column (Transgenomic). With ultrapure water, elution was isocratic. Total sugar,

fructose, and sucrose concentrations were calculated using respective standards and expressed as a percentage of fresh weight (FW).

Statistical Analysis

The trial had three replicates and was completely randomized. The statistical analyses were carried out using the SAS Institute's JMP statistical program 22.0.0 (1 (North Carolina, USA).) The data were subjected to analysis of variance, and Duncan's multiple range test was used to separate the means at a significance level of 0.05.

Results and Discussion

Phenols are crucial healthy components of fruits as they have antioxidant effects by inactivating free lipid radicals or controlling the decompositon of hydroperoxides to free radicals (Maisuthisakul et al., 2007) As a result of this study, it was found that the percentage DPPH radical scavenging and total phenolic content of banana samples (mg/ GAE 100 g) have changed in different ripening periods. In addition, the statistical differences in the results of TPC and DPPH radical scavenging activities ($P < 0.05$) between banana samples were found to be significant ($P < 0.05$). Total phenol was found to be 10.54 mg GAE /100g in green banana (first stage), 9.03 mg GAE /100g in medium ripe (fourth stage) banana and 13.46 mg GAE /100g (seventh stage) in over ripe banana. When antioxidant contents were compared, the highest %DPPH radical scavenging value was found in the fruit of the ripe banana (51.64%), while the lowest %DPPH radical scavenging value was found in the fruit of the medium ripe banana (17.06%). The determination of the efficiency of antioxidant compounds is generally used FRAP methods in plants that compete with the reagent FRAP and reduce the ferric to ferrous iron. Antioxidant compounds that can act in this method are classified as secondary antioxidants because they repress the formation of radicals and control oxidative damage. In addition, secondary antioxidants also have the function of metal chelators and oxygen scavengers. Decreasing the iron content(III) leads to the formation of a blue coloured product, ferrous-TPTZ(2,4,6-tris(2-pyridyl)-s-triazine) complex in the reagent FRAP. In our study, it was found that the highest content of Trolox equivalents (TE) in the overripe banana (1.24 mg TE / g FW), followed by the medium ripe (0.63 mg TE / g FW) and green banana (0.56 mg TE / g FW) in FRAP experiment (Table 1).

In the FRAP method, the chloroform extraction method yielded the highest activity in the dried pulps of the Awak and Berangan banana varieties and the dried peels of the Rastali variety. The values were 22.57±0.13 (Awak variety), 22.53±0.12 (Berangan variety), and 21.63±0.42 mg TE /g d.w (Rastali variety) (Sulaiman et al., 2011). Besides, Alothman et al., (2009) found that mas banana variety includes 0.59-3.30 µmol ferum (II) /g FW in FRAP method. Authours also found that the highest content of trolox equivalents (TE) in the raja banana variety namely 0,140.8-0,1607 g TE /100 g FW, followed by mas (233.6-485.8 mg TE /100 g FW) and Beranganese (39.4-403.7 mg TE /100 g FW), which depending on the types of solvent used (Shian & Abdullah, 2012). Different extraction procedures and solvents may have resulted in different outcomes (Chirinos et al., 2007). In our study, we also found that the changes in total antioxidant content were well related to the changes in antioxidant activities. We determined that green bananas have lower total phenolic content than over ripe fruits. Fatemeh et al., (2012) also showed that green bananas have lower total phenolic content than ripen fruits The extracts' radical scavenging abilities (DPPH inhibition) ranged from 26.55 to 52.66 percent (first stage to seventh stage). González-Montelongo et al., (2010) compared different solvents for their DPPH scavenging activity. They revealed that acetone:water extracts had the highest antioxidant activity compared to the other solvents studied, with a factor of 1.3-1.9 (methanol) and 25-35 (acetone) for the DPPH assay and a factor of 2-4 (methanol) and about 10-35 (ethanol, acetone, and water in the banana variety "Grande Naine" and ethanol, acetone, and water in "Gruesa") for the the ABTS+ assay. The extracts produced with acetone:water have greater radical scavenging activity than 1.8 g TE or AE /100 g freeze-dried residue powder. Although ethanolic extracts inhibited DPPH more than acetone extracts (by 4.5 to 5.5 times), their actions against ABTS+ radicals were quite similar. In another study, Fernando et al., (2014) investigated the changes in total phenolic content in banana cultivar 'Khai' during storage. They found that it decreased in the first two days of storage and then significantly increased until the 6th day of storage. Total antioxidant activity increased with ripening and decreased rapidly with senescence in the banana cultivars studied. Ngoh Newilah et al., (2008) documented similar results in hybrid bananas, where phenolic content increased during ripening before decreasing at the fully ripe stage. The results of our study are comparable to values reported in the literature. In our study, we also found that the changes in total antioxidant content were well related to the changes in antioxidant activities. A direct relationship was also found in other fruits (Baskar et al., 2011; Patthamakanokporn et al., 2008; Sulaiman et al., 2011).

Table 1. At different stages of ripeness, total phenolic content (mg/ GAE 100g) and DPPH radical scavenging values percent of banana samples (mean SD,n=3)

	Total Phenol	DPPH radical scavenging%	Frap
Green (First Stage)	10.54±1.57b	45.25±1.68b	0.56±0.01c
Medium Ripe (Fourth Stage)	9.03±3.67b	17.06±0.75c	0.63±0.01b
Over Ripe Banana (Seventh Stage)	13.46±1.42a	51.64±0.97a	1.24±0.06a

The HPLC sugar profiles show that sucrose is the major sugar, followed by fructose and glucose in all banana samples (Table 2). The content of soluble sugars (sucrose, glucose and fructose) in the 'Grand Naine' banana increased with fruit ripening (Table 2). 'Grand Naine' showed increasing sugar content as ripening progressed, being highest when the fruit was fully ripe. Fernando et al., (2014) reported that in the Khai banana cultivar, sugar content increased as ripening progressed and was highest after 8 days of storage. They also reported the sugar content leveled off two days later when the fruits were overripe. In contrast, sugar content of the Hom Thong banana cultivar increased during the first 4 days of storage. During ripening, the Hom Thong banana cultivar showed the same characteristics as the Grand Nine variety during the ripening process. The increase in sugar content is a typical characteristic of ripening bananas due to the increased conversion of starch to sugar (Valerio-Traya et al., 2002). Cordenunsi & Lajolo, (1995) also noticed a significant drop in starch content, which was accompanied by a rise in sugar content. In our study, fructose concentration was lowest and sucrose content was highest during the green and medium ripening stages, implying that sucrose dominates over glucose and fructose as the peel matures. (Soares et al., 2011). On the other hand, the differences between the contents of glycose, fructose and sucrose are very small at the stage of full maturity.

Table 2. Shows the results of free sugars in banana samples (mg per 100 g fresh weight) (mean SD, n=3).

	Free Sugar			Total Sugars
	Sucrose	Glucose	Fructose	
Green (First Stage)	1341.6±70,4^{a}	21.73±0,2^{b}	13.06±1,4^{c}	1375±71.6^{b}
Medium Ripe (Fourth Stage)	408,6±4,32^{a}	165.84±15,55^{b}	84.6±7.49^{c}	667±18.71^{c}
Over Ripe Banana (Seventh Stage)	2444.07±55.44^{a}	2189.22±68,7^{a}	2578.58±7.67^{a}	7220±131.82^{a}

Conclusions

The following are some of the study's findings:

- Total phenolic content and DPPH scavenging activity decrease with medium ripe and then decrease when fruits are overripe.
- The content of soluble sugars (glucose, fructose) increased with ripening.
- When the fruit ripens, the content of glucose, and total sugars increases sharply.

– HPLC profile provides valuable information on sugar composition and fruit quality to evaluate the influence of technological processes.

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Statement of Conflict of Interest
The author(s) declare no conflict of interest for this study.

Author's Contributions
The contribution of the authors is equal

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