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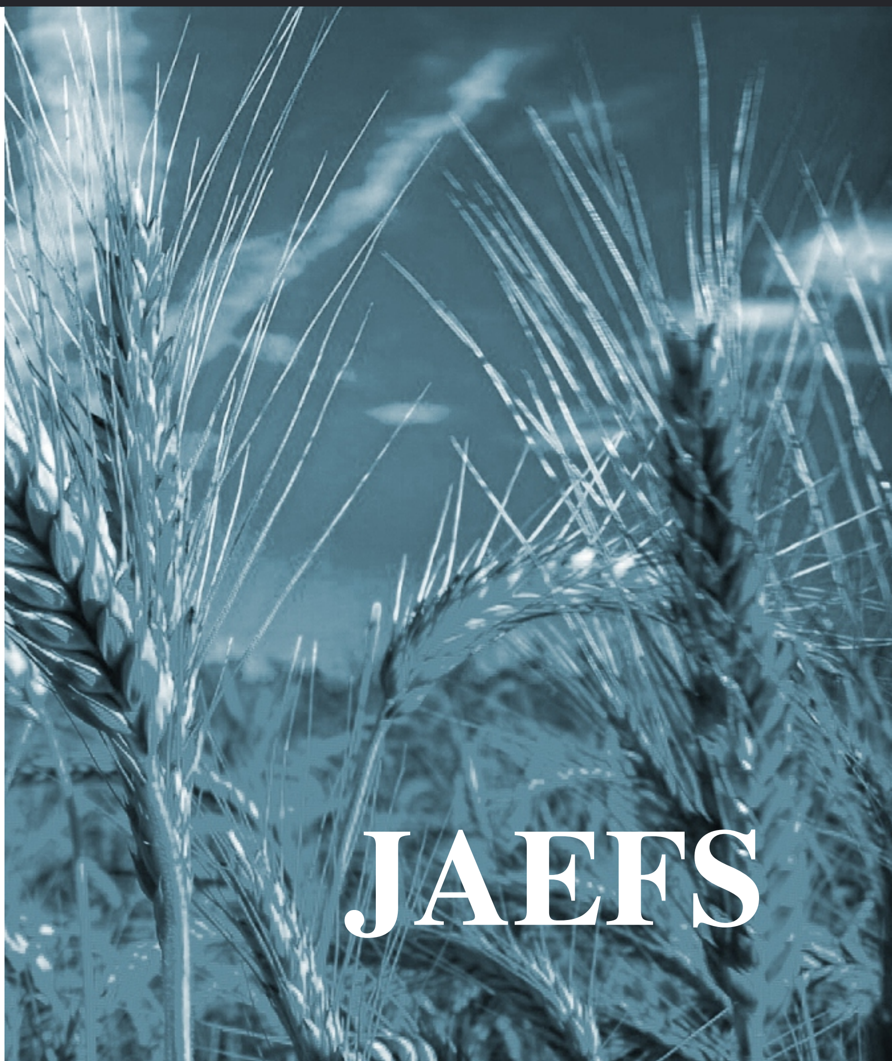

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Research Article Int J Agric Environ Food Sci 4 (2): 130-133 (2020)

The effects of thermal process solid product on development of "Cicer arietinum L." and "Allium ascalonicum"

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Abstract

Today, the number of wastewater treatment plants is rapidly increasing. Accordingly, there is a large increase in the amount of sewage sludge. The sewage sludge obtained should be disposed of in such a way as not to damage the environment. Among these methods, disposal of sewage sludge by landfill is one of the most suitable methods in terms of environment and economics. In this study, the effects on the development of "Cicer Arietinum" and "Allium Ascalonicum" plants were investigated by adding municipal solid waste compost, brewery sludge, pyrolysis solid product of brewery sludge and chemical fertilizer at certain rates to the soil in order to improve the soil and increase the yield. As a result, it can be seen that the materials used can be used for remediation of the soil and contribute to the development of the plant. As a result of the study, it was observed that the soil mixture obtained by mixing the pyrolysis solid product with soil at certain ratios positively affected plant growth. Soil and pyrolysis solid product (25%) provided the highest yield for "Cicer Arietinum".Soil and brewery sludge (25%) provided the highest yield for "Allium Ascalonicum".

Keywords: Waste water treatment sludge, Pyrolysis, Plant growth

Introduction

Sewage sludge is a type of solid waste, and it contains 0.25-12% solids by weight, depending on the treatment applied, which is odorous, semi-solid in the liquid formed as a result of wastewater treatment. The micro and micro nutrients in the sludge that emerged as a result of the treatment are a beneficial fertilizer to this waste; and organic substances provide a good soil improvement feature, many authorities support the use of these products in agriculture and applications are spreading in many countries (Strauch,1991; Düring and Gäth, 2002). Within the framework of the European Union Directives; composting, biometanization and landmass application with energy recovery and recovery methods to reduce the storage of biodegradable waste in landfills. It is also appropriate to use treatment sludge not only for agriculture but also for green space, land recreation and urban landscape (Debosz et al., 2002). Nowadays it is a very common practice to supply the treatment sludges having suitable properties to agricultural areas and to use them. Both the final disposal and the plant nutrients in the sludge become natural cycles of the soil (Kocaer et al., 2003) by applying the treatment sludges to the soil in accordance with the agriculture. Waste incineration / gasification (thermal conversion) is the process of converting combustible waste to an inert residue (ash, slag) at high temperatures. By means of the method, while the space required for the storage of solid wastes is reduced, energy recovery is achieved by using the heat that is present in the waste and which is produced as a result of the treatment (Öngen et al., 2019). Pyrolysis is a process based on thermal decomposition of waste in a completely oxygen-free environment. With the pyrolysis method, coke, tar, volatile oils, condensable hydrocarbons, water and pyrolysis gases are released as a result of disposal of wastes. In the gasification method, a certain amount of air is given but the amount of oxygen in the environment is kept below the stoichiometric ratio (Saltabaş et al., 2011). Biochar produced by carbonization of biomass with pyrolysis are used for

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soil remediation and as a plant fertilizer to store carbon in the soil, increase soil fertility, reduce climate change (reduce CO_2 and CH_4 emissions), dispose of waste causing environmental pollution in waste management and generate energy (Akgul, 2017). In this study, the effect of compost, brewery sludge, biochar obtained by pyrolysis of brewery sludge and chemical fertilizer on the development of "*Cicer Arietinum"* and "*Allium Ascalonicum"* plants was investigated.

Materials and Methods

In this study, Tropical brand 100% organic soil was used. Compost which is recycled from the domestic solid waste obtained from Istac Kemerburgaz Recovery and Compost Facility. Ammonium sulphate containing 21% Nitrogen obtained from Gübretaş as chemical fertilizer. Treatment sludge (from a brewery waste water treatment plant) and heat treatment solid product (pyrolysis product of brewery sludge) were used. These contents mixed at the ratios indicated in Table 3 and placed in a pot. As a plant material, natural field crop "*Cicer Arietinum*" (chickpeas) and "*Allium Ascalonicum"* (shallot) were used. Soil samples were prepared to contain 25% compost, 50% compost, 1.5% chemical fertilizer, 2.5% chemical fertilizer, 10% brewery sludge, 25% brewery sludge, 10% biochar, 25% biochar, mixture of 10% biochar and 25% compost. All of the percentages are as volume/volume (v/v).

Prior to addition of chemical fertilizer to samples, a two-week waiting period was applied to obtain the reaction between the soil and compost for reclamation. Only irrigation was employed at this stage. Also, plant seeds were planted in the soil with no additives. The prepared pots were placed under artificial light and constant temperature environment with timer control, for 16 hours day and 8 hours night. The change in the size of the plant (body and root), weight of the plant were measured after a period of 40 days. At the beginning and end of the experiment, the pH values of the mixtures in the pots were measured.

For determining the pH values of the samples, for compost pH; distilled water was added to the samples at a 5:2 (v/w) ratio (Page et al. 1982), for soil pH; solution KCl 0.1 N was added to soil samples at a 1:2.5 (v/w) ratio (Paradelo et al. 2011).Then mixed by a magnetic stirrer for 10 minutes. The pH values were measured using a Jenway 3040 Ion Analyzer.

For Elemental analysis of the compost and soil samples (C, H, N, S determination) Thermo-Flash 2000 CHN-S elemental analyzer in the laboratory of Istanbul University-Cerrahpasa, Environmental Engineering Department was used (ASTM-D5373 (2016) method). The organic matter (%) determination of the compost samples was performed in Halic Cevre Laboratory.

For the determination of the total metal concentrations and some elements (Mg, Ca, Na and K), the samples from the soil and the compost were thoroughly ground with porcelain mortar. Then, 0.5 g of air-dried sample was placed in microwave tubes and mixed with 9 mL of concentrated HNO_3, 3 mL of concentrated HCl, 2 mL of concentrated HF and microwaved (Berghof MWS-2 microwave-system) (EPA Method 3051A, 2013). During the digestion of the microwaves, the temperature was gradually increased and 10 minutes were left at 185 ° C. After digestion, the samples in Teflon containers were filtered (MN 640 de, 125 mm Macherey-Nagel fitler paper) and the filtrate was taken to HDPE containers and their volumes were completed to 50 mL. The determination of concentrations of metals and some elements was measured using ICP optical emission spectrometer (Perkin Elmer Optima 7000 DV) combined with autosampler (Perkin Elmer S10 Autosampler) in Bahçeşehir University Environmental Engineering laboratory. Using the reference soil, the accuracy of the measurement results was checked. NCS Certified Reference Material NCS ZC73002 was used as reference soil.

Results

Table 1 shows the composition of the soil and compost used in the study. When the results in Table 1 were examined, it was found that the pH of the soil was slightly acidic. The pH value of the compost is a slightly basic value.

Table 1. Composition of soil and compost

Parameters	Soil	Compost
pH	5.5-6.5	7.9
NO_3	50-100 ppm	-
EC	0.5-0.8 mmhos/cm	-
Ca	30-50 ppm	22727 mg/kg dryweight
P	10-20 ppm	-
K	40-100 ppm	7995 mg/kg dryweight
OM(%)	-	1.36
Na	-	17525 mg/kg dryweight
Mg	-	5100 mg/kg dryweight
S(%)	-	0.17
C(%)	-	11.07

Table 2 shows the characterization of the brewery sludge and biochar used in the study. When the results in Table 2 were examined, it was seen that the C (%) and N(%) contents in the brewery sludge not subjected to pyrolysis were higher than the C (%) and N (%) contents of biochar obtained from pyrolysis sludge.

Table 2. Characterization of brewery sludge and biochar

Parameters	Brewery Sludge	Biochar
C	17	9.30
H	3.15	0.20
N	3.20	0.12
S	-	-
Calorific Value (kcal)	1470	720

Table 3. Plant height, root length, weight and pH measurements at the end of the study for *Cicer Arietinum*

Soil mixture	pH	Body height (cm)	Root lenght (cm)	Weight (gr)
Soil	6	19	9	0.11
Soil+ 25% compost	7	19.25	5.5	0.88
Soil+ 50% compost	7.5	13.5	6.5	0.92
Soil +1.5% chemical fertilizer	5.5	-	-	-
Soil+2.5% chemical fertilizer	6	-	-	-
Soil+10% brewery sludge	5	19.75	9	0.64
Soil +25% brewery sludge	5.5	16.25	5.85	0.3
Soil +10% biochar	7	23.5	13.25	1.07
Soil + 25% biochar	7	25.5	11	1.0595
Soil+ 10% biochar + 25% compost	7	21	9	0.95

When the changes in pH values of soil mixtures were examined, it was seen that the addition of compost and biochar to soil increased soil pH slightly (Table 3 and 4). When the results in Table 3 are examined, it is seen that the best plant growth for *Cicer Arietinum* plant is in biochar added soil mixtures. *Cicer Arietinum* plant growth was not observed in pots where chemical fertilizer was added. When the development of *Cicer Arietinum* plant was evaluated, it was observed that the results were similar for plant development as a result of using compost and brewery sludge (Table 3).

Table 4. Plant height, root length, weight and pH measurements at the end of the study for *Allium Ascalonicum*

Soil mixtures	pH at the end of study	Body height (cm)	Root lenght (cm)	Weight (gr)
Soil	6	25.75	9	1.6
Soil + 25% compost	7	29.9	12.15	2.4
Soil + 50% compost	7.5	18.5	11.75	1.23
Soil + 1.5% chemical fertilizer	5.5	27.75	5.85	2.038
Soil + 2.5% chemical fertilizer	6	13.85	1	0.7
Soil + 10% Brewery sludge	5	28.25	7	2.75
Soil + 25% Brewery sludge	5.5	31	2.5	3.5
Soil + 10% biochar	7	36.5	17	2.829
Soil + 25% biochar	7	-	-	-
Soil + 10% biochar + 25 % compost	7	2.09	9.5	1.5

In the study, when the results of the experiments related to the growth of *Allium Ascalonicum* plant were examined, it was seen that 10% compost added soil growth in the soil was better than no remedial soil. Best plant growth was observed in soil mixtures with 10% biochar added. It was observed that plant *Allium Ascalonicum* did not develop in soil added to 25% biochar. It was observed that *Allium Ascalonicum* plant growth was also very good in the soil mixtures where brewery sludge was added. It can be said that *Allium Ascalonicum* plant root growth and plant weight in soil mixtures with 25% brewery sludge is better than the growth of plants growing in soil mixtures using 10% biochar (Table 4).

According to Lehmann and Joseph (2009), biochar not only enhances water and nutrient retention properties, but also contributes to creating favorable micro-environments to accommodate microorganisms. Addition of biochar in soil may affect soil composition, soil diversity and microbial activity (Doan et al., 2014; Purakayastha et al., 2015; Wang et al., 2015; Pan et al., 2016). In this study, it was found that adding 10% biochar to the soil positively affects plant development. The reason for this is thought that the biochar added to the soil increases the water and nutrient retention properties of the soil as stated in the studies in the literature.

Conclusions

In this study, it has been determined that the waste sludge from the brewery's wastewater treatment plant can be used as a soil conditioner in the agricultural sector. While the yield is highest in the soils where the heat treatment is included in the solid product (biochar), it is followed by beer sludge which is included raw in terms of yield. Thus, the use of so-called waste sludges as soil conditioners after pyrolysis is considered to be good in terms of "zero waste" approach. However, it is thought that it is necessary to deepen the research with laboratory scale studies where more parameters related to soil and plant are observed before the implementation.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

The contribution of the authors is equal.

All the authors read and approved the final manuscript.

All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Growth, root morphology and leaf physiology of watermelon as affected by various rates and forms of nitrogen in the hydroponic system

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Abstract

The aim of this study was to determine the effects of different rates and forms of nitrogen (N) on shoot growth and root morphological and leaf physiological responses of watermelon (cv. Crimson Tide F1) under hydroponic growth condition. The nutrient solution experiment was conducted between January - March in 2018 by using an aerated Deep Water Culture (DWC) technique in a fully automated climate room placed in the Plant Physiology Laboratory of Erciyes University, Faculty of Agriculture, Kayseri in Turkey. Plants were tested under two N-Rates (N1: 1000 and N2: 2000 µM N) and three different N-Forms (Am-N: $NH4^+$, Nit-N: $NO3^-$, 50% mixture of both N-Forms Mix-N: NH4+NO3) by growing in 8 L pots filled continuously aerated nutrient solution (modified Hoagland). The experiment was conducted with a completely randomized block design with four replications. From each pot two plants were harvested 42 days after treatment (DAT) by separating into stem, leaf and root fractions. The results indicated that shoot growth, root morphological and leaf physiological responses were significantly ($p<0.001$) affected by N-Rate, N-Form and N-Rate x N-Form interaction. The lowest performance under sole Am-N supply was achieved, since it severely reduced shoot and root growth and leaf area development as compared to sole Nit-N and Mix-N treatments. Irrespective of N rates, best growth performance in shoot growth was achieved under Mix-N supply, while root growth significantly improved under sole Nit-N supply. All these clearly indicate that the application of sole ammonium (1000 µM N) is detrimentally toxic for hydroponically grown watermelon plants. On the other hand, a 50% mixed of ammonium with nitrate even at a higher dose (N2: 2000 µM ammonium N) can be more advantageous for the growth and development of watermelon plants grown in the hydroponic system. Furthermore, our study showed that the effects of N-Form (Nit-N and Mix-N) on the improvement of shoot growth, root morphology and leaf physiological development and photosynthesis were significantly higher than the effects of N-Rate. Therefore, the application of nitrogen fertilizers in the form of Mix-N could be a useful N management strategy for growth and yield of watermelon plants under hydroponic conditions.

Keywords: Nitrogen, Watermelon, N-form, Ammonium toxicity, Photosynthesis

Introduction

In crop production nitrogen (N) is the most common and widely used fertilizer nutrient. Due to be an important resource and essential input for crop growth and yield although, the available N is more often a limiting factor influencing plant growth than any other nutrient in both high-input and low-input agriculture systems (Grindlay, 1997). Plants may prefer the ammonium (NH_4^+) or the nitrate (NO_3^-) as the N source in the soil. There are several chemicals, physical and biotic soil factors determine which of the two forms prevails (Kinzel, 1983). Under favorable soil conditions NO_3 is usually the major N-Form in the soil solution and is therefore, supposed to be the most important N-source for crop growth and yield. Because, under favorable soil conditions, NH_4^+ is readily converted to

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NO_3^- by soil microorganisms through the nitrification process. However, this may be different when adverse soil conditions, in particular low oxygen and low pH, hamper the activity of nitrifying bacteria. For instance, agricultural practices such as the application of NH4 fertilizers or urea along with nitrification inhibitors or the creation of soil zones with high NH4 concentrations by specific application techniques (CULTAN technique: Sommer, 1994) may result in an enhanced NH4 supply. The N-Form and N-concentration have pronounced effects on plant growth (Wilcox et al., 1985). The response of plant species or cultivars to NH4 or NO3 nutrition varies widely. Some shows better growth with NH4, others with NO3. Beneficial effects of enhanced NH4 supply on yield (Huffman, 1989), specific yield components (Wiesler, 1997) and yield responses among genotypes (Below, 1987) were clearly demonstrated. However, the question remains unanswered as to which form of nitrogen, NO3 or NH4, or which combinations of these forms, is superior for obtaining maximum crop productivity. Moreover, in the literature, there is not enough information or conducted studies on the physiology of horticultural crops related to N-rates or N-forms in hydroponic growth systems. Therefore, the aim of this study was to assess the effects of different rates and forms of nitrogen on shoot growth and root morphological and leaf physiological responses of watermelon under hydroponic growth conditions.

Materials and Methods

Plant Material

In the present study, to obtain a high germination rate and also homogenous seedlings for hydroponic growth system, a well-known commercial watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] cultivar (Crimson Tide F1) was used as plant material.

Experimental Site and Plant Growth Conditions

An experiment was conducted between January - March in 2018 by using an aerated Deep Water Culture (DWC) technique in a fully automated climate room placed in the Plant Physiology Laboratory of Erciyes University, Agriculture Faculty, Kayseri in Turkey. During the vegetation period, the room temperatures (day/night) were 25/22 °C while the relative humidity was almost 65-70%. The supplied light intensity was almost 350 µmol m^{-2} S^{-1} photon flux of 16/8 h of light/dark photoperiod regimes. The watermelon seeds were sown in a mixture of peat (pH: 6.0-6.5) and perlite contained media with a ratio of 2:1 (v:v) for two weeks in the plastic multi-pots. The seedlings almost developed two to three true leaves, were carefully freed from peat-perlite medium by root washing and then transferred into 8 L plastic pots. Two plants were grown in each pot filled with 8 L nutrient solution (modified Hoagland). The solution was continuously aerated by an air pump to supply sufficient dissolved oxygen (8.0 mg/ L). The experiment was conducted with completely randomized block design with four replications. In the hydroponic experiment the total vegetation period from transplanting into 8 L plastic pots up to the final harvest was almost 42 days.

In this study, the basic nutrient solution was prepared regarding to Hoagland (modified) formulation. During hydroponic study only distilled water with analytical grade (99% pure) chemicals contained were used. In this hydroponic experiment, ammonium sulfate ($(NH_4)_2SO_4$) and clacium nitrate ($Ca(NO_3)_2$) were used as the N sources in two different N-Rates (N1: 1000 and N2: 2000 µM N) and three different N-Forms (sole nitrate: Nit-N, sole ammonium: Am-N and 50% mixture of both N-Forms: Mix-N). The pH of nutrient solution was maintained neutral by adding $CaCO_3$ and to hamper the nitrification process, a nitrification inhibitor (DMPP: 3,4-dimethylpyrazole phosphate) was applied to the solution. Furthermore, basic nutrient solution had the following composition (µM): K_2SO_4 (500); KH_2PO_4 (250); $CaSO_4$ (1000); $MgSO_4$ (325); NaCl (50); H_3BO_3 (8.0); $MnSO_4$ (0.4); $ZnSO_4$ (0.4); $CuSO_4$ (0.4); $MoNa_2O_4$ (0.4); Fe-EDDHA (80). All nutrients were replaced when the N concentration of the nutrient solution in the 2.0 mM N rate pots fell below 1.0 mM, as measured daily with nitrate test strips (Merck, Darmstadt, Germany) by using a NitracheckTM reflectometer. Distilled water was added every two days to replenish the water lost to evaporation, and the solution was changed weekly.

Harvest, Shoot- Root Fresh and Dry Weight, Root: Shoot Ratio Measurements

At the end of the experiment (42 DAT) watermelon plants were harvested by separating them into the leaf, stem and roots for the fresh weight determination. After measuring the fresh weights of each shoot and root fraction, samples were stored separately in paper bags and dried in a ventilated oven at 70 °C for 72 hours. Root: shoot ratio was calculated from the dry weight.

Root Morphological Measurements

In the hydroponic experiment, the plant root morphological parameters such as root length (m), root volume (cm^3) and root diameter (mm) of the plants were measured by using a special image analysis software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada) in combination with Epson Expression 11000XL scanner. From harvested fresh root samples of watermelon plants, almost 5.0 g sub-samples were taken. The samples were each (one after the other) placed in the scanner's tray.

Water was added and with the aid of a plastic forceps, the roots were homogenously spread across the tray; and the scanning and analysis have done from the WinRhizo system's interface on a computer connected to the scanner. The total plant root length and volume was then determined as the ratio of sub-sampled root fresh weight to the total root fresh weight.

Main Stem Length and Leaf Physiological Measurements

In the hydroponic experiment some of the leaf physiological parameters were determined destructively at harvest while some of them were measured non-destructively prior to the harvest. The main stem length was measured non-destructively prior to the harvest by using a ruler and it was recorded in centimeter (cm). The total leaf area of harvested plants was measured destructively with a leaf area measuring device (LI-COR LI-3100C, Inc., Lincoln, NE, USA). The measurements were recorded in centimeter square (cm^2).

On the other hand, the leaf chlorophyll index (SPAD) was determined non-destructively by using a portable chlorophyll (SPAD) meter (Minolta SPAD-502). During the growth period, SPAD readings were performed on 3th and 4th week of the vegetation period at the center of the leaves on the fully expanded youngest leaf of whole plants for each treatment.

The leaf-level CO_2 gas exchange (μmol CO_2 m^{-2} s^{-1}) measurements were done non-destructively in a controlled growth chamber by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). The leaf photosynthesis measurement was performed on the most recent fully expanded leaves, using four replicate leaves per treatment on 3th and 4th week of the vegetation period.

Shoot and Root Nitrogen Analysis

After grinding shoot (leaf) and root dry materials, almost 200 mg from each dry plant samples were taken to analyze the shoot and root N concentration (mg N g^{-1} d.w.) by using Kjeldahl Nitrogen Determination Method, introduced by Johan Kjeldahl in 1883 (Labconco, 1998). After the determination of shoot and root N concentration, the value was multiplied by total shoot or root dry matter in order to calculate the total shoot and root N content (N uptake) of a whole plant (mg N $plant^{-1}$).

Statistical Analysis

Statistical analysis of the nutrient solution experiment data was performed using the PROC GLM procedure of SAS Statistical Software (SAS for Windows 9.1. SAS Institute Inc., Cary, NC.). A two-factorial analysis of variance was performed to study the effects of N-Rate and N-Form and N-Rate x N-Form interactions on the plants. Levels of significance are represented by $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and ns means not significant (F-Test). Differences between the treatments were analyzed using Duncan's Multiple Test ($p < 0.05$).

Results and Discussion

Shoot and Root Biomass Production and Partitioning

Results obtained from hydroponic experiment indicated that shoot and root fresh weight, main stem length (Table 1), shoot and root dry weight and root:shoot ratio (Table 2) of watermelon plants were significantly ($p < 0.001$) affected by N-Rate, N-Form and N-Rate x N-Form interaction. Plants under N2 supply showed usually a higher performance in shoot and root growth and biomass production than plants grown under N1 supply, but only when the nitrogen was applied in the form of sole nitrate (Nit-N) or a mixture of both N-Forms (Mix-N) (Table 1 and 2). On the other hand, a significant decline in shoot and root growth was recorded when the nitrogen was increased in the form of sole ammonium (Am-N) from N1 to N2 level. Increasing N supply from 1000 μM (N1) to 2000 μM (N2), led to an increase in shoot fresh weight by almost 19.9% and 23.2%, in root fresh weight by almost 35.6% and 36.1% and in main stem length by almost 26.2% and 16.4%, respectively, under supply of Nit-N and Mix-N (Table 1). In contrast, the reduction in shoot and root fresh weight and main stem length was almost by 31.2%, 24.9% and 28.1%, respectively, under the supply of sole Am-N.

Besides substantial nitrogen effects on the shoot and root growth of watermelon, also highly significant differences were found between N-Forms in shoot and root growth under N1 and N2 nitrogen levels (Table 1 and 2). As compared to both Nit-N and Mix-N forms, significantly lowest shoot and root fresh biomass and main stem length were recorded under the supply of sole Am-N at N1 and N2 levels (Table 1). Averaged over N-Rates, shoot fresh weight (SFW), root fresh weight (RFW) and main stem length (MSL) were increased almost by 167.3%, 190.3% and 93.5%, respectively at Nit-N, and almost by 196.5%, 165.6% and 114.2%, respectively at Mix-N, as compared to sole Am-N supply. Interestingly, this result clearly indicated that the effects of N-Forms (Nit-N and Mix-N) on the enhancement of SWF, RFW and MSL were substantially higher than the effects of N-Rates (Table 1 and 2).

In agreement with several studies (Wilcox et al., 1985; Sattelmacher et al., 1990; Schulte Auf'm Erley et al., 2007; Ulas et al., 2012; 2013; 2019), our results clearly demonstrated that nitrogen rates and forms have pronounced effects on the shoot and root growth of watermelon. Moreover, our results

Table 1. Shoot and root fresh weight and main stem length of watermelon as affected by different N-Rates (N1: 1000 and N2: 2000 μM) and N-Forms (Nit-N: NO_3^-, Am-N: NH_4^+ and 50% mixture of both N-Form; Mix-N) in hydroponic system

	Shoot fresh weight (g $plant^{-1}$)		Root fresh weight (g $plant^{-1}$)		Main stem length (cm $plant^{-1}$)	
N-Form	N1	N2	N1	N2	N1	N2
Am-N: NH_4^+	26.42 c	18.18 C	4.22 c	3.17 C	9.60 c	6.90 B
Nit-N: NO_3^-	54.20 b	65.00 B	9.10 a	12.34 A	13.93 b	18.00 A
Mix-N: NH_4+NO_3	59.27 a	73.00 A	8.30 b	11.30 B	16.33 a	19.00 A
<u>F-Test</u>						
N-Rate	***		***		***	
N-Form	***		***		***	
N-Rate x N-Form	***		***		***	

Values denoted by different letters (lower and upper case letters for N1 and N2, respectively) are significantly different between N-Forms within columns at $p < 0.05$. ns, non-significant. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

are highly corroborating with the results of cereal crop experiments which indicated that the best growth was obtained when a mixture of both N-Forms (ammonium and nitrate) compared to sole nitrate was supplied to the plants (Hageman, 1984; Huffman, 1989). Furthermore, our results clearly showed that sole ammonium supply (Am-N) is also detrimental for the watermelon either grown under N1 or N2 nitrogen levels. This could be the result of supra-optimum ammonium (NH_4^+) uptake of plant roots which might be caused a growth reduction in this treatment.

Since ammonium-fed plants are not able to store excess nitrogen in the form of nitrate (NO_3^-), and hence the assimilation capacity of roots and accumulation capacity of shoot might be exceeded due to free ammonium/ammonia (Takács and Técsi, 1992).

In agreement with our results, similar growth inhibition was often observed when the plants (*Zea mays* L., Bennett et al. 1964; *Solanum lycopersicon*, Ganmore-Neumann and Kafkafi 1980; *Solanum lycopersicon*, Magalhaes and Wilcox 1983; *Phaseolis vulgaris*, Chaillou et al. 1986) were fed exclusively with sole ammonium in previous studies.

As similar as shoot and root fresh weight (Table 1), N-Rates and N-Forms were differed significantly (p <0.001) in shoot and root dry matter productions and their partitioning (root:shoot ratio) under both N1 and N2 nitrogen levels (Table 2). Although showing similar dry matter partitioning as Nit-N, the shoot and root dry matter accumulation were significantly lowest under sole Am-N as compared to both Nit-N and Mix-N forms under N1 and N2 supply. This clearly indicates that the root growth of ammonium-fed plants was relatively less reduced than shoot growth. Similar results have been observed by Atkinson (1985), De Viesser (1985) and Haynes and Goh (1978). The highest shoot dry matter was produced under Mix-N form while the highest root dry matter was produced under Nit-N form at both N1 and N2 nitrogen levels. This also clearly explaining why the dry mater partitioning between shoot and root was significantly higher at Nit-N than at Mix-N form at both N1 and N2 nitrogen levels. This might be due to a high assimilate allocation from shoot to roots under sole nitrate supply. Similar and confirmative results have been demonstrated in the studies of Feil (1994) and Ulas et al. (2013).

Table 2. Shoot and root dry weight and root:shoot ratio of watermelon as affected by different N-Rates (N1: 1000 and N2: 2000 µM) and N-Forms (Nit-N: NO_3^-, Am-N: NH_4^+ and 50% mixture of both N-Form; Mix-N) in hydroponic system

	Shoot dry weight (g plant^{-1})		Root dry weight (g plant^{-1})		Root: shoot ratio (g g^{-1})	
N-Form	N1	N2	N1	N2	N1	N2
Am-N: NH_4^+	2.42 c	1.52 C	0.27 c	0.18 C	0.11 b	0.12 A
Nit-N: NO_3^-	4.89 b	6.34 B	0.58 a	0.77 A	0.12 a	0.12 A
Mix-N: NH_4+NO_3	5.96 a	7.15 A	0.52 b	0.66 B	0.09 c	0.09 B
F-Test						
N-Rate	***		***		***	
N-Form	***		***		***	
N-Rate x N-Form	***		***		***	

Values denoted by different letters (lower and upper case letters for N1 and N2, respectively) are significantly different between N-Forms within columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

Shoot and Root N Concentration and Total Plant N Uptake

Results indicated that highly significant (p <0.001) differences between N-Rates and N-Forms were found in shoot and root N concentrations and in total plant N uptake of watermelon plants (Table 3). The growth response to supplied N, i.e., the interaction between N-Rate and N-Form was also significant but only in shoot N concentration and total plant N uptake. Plants grown either under sole ammonium (Am-N), sole nitrate (Nit-N) or a mixture of both N-Forms (Mix-N) exhibited a significant increase in shoot (13.3% by Am-N, 13.6% by Nit-N and 11.7% by Mix-N) and root (31.5% by Am-N, 26.6% by Nit-N and 20.8% by Mix-N) N concentrations when the nitrogen was increased from N1 to N2 level. Interestingly, the highest shoot N concentrations at both N levels were found under sole Am-N, while the lowest was shown under sole Nit-N supply. However, the highest shoot N concertation under sole Am-N supply is contrasting with the lowest shoot fresh (Table 1) and dry (Table 2) matter production of the same watermelon plants. This can be explained by the detrimental effects of ammonium toxicity that occurred in watermelon plants grown under both 1000 µM (N1) and 2000 µM (N2) nitrogen levels supplied as the form of sole ammonium (Am-N). This might be due to a low leaf area formation which usually leads to an increase in the amount of N accumulated per unit of leaf area (Hirasawa and Hsiao, 1999). Similar and corroborative results were demonstrated by the study of Ulas et al. (2013) when the oilseed rape plants are grown under hydroponic sole ammonium supply at a rate of 1000 µM N. Moreover, our watermelon plants showed an intermediate shoot N concentration under Mix-N supply at both N1 and N2 levels (Table 3). On the other hand, the highest root N concentrations were found under Mix-N supply, while the lowest and similar root N concentrations were recorded under sole Am-N and Nit-N supply at both N1 and N2 levels.

Table 3. Shoot and root nitrogen concentration and total plant nitrogen uptake of watermelon as affected by different N-Rates (N1: 1000 and N2: 2000 µM) and N-Forms (Nit-N: NO_3^-, Am-N: NH_4^+ and 50% mixture of both N-Form; Mix-N) in hydroponic system

	Shoot N concentration (mg g dw.$^{-1}$)		Root N concentration (mg g dw.$^{-1}$)		Plant total N uptake (mg plant^{-1})	
N-Form	N1	N2	N1	N2	N1	N2
Am-N: NH_4^+	36.77 a	41.67 A	18.14 b	23.75 B	93.72 c	67.76 C
Nit-N: NO_3^-	29.49 c	33.47 C	19.17 b	24.33 B	155.43 b	230.90 B
Mix-N: NH_4+NO_3	31.62 b	35.28 B	22.55 a	27.29 A	200.06 a	270.30 A
F-Test						
N-Rate	***		***		***	
N-Form	***		***		***	
N-Rate x N-Form	*		n.s		***	

Values denoted by different letters (lower and upper case letters for N1 and N2, respectively) are significantly different between N-Forms within columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

Leaf Physiological Development and Photosynthetic Activity of Leaves

In this study, the total leaf area, leaf chlorophyll index (SPAD) and photosynthetic activity of leaves were significantly (p <0.001) affected by N-Rate, N-Form and N-Rate x N-Form interaction (Table 4). As similar as shoot and root biomass production (Table 1 and 2), the plants grown under N2 supply showed usually a higher performance in leaf area formation, SPAD value and photosynthesis than plants grown under N1 supply, but only when the nitrogen was applied in the form of sole nitrate (Nit-N) or a mixture of both N-Forms (Mix-N) (Table 4). These results clearly explaining which factors are primarily contributing to the shoot and root growth of watermelon plants under increased N supply of both N-Forms. Increasing N supply from N1 to N2 level, led to an increase in total leaf area by almost 37.1% and 22.8%, in SPAD value by almost 21.4% and 21.3% and in photosynthesis by almost 48.0% and 46.4%, respectively, under supply of Nit-N and Mix-N (Table 4). However, the opposite is also true, since significant reductions in total leaf area and photosynthesis were recorded when the nitrogen was increased in the form of sole ammonium (Am-N) from N1 to N2 level.

Table 4. Total leaf area, leaf chlorophyll index (SPAD) and photosynthesis of watermelon as affected by different N-Rates (N1: 1000 and N2: 2000 µM) and N-Forms (Nit-N: NO_3^-, Am-N: NH_4^+ and 50% mixture of both N-Form; Mix-N) in hydroponic system

	Total leaf area (cm^2 plant^{-1})		Leaf chlorophyll index (SPAD)		Photosynthesis (µmol m^{-2} s^{-1})	
N-Form	N1	N2	N1	N2	N1	N2
Am-N: NH_4^+	303.74 c	229.81 C	50.61 a	52.28 C	6.33 c	4.77 C
Nit-N: NO_3^-	738.91 b	1012.74 B	48.58 b	59.00 A	9.19 b	13.58 B
Mix-N: NH_4+NO_3	914.36 a	1122.18 A	45.54 c	55.18 B	10.60 a	15.52 A
F-Test						
N-Rate	***		***		***	
N-Form	***		***		***	
N-Rate x N-Form	***		***		***	

Values denoted by different letters (lower and upper case letters for N1 and N2, respectively) are significantly different between N-Forms within columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

The reduction in total leaf area and photosynthesis was almost by 24.4% and 24.2%, respectively, under the supply of sole Am-N. All these results are contrasting with the generally positive effects of nitrogen on plant growth and development.

However, similar negative effects of sole ammonium supply were demonstrated in several experiments that were conducted on various plant species (Bennett et al. 1964; Ganmore-Neumann and Kafkafi 1980; Magalhaes and Wilcox 1983; Chaillou et al. 1986, Ulas et al., 2013). However, a small increase in SPAD value by almost 3.4% under sole Am-N was recorded when the nitrogen was increased from N1 to N2 level. This is highly corroborating with the result of high shoot nitrogen concertation under sole Am-N supply (Table 3).

Highly significant differences were found between N-Forms in total leaf area, SPAD value and photosynthetic activity of leaves under both N rates (Table 4). As compared to both Nit-N and Mix-N forms, significantly lowest total leaf area and photosynthesis were recorded under the supply of sole Am-N at N1 and N2 levels. Averaged over N-Rates, total leaf area and photosynthesis were increased almost by 228.9% and 105.4%, respectively at Nit-N, and almost by 281.7%, and 135.7%, respectively at Mix-N, as compared to sole Am-N supply. Our results indicated clearly again that the effects of N-Forms (Nit-N and Mix-N) on the enhancement of leaf area and photosynthetic activity of leaves were substantially higher than the effects of N-Rates on both parameters.

Root Morphological Development and Root Architecture

The results indicated that total root length, total root volume and average root diameter of watermelon plants were significantly ($P<0.001$) affected by N-Rate, N-Form and N-Rate x N-Form interaction (Table 5). Highly significant increases in total root length and root volume, but oppositely significant reductions in average root diameter were recorded when the nitrogen was increased from N1 to N2 level in the form of sole nitrate (Nit-N) or a mixture of both N-Forms (Mix-N). On the other hand, significant reductions in total root length and root volume, but oppositely significant increases in average root diameter were recorded when the nitrogen was increased in the form of sole ammonium (Am-N) from N1 to N2 level. All these indicate that there is a negative relationship between total root length and average root diameter, irrespective of the N-rates.

Increasing N supply from 1000 µM (N1) to 2000 µM (N2), led to an increase in total root length by almost 38.9% and 48.1%, in root volume by almost 27.5% and 36.7%, respectively, while the average root diameter was declined almost by 4.2% and 4.0%, respectively, under supply of Nit-N and Mix-N (Table 5). In contrast, the reductions in total root length and root volume were almost by 30.4%, and 23.1%, respectively, while the average root diameter was increased almost by 3.7% under the supply of sole Am-N. Similar to shoot fresh and dry weight (Table1 and 2) sole ammonium supply also reduced root dry weight (Table 2), total root length and volume (Table 5). However, ammonium-fed plants showed a higher root:shoot ratio than ammonium-nitrate-fed plants (Table 2) indicating that the root growth of ammonium-fed plants was relatively less reduced than shoot growth. Similar results have been observed by Atkinson (1985), De Viesser (1985) and Haynes and Goh (1978).

The reduced growth of ammonium-fed plants might also have been caused by enhanced C demand for ammonium assimilation followed by deprivation of nonstructural carbohydrates exclusively in the roots (Blacquiere et al., 1987; Chaillou et al., 1991) or both, in the roots and shoots (Raab and Terry, 1995). The high demand on intermediates from the TCA cycle for ammonium assimilation and the need to stabilize cytosolic pH by decarboxylation of organic acids (Marschner, 1995) may also cause a deprivation of carboxylates in ammonium fed plants.

Table 5. Total root length, volume and root diameter of watermelon as affected by different N-Rates (N1: 1000 and N2: 2000 µM) and N-Forms (Nit-N: NO_3^-, Am-N: NH_4^+ and 50% mixture of both N-Form; Mix-N) in hydroponic system

	Total root length (m plant^{-1})		Total root volume (cm^3 plant^{-1})		Average root diameter (mm)	
N-Form	N1	N2	N1	N2	N1	N2
Am-N: NH_4^+	9.50 c	6.61 C	2.12 c	1.63 C	0.27 a	0.28 A
Nit-N: NO_3^-	21.30 a	29.58 A	3.85 a	4.91 A	0.24 c	0.23 B
Mix-N: NH_4+NO_3	16.69 b	24.72 B	3.27 b	4.47 B	0.25 b	0.24 B
F-Test						
N-Rate	***		***		n.s	
N-Form	***		***		***	
N-Rate x N-Form	***		***		**	

Values denoted by different letters (lower and upper case letters for N1 and N2, respectively) are significantly different between N-Forms within columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

The N-Forms differed significantly in total root length, root volume and average root diameter under both N rates (Table 5). As compared to both Nit-N and Mix-N forms, significantly lowest total root length and root volume were recorded under the supply of sole Am-N at N1 and N2 levels. Averaged over N-Rates, total root length and root volume were increased almost by 215.9% and 134.2%, respectively at Nit-N, and almost by 157.0%, and 106.9%, respectively at Mix-N, as compared to sole Am-N supply. Moreover, the average root diameter was reduced almost by 13.91% and 10.3%, respectively at Nit-N and Mix-N forms, as compared to sole Am-N supply. Our results indicated clearly again that the effects of N-Forms (Nit-N and Mix-N) on the enhancement of total root length and root volume were substantially higher than the effects of N-Rates on both parameters.

Conclusion

Usually, nitrogen concentration has pronounced effects on plant growth and development. However, the effects of different N-forms (ammonium or nitrate) on the response of different plant species or cultivars varies widely. Some showing better growth with nitrate while some oppositely with ammonium. Most of the studies revealed that best growth performance was often obtained when a mixture of both N-forms was supplied. Our study indicated that shoot growth, root morphological and leaf physiological responses were significantly (p<0.001) affected by N-Rate, N-Form and N-Rate x N-Form interaction. A lowest performance under sole Am-N supply was achieved, since it severely reduced shoot and root growth and leaf area development as compared to sole Nit-N and Mix-N treatments. Irrespective of N rates, best growth performance in shoot growth was achieved under Mix-N supply, while root growth significantly improved under sole Nit-N supply. All these clearly indicate that the application of sole ammonium (1000 µM N) is detrimentally toxic for hydroponically grown watermelon plants. On the other hand, a 50% mixed of ammonium with nitrate even at a higher dose (N2: 2000 µM ammonium N) can be more advantageous for the growth and development of watermelon plants grown in the hydroponic system. Furthermore, our study showed that the effects of N-Form (Nit-N and Mix-N) on the improvement of shoot growth, root morphology and leaf physiological development and photosynthesis were significantly higher than the effects of N-Rate. Therefore, the application of nitrogen fertilizers in the form of Mix-N could be a useful N management strategy for growth and yield of watermelon plants under hydroponic conditions.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

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Data availability

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Research Article

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GIS-based assessment for trace metal pollution: case study on lake Uluabat

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Abstract

This study was carried out to investigate distribution of Cr and Ni concentrations in water and sediment of Lake Uluabat, in Bursa (Turkey). The samples were collected from 10 sites and monitored monthly from August 2013 to July 2014. Data were mapped in ArcGIS 10.1 software and metals in water were assessed according to Turkish Water Pollution Control Regulations (TWPCR), while in sediment were assessed according to American National Oceanic and Atmospheric Administration's (NOAA) criteria. As a result, Lake Uluabat was determined 4th class water, in terms of dissolved forms of Cr, while the lake was determined 3th class water quality in terms of dissolved Ni forms. Trace metals monitored were found above upper threshold value in the lake sediment. These results showed the importance and the need for hard control of pollution loads for the protection of the Lake's sediment and water quality. It is recommended to control and monitor all pollutant sources for ecological sustainability in the lake.

Keywords: Trace Metals, Geographic Information Systems (GIS), TWPCR, Uluabat Lake, NOAA

Introduction

Great amount of trace metals are being discharged into the ecosystems with the rapid industrial and economic development (Liu et al. 2014). Also mining and geochemical structure of soil compose eventual sources of heavy metal pollution into the ecosystems. Trace metals may accumulate up to toxic concentration and result in ecological damage, in these environmental conditions (Hu et al. 2011, Castillo et al. 2011). Trace metal contamination has become a topic of many studies in recent literatures because of the rapid development of industrialization and urbanization (Liu et al. 2018, Gür and Özan 2017, Liu et al. 2014, Yang et al. 2014, Gao and Li 2012, Varol and Şen 2012, Katip et al. 2012). These toxic metals be formed in water systems in soluble, in suspension, in colloidal and in bottom sediments. Geochemical structure of bottom sediment is a very useful indicator for surface water quality, principally regarding heavy metal contents. Also physicochemical composition of lake water is the same significance (Katip 2010). Lake Uluabat forms a significant part of the Susurluk Basin in Marmara Region (42°12′ N, 28°40′ E), where economy, industry and population are developing quickly (Figure 1). The Lake is also one of Turkey's richest lakes in terms of aquatic plants as well as birds and fish populations. Lake Uluabat is under natural and anthropogenic pressure due to its position (Katip et al. 2013).

Consequently, Lake Uluabat has been contaminated for many years by anthropogenic sources such as domestic, agricultural, industrial waste waters, contaminated rain water, etc. The results of many studies that have been made Lake Basin indicate that erosion, eutrophication and heavy metal pollution continues in the lake (Aksoy and Özsoy 2002, Elmacı et al. 2007, Kazancı et al. 2010, Katip 2010, Akdeniz et al. 2011, Katip et al. 2013, Liu et al. 2018). This situation preoccupies the effectiveness of the management plan for the basin which was completed in 2002. To increase the effectiveness of current management plan, GIS utilized from different management plan tools, which widely used in the management of water quality recent years. This paper describes research undertaken

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to explore the degree of metal pollution in Lake Uluabat. Cr and Ni elements were found to be important in previous studies in the lake (Katip 2010, Katip et al. 2012, Katip et al. 2016), therefore in this study these metals were studied.

Materials and Methods

Study Site and Sampling Strategy

Lake Uluabat is a freshwater lake in the city of Bursa, west part of Turkey (42°12’ N and 28°40’ E). The lake is important part of Susurluk basin. It is a large lake, covering an area of between 135 and 160 km^2 depending on the water level, but very shallow, being only 3 m deep at its deepest point. The lake is fed by the Mustafa Kemal Paşa Brook from the southwest. Water leaves the lake by way of the Kocasu Brook in the northwest. A map of Lake Uluabat showing sampling sites is presented in Figure 1. The locations of the sampling sites determined using global positioning system tool (GPS). Water and sediment samples were taken from ten different sites in the lake. Samples were taken monthly and synchronously for a year, from Aug 2013 to July 2014.

Water samples were taken from 0.5 m below the water surface by using a Hydro-Bios brand standard sampler. The samples were transferred to dark polythene bottles (APHA 1998, Burton and Pitt 2002). Sediment samples were taken from the 5 cm layer of the surface sediment by Ekman grab sampler. The samples were carried into the laboratory in plastic bags, and then air-dried for 4 days for stable weighing. Water samples were filtered through a milipore filter paper with preweighed 0.45 µm pore-size. The filtered water samples were acidified with 0.2% (v/v) concentrated HNO_3 and kept in glass bottles. The filter papers containing the suspended solids were air dried and reweighed again. They were digested with 4/1 (v/v) HNO_3/HCl mixture using a microwave device. After cooling, digestions were diluted to 30 ml with mili-Q water. Sediment samples were air dried and then sieved through a 0.2 mm mesh. These samples were digested with Aqua Regia solution 3/1 (v/v) HCl/HNO_3 in microwave device. Then diluted to 50 ml with mili-Q water. Samples were placed in teflon cups and digestion operations were performed in a CEM brand Mars 5 model microwave device (Katip et al. 2013). Trace metals (Cr and Ni) were determined using the VISTA-MPX model of the VARIAN brand ICP-OES device.

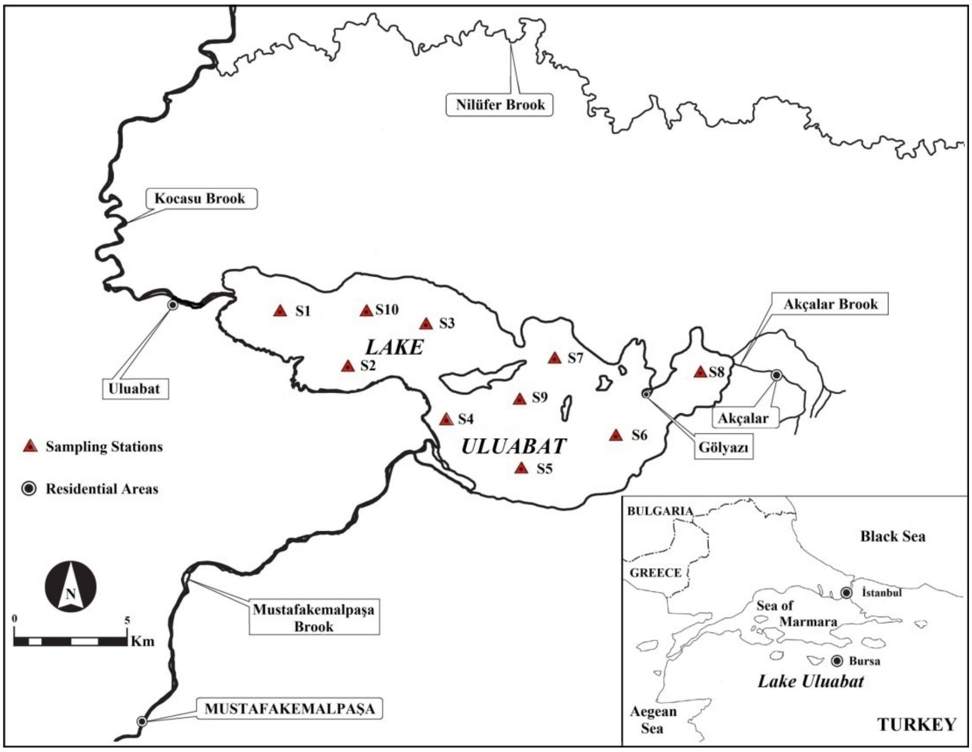

Figure 1. Sampling Sites in Lake Uluabat

Mapping Study

ArcGIS is a worthful tool for interpreting spatial diversity and environmental monitor. Interpolation is used to predict the values of cells at locations that lack sampled points. Inverse distance weighting (IDW) and kriging are two interpolation methods applied widely to clarify spatial variation and distribution of many parameters including heavy metals (Liu et al. 2018). Kriging interpolation refers to a group of spatial interpolation methods for assigning a value of a random field to an unsampled location based on the measured values of the random field at nearby locations (Li and Heap 2008, Xie et al. 2011). IDW assumes that the predictions are a linear combination of available data, and greater weighting values are assigned to values closer to the interpolated point (Hacısalihoğlu et al. 2016). The formula for the IDW method is presented below;

$$z(x_0) = \frac{\sum_{j-1}^{m} z(x_j) d_{ij}^{-k}}{\sum_{j-1}^{m} d_{ij}^{-k}} \quad \text{(Eq.1)}$$

z (x_0) : The linear interpolator weights the interpolated data,
z (x_j) : The using parameter at the location j,
m : The number of neighboring sampling locations,
x_0 : Non-sampling location,
k : The distance influence coefficient, which is usually 1 or 2,
d_{ij} : The distances between the unsampling location i(x_0) and the sampling locations j(x_j) (Hacısalihoğlu et al. 2016, Mantzafleri et al. 2009).

ArcGIS 10.1 software, spatial analyst extension, and IDW interpolation methods were applied in this study. The locations of the sampling sites determined with global positioning system tool (Magellan XL-GPS) using the Europe 1950 UTM coordinate system. The annual average results of trace metals were recorded vector maps attribute tables. These results were interpolated by the method of IDW and were created layers of grid format. In this way the data point transformed into spatial map data and the distribution of pollution maps in the lake have been obtained (Mantzafleri et al. 2009). These maps were evaluated in comparison with national and international standards.

Results and Discussion

The ranges and mean concentrations of trace metals obtained as a result of the study were given in Table 1. The accumulation order of these metals was found to be Cr>Ni for dissolved form, Ni>Cr for metals in the particulate form and Ni>Cr for those in sediment as seen on Table 1. As shown in Table 1, in particulate and sediment form of Ni while the maximum, in dissolved, concentration of Cr is maximum. These elements had high concentrations in summer and in September, also low concentrations in spring and in winter. Because concentration was diluted during rainy periods.

Table 1.Trace Metal Concentrations of Lake Uluabat

Metal	Mean-SD	Maximum-Minimum
Dissolved form (mg/l)		
Cr	0.022 ± 0.03	0.249 – 0.0
Ni	0.012 ± 0.01	0.091 – 0.0
Particulate form (mg/kg)		
Cr	10.53 ± 8.50	42.2 – 0.95
Ni	20.77 ± 32.1	159.3 – 1.2
Sediment(mg/kg)		
Cr	119.74 ± 34.70	210.52 – 1.02
Ni	196.27 ± 52.32	310.28 – 2.05

Spatial distributions of pollution are effective method to identify 'hot points' areas with high contents (Zhou et al. 2007). The concentrations of Cr and Ni are not uniform at all sites. Distribution patterns of Cr and Ni in dissolved, particulate, sediment forms are presented in Figure 2. As shown in Figure 2, dissolved metal concentrations are very high in the site 1st.When the particulate metal distribution maps are analyzed, pollution has been observed intensively in Brook Mustafa Kemal Paşa (MKP) and Brook Kocasu where it is input-output of the Lake. At the sites 6th, 7th and 8th which are more stable regions, metal pollutions are very low. The maximum concentrations of Cr and Ni were detected at 2nd, 4th and 5th sites. This is due to the fact that the area where these stations are located is exposed to too much wind causing water turbulence. In water column, winds cause vertical mixing of bottom sediment. This situation encourage bonding between dissolved trace metals and particulates (Singh et al. 2008).When the sediment metal distribution maps are analyzed, the maximum values of Cr were observed at the 2nd and 3rd sites, respectively. Katip et al. (2016) were found that Cr and Ni concentrations are higher than the other metals such as As, Cu, Zn, Pb etc. Additionally, the high concentrations of Ni are based on the configuration of soil (Başar et al. 2004).

After the creation of metal pollution distribution maps, water pollution of the Lake was assessed in conformity with TWPCR, sediment pollution of the Lake was assessed in according to NOAA international criteria. TWPCR are generally used in Turkey as a pragmatic technique for following the pollution problem in water. The measured parameters were classified according to "Quality Classification of Continental Surface Water (Water Quality Index)" tables in standard. The standard was given in Table 2. According to Table 2, 1st class water refers to high quality water, 2nd class water refers to less contaminated water, 3rd class water refers to dirty water and 4th class water refers to very dirty water.

Table 2 valid for dissolved metals in water. The annual average values of the measured metals were used to decide quality classes depending on metal concentrations. Spatial distribution maps of these metal classes were given in Figure 3.

Table 2. Turkish Water Pollution Control Regulation, Quality Classification (Water Quality Index) (Anonymous, 2004)

(mg/l)	TWPCR				This Study
Metals	Class I	Class II	Class III	Class IV	Mean – SD
Cr	0.02	0.05	0.2	> 0.2	0.0848 ± 0.2092
Ni	0.02	0.05	0.2	> 0.2	0.0304 ± 0.0416

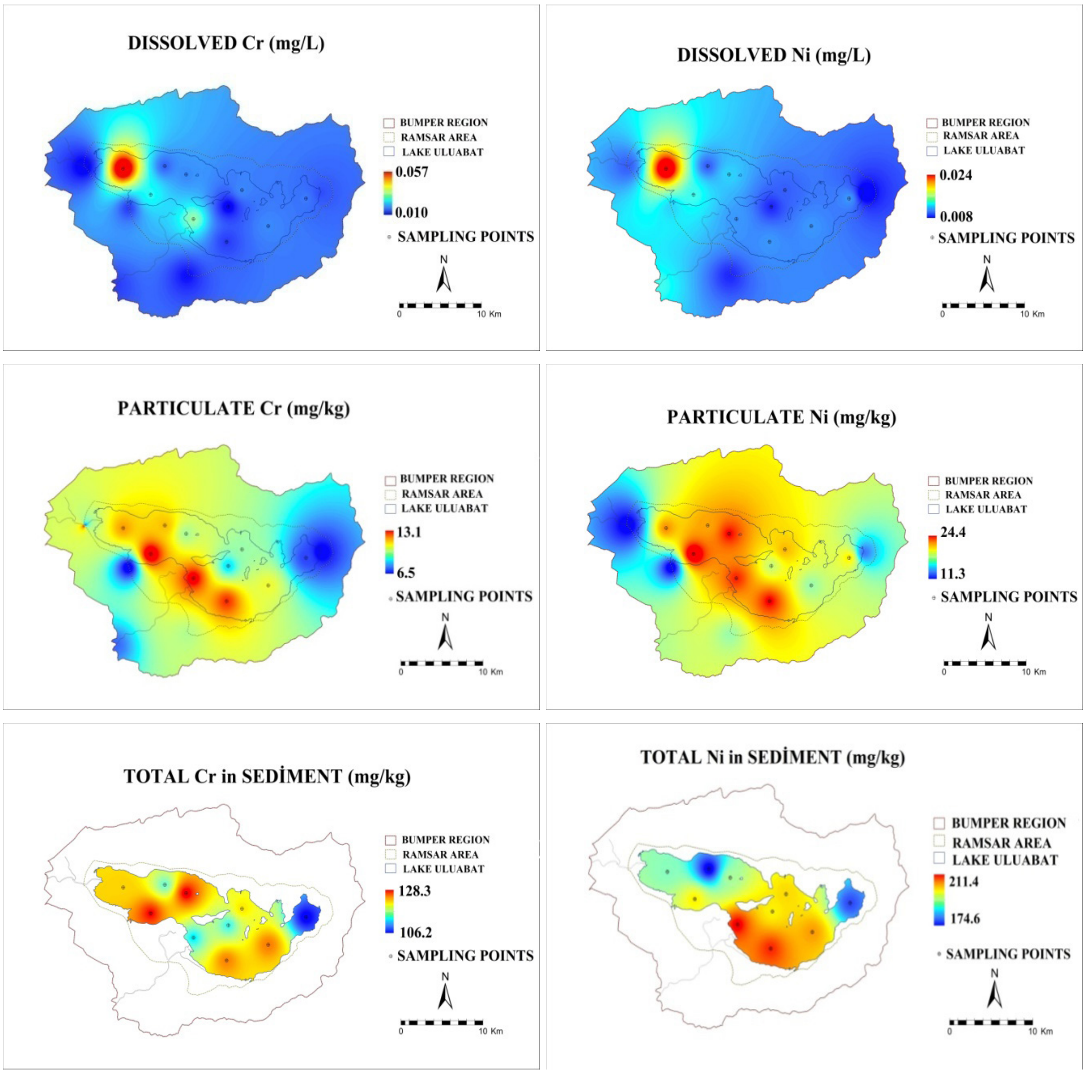

Figure 2. Pollution Distribution Maps of Trace Metals in Lake Uluabat

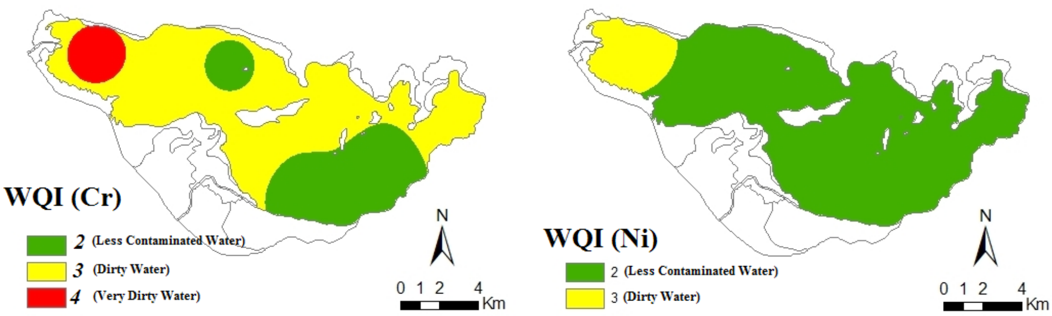

Figure 3. Spatial distribution maps of Cr and Ni according to WQI (in dissolved form)

According to Figure 3, the study area was classified into three zones according to Cr values, that is, class 2 (3rd, 5th, 6th sites), class 3 (2nd, 4th, 7th, 8th, 9th, 10th sites), class 4 (1st site). Also, the lake was classified into two zones according to Ni concentrations, that is, class 3 (only 1st site) and class 2 (all sites). The two metals were determined higher at site 1 than all of the lake, which is closest to the lake exit. Some another pollutant sources enter the Lake, such as reverse current. This current occurs in the Kocasu Brook, because its rate of flow is raised by rainfall during winter (Katip et al. 2013). Another sources of pollutant is Creek Akçalar, which carries domestic and industrial wastewaters of Akçalar village. The irrigation canals of the General Directorate of State Hydraulic Works, which drains wastewaters from agricultural irrigation and neighboring industries are some of the other pollutant sources (Katip et al. 2012). Sediment is an important layer in which water contaminants can be caught like heavy metals. The level of pollution in water resources can be measured by detailed analysis of water, sediment and aquatic organisms living in that area (Goher et al. 2014). Sediment quality guidelines are important tools for assessing the level of contamination in biologically important water resources (Hacısalihoğlu and Karaer 2016, Zhou et al. 2007). Sediment pollution of the Lake was assessed in according to American National Oceanic and Atmospheric Administration (NOAA) international criteria. This criteria was given in Table 3.

Table 3. Concentrations of metals in Lake Uluabat sediment and the toxicological reference values for sediments according to NOAA (Burton and Pitt, 2002)

	Limits	Cr	Ni	
(mg/kg) dryweight	PEL	90	36	PEL : Probable Effect Level
	SEL	110	75	SEL : Severe Effect Level
	TET	100	61	TET : Toxic Effect Threshold
	ERM	145	50	ERM : Effect Range-Median
	UET	95 H	43 H	UET : Upper Effect Threshold
This Study		131.627	220.032	H : Hyalella azteca test

As seen in Table 3, according to the guideline, Cr and Ni values were defined above from all levels (PEL, SEL, TET, ERM, UET). These metals were determined to be higher than upper effect threshold (UET) concentrations. The accumulation of heavy metals in environmental samples affects both human health and the entire ecosystem through the food chain (Castillo et al. 2011). Also, industrial activities, traffic emissions, air pollution can cause contamination of water bodies by heavy metals. Furthermore, rain water coming from runoff carries heavy metals in water resources. These metals, which are found in surface waters, are deposited in the sediment layer. (Katip et al. 2012). In a study conducted by Başar et al. (2004), it was found that the concentrations of Cr, Pb and Ni metals in the soils in the South Marmara Region exceeded the relevant limits. It is possible that these metals deposits in sediment after runoff from the basins of MKP Brook. In addition, the high Ni concentration is due to the general structure of the soil (Başar et al. 2004). Although the metals may remain in the sediment for a long time, they may become free by degradation under oxidizing conditions. This is explained by the fractions of metals. According to the NOAA criteria metal distribution maps for Cr and Ni in sediment was given in Figure 4.

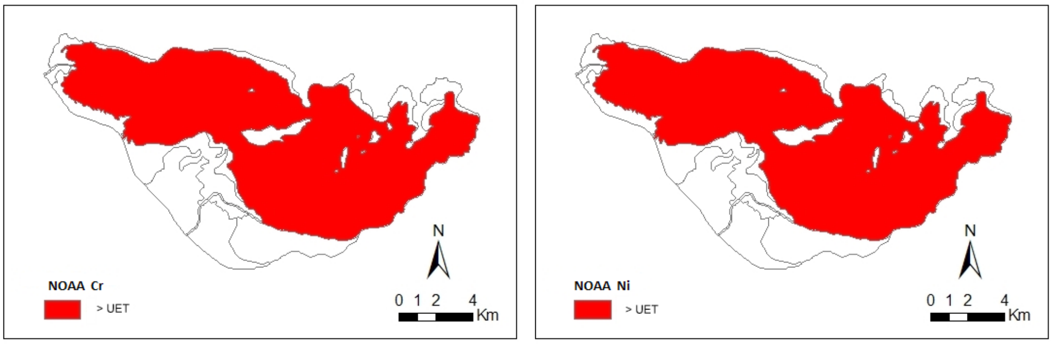

Figure 4. Spatial distribution maps of Cr and Ni according to NOAA (in sediment)

As shown in Figure 4, metal concentrations of the lake sediment were found higher than UET (upper effect threshold) limits. The two metals were determined very high all of the lake sediment. Difference methods have been produced for the risk assessment of heavy metals in sediment; one of them is pollutant load index (Goher et al. 2014). Ecological risk management supply systematic methods that can apprise decision making. Heavy metals are non-biodegradable pollutants. These pollutants do not remove by self purification, on the contrary they cause accumulation, finally enter the food chain (Goher et al. 2014).

Conclusions

Contamination of toxic metals has attracted global notice owing to its persistence, bioaccumulation, and toxicity. The results of this research provide valuable data about Cr and Ni contents of water and sediment from different sites on Lake Uluabat. The concentrations of these metals in water and sediment undergo seasonal changes. Especially concentrations are generally higher during summer. In addition, the quality status of the lake was determined by overlapping water and sediment quality indices with GIS. GIS is widely used for water resources management as well as for many purposes. Using GIS modeling software spatial distribution of numerous water and sediment quality parameters were prepared and analyzed. More intense pollution load were observed at the sites of 1st and 5th. The present situation of the lake compared with WQI for dissolved metals in water and compared with NOAA for metals in sediment. The results show that according to Cr concentrations the lake was classified three zones; class 2 (less contaminated water), class 3 (dirty water), class 4 (very dirty water). Also according to Ni concentrations the lake was classified into two zones that is, class 3 (dirty water) and class

2 (less contaminated water). The two metals were determined higher at site1 than all of the lake, which is near the outlet of the lake. In the Lake sediment, heavy metals were found to be over upper effect threshold value. Lake Uluabat which its importance is high lightened at the global status, have been exposed to heavy metal pollution. So, this study suggested that heavy metals should be monitored regularly in the water and sediment of the lake. Domestic, industrial and agricultural discharges in the lake should be avoided, considering the danger of metals to human health and ecosystem.

Finally, if the necessary precautions are not taken, these metals will participate the human food chain and affect human health.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Determination of physicochemical characteristics, organic acid and sugar profiles of Turkish grape juices

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Abstract

This study investigated the physicochemical properties, sugar and organic acid profiles of 21 grape juice and 3 sour grape juice samples in Turkey. The pH, acidity and soluble solids were ranged from 2.64 to 4.19, 3.58 to 30.75 g L^{-1} and 5.45 to 25.45 °Bx, respectively. The turbidities varied between 1.59 and 109.50 NTU and the lowest value was in the Sultani Çekirdeksiz sour grape juices. The Denizli Karasi sample had the highest color index. The tartaric and malic acid amounts of the samples ranged from 0.53 to 13.16 g 100^{-1} g^{-1} and 0.45 to 30.80 g 100^{-1} g^{-1}, respectively. The major acid was malic acid in the sour grape juice samples and tartaric acid in the grape juice samples. For all samples, glucose and fructose constituted a great part of total sugars. The glucose, fructose and total sugar contents changed from 28.45 to 48.00 g 100^{-1} g^{-1}, 15.88 to 48.75 g 100^{-1} g^{-1} and 53.67 to 97.27 g 100^{-1} g^{-1}, respectively. The highest sugar content was observed in Kara Erik and the lowest in Yediveren. As a result; some physiochemical characteristics, sugar and organic acid contents of the examined 24 grape juice samples were revealed by the current work.

Keywords: Grapes, Juices, Physicochemical characteristics, Organic acids, Sugars

Introduction

Turkey has approximately 435,000 ha vineyards and produces 4 million tons of grapes annually (Faostat, 2018). Most of the grapes used for table and drying. A small part of the production are processed to wine, molasses, grape juice and other grape based traditional foods. Grape based traditional products such as grape juice (clarified or unclarified) and sour grape juice have been processed for a long time. In the last years, the consumer demand for these grape products has increased with emergence of benefits of them on human health. For this reason, production amounts of them have tended to upward especially last decade.

Organic acid quantity and composition are important parameters indicating the quality of grape juice. These compounds affect taste balance, chemical stability, and pH values with organoleptic features, such as flavor, taste, color, and aroma in grape juice products (Lima et al., 2014; Nascimento Silva et al., 2015). Additionally, they can affect stability in juice and can be used as microbiological indicators in beverages. Especially acetic acid is utilized as an indicator to detect undesired microbiological activities in beverages (Ali et al., 2010). Major organic acids are tartaric and malic acid in grape juice, and citric and succinic acid are also present, albeit in lower amounts (Soyer et al., 2003; Ali et al., 2010). Additionally, in a previous study, Lima et al (2014) detected lactic and acetic acid in grape juice samples.

Sugar is one of the main components of grape juice and it is very important for taste balance. Glucose and fructose are the major sugars in Vitis vinifera grapes, but sucrose and other sugars are rarely found (Ali et al. 2010). Furthermore, Coelho et al (2018) reported the detection of maltose and rhamnose in addition to glucose and fructose in Vitis labrusca L. grape juice samples.

The physicochemical features, aroma, phenolic compounds, organic acid, and sugar compositions of the grapes effect on the grape juice quality. The functional properties of the

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grape juices are directly related with their bioactive compound profiles and ingredients, especially phenolic acids (Margraf et al., 2016). Additionally, growing conditions and location, agricultural applications, climate characteristics, and maturity level and variety of grapes affect to quality also (Granato et al., 2015; Yamamoto et al., 2015; Sabir et al., 2010).

The objective of the current study was to determine physicochemical features, sugar and organic acid profiles of the 24 grape and sour grape juices that were processed with different techniques. Three of the 24 samples were un-processing sour grape juice, eight of them were processed grape juice and the others were also un-processing grape juices. The un-processing grape juices of some native grape varieties have been made for a long time in Turkey. Therefore, the traditional technique was performed for native grapes and industrial technique was for the others.

Material and Method

Chemicals

Acetonitrile and malic acid were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). Waters RS for HPLC Plus were obtained from Carlo Erba (Carlo Erba Reagents S.A.S., Val de Reuil, France). D-fructose, D-glucose and L-(+)-tartaric acid were obtained from Extrasynthese (Lyon, France).

Grape samples and juice processing

The grapes and sour grapes (V. *vinifera* L.) samples were picked from Manisa Viticulture Research Institute vineyards, approximately 10 kg were used for each samples, and they were presented in Table 1. After harvest, the samples were transferred to grape processing unit of the institute. Firstly, grapes were washed and passed through a destemmer-crusher machine (Türköz Metal Makine, Turkey). The obtained grape mash samples were used to produce grape juices with traditional or industrial techniques. The grape mash was heated up to 50 °C and were kept at this temperature for 60 min for red and 30 min for white varieties. Then, they were pressed and blurred grape juice samples were obtained. Must yield of traditional produced grape juice samples has changed between 39 and 63 %. Besides, must yield was determined approximately 75 % and 40 % for industrial produced grape juice samples and SGJ, respectively. For the production with the traditional technique, this blurred juice was put into 250 mL glass bottles and pasteurized. Thus, un-processing grape juices were obtained. To manufacture processing grape juice and sour grape juice (SGJ) with industrial technique, pectolytic enzyme (Pectinex XXL, 10000 PECTU/mL) was applied (1mL L^{-1}) for 60 min at 50 °C. Bentonite (SIHA Puranit UF, Germany) was used 1.2 g/L at 50 °C for 60 min, gelatin (SIHA Gelatin Fine Granules, 80-100 Bloom, Begerow, Germany) was applied 0.2 g/L for 120 min, and kieselsol (Levasil 200 /30/FG, HC Starck, Germany) was used fivefold of the gelatin amount for 60 min. Then, the samples filtered using by a plate filter (Europor K 3 filter sheets, 10.4 gpm/ft2; 40x40 Plate filter, Turkey). After filtering, processed grape and SGJ juices was filled into 250 mL glass bottles. The un-processed and processed grape juice and SGJ samples were pasteurized in the 85 °C of water for 20 min and immediately cooled to room temperature.

Table 1. The sample properties, codes and harvest dates

No.	Code	Varieties	Color	Harvest Date	Processing techniques
1	M1	50% Hamburg muscat + 50% Siyah Dimrit	Red	08.08.2017	Processing with industrial techniques: clarified grape juice
2	M2	20% Hamburg muscat + 80% Siyah Dimrit	Red	15.08.2017	
3	M3	85% Royal + 15% Italia	Red	01.09.2017	
4	MH1	Mixed grape hybrids	Red	13.09.2017	
5	MH2	Mixed grape hybrids	Red	20.09.2017	
6	OKG	Öküzgözü	Red	28.09.2017	
7	SCGJ	Sultani Çekirdeksiz	White	15.08.2017	
8	IT	Italia	White	11.08.2017	
9	CS	Cabernet Sauvignon	Red	25.06.2017	Processing with industrial techniques: clarified sour grape juice (SGJ)
10	SC	Sultani Çekirdeksiz	White	25.06.2017	
11	YD	Yediveren	White	20.06.2017	
12	BL	Bulama	White	07.08.2017	Processing with traditional techniques: blurred grape juice
13	EXL	Exalta	White	07.08.2017	
14	KH	Kanon Harabı	White	06.08.2017	
15	KY	Köy Yeri	White	06.08.2017	
16	TG	Tergöynek	White	12.08.2017	
17	KO	Koca Osman	Red	11.08.2017	
18	CU	Çilek Üzümü	Red	10.08.2017	
19	YDM	Yerli Dimrit	Red	04.08.2017	
20	BK	Balçova Karası	Red	07.08.2017	
21	ED	Erkenci Dimrit	Red	04.08.2017	
22	KE	Kara Erik	Red	08.08.2017	
23	DK	Denizli Karası	Red	03.08.2017	
24	KK	Katı Kara	Red	07.08.2017	

Determination of physicochemical properties

The pH, titratable acidity (TA) and soluble solid (SS) analyses were conducted as described by William (2005). The pH was measured using a calibrated pH meter (Sartorius Docu-pH meter, Germany). The TA analysis was performed using the potentiometric titration method, and the results were obtained as tartaric acid equivalent (g/L). A portable refractometer (Hanna HI 96801, USA) was used to measure SS (°Bx).

The absorbance values of the samples were measured using a spectrophotometer (Thermo scientific, Multiskango, Finland) at 420, 520 and 620 nm, and the color intensity (CI) values were calculated using the formula below (1).

$$CI=A420+A520+A620 \qquad (1)$$

The turbidity values of the samples were measured using a portable turbidimeter (Hach 2100Q Portable Turbidimeter, China), and the results were expressed in the nephelometric turbidity unit (NTU)

Sugar compositions of the samples

The sugar profiles of the samples were determined with slight modifications to the method described by Xu et al (2015). First, the samples were diluted with distilled water and passed through a PTFE 0.45 µm syringe filter (Sartorius). Then, they were injected into the HPLC system (Agilent 1260 infinity) for analysis. The detector was selected as the refractive index (RID), and the column was NH_2 250 x 4.6 mm, 5µm (Inertsil). The column temperature was set to 30 °C, and a 20 µL injection volume was used. The flow was isocratic, the flow rate was 1.5 mL min^{-1}, and the elution time was 20 min. Acetonitrile and distilled water (80:20; v:v) were used as the mobile phase. The R^2 values were 0.9996 and 0.9928 and detection limits (LOD) were $6.28x10^{-8}$ and $4.68x10^{-7}$ mg/L for fructose and glucose, respectively. The results were expressed as g in 100 g DW.

Organic acid compositions of the samples

The chromatographic organic acid analyses were performed according to Reuter & Shelton (2015). The samples diluted with distilled water to a certain ratio were filtered (PTFE 0.45 µm syringe filter) and injected into the HPLC instrument (Agilent 1260 infinity). The injection volume was 20 µL, and the flow rate was 1.5 mL/min. The measurements were undertaken using a diode-array detector (DAD; Agilent 1260 infinity) with the following parameters: wavelength 210 nm, column C18 ODS 250 x 4.6 mm, 5 µm (Agilent), and column temperature 30 °C, flow isocratic, and elution time 8 min. An acidified 25 mM KH_2PO_4 buffer (to pH 2.4 with H_3PO_4) was used as the mobile phase. For tartaric and malic acids, the R^2 and LOD values were 0.9998-0.9997 and 0.015-0.037 mg/L, respectively. The results were calculated according to a calibration curve and given as g in 100 g DW.

Statistical analysis

In this study, all analyses were performed in triplicate, and the results were given with standard deviations. The obtained results were subjected to an analysis of variance (ANOVA), and the Duncan multiple comparison test was used to determine the differences between the samples.

Results and Discussion

Physicochemical properties of the samples

The physicochemical properties, CI and turbidity values of the samples are presented in Table 2. The differences between the pH values of the samples were statistically significant ($p \leq 0.05$), ranging from 2.64 to 4.19 in grape juice and SGJ samples. The highest value was found in the KK sample and the lowest in YD. At the same time, the pH values of the SGJ samples were lower than those of the grape juice samples, as expected. The pH of grape juice has an important effect on many parameters, such as conservation, storage, color and characteristics of the product. Soyer et al (2003) reported that the pH of the grape juice products in Turkey varied between 3.3 and 4.0. In other studies, the pH values of the grape juice samples were found between 3.02 and 3.90 (Matos et al., 2017; Margraf et al., 2016; Yamamoto et al., 2015; Lima et al., 2014; Toaldo et al., 2014).

While the SS values of the grape juice samples ranged from 16.15 to 25.45 °Bx, these values were between 5.45 and 7.45 °Bx in the SGJ samples. In different studies, the SS values of grape juice and SGJ samples were reported to vary between 4.50 and 22.6 °Bx (Margraf et al., 2016; Öncül and Karabıyıklı, 2015; Yamamoto et al., 2015; Lima et al., 2014; Hayoglu et al., 2009). The results obtained from the current study in relation to the pH and SS values were similar to those reported in the literature.

TA is very important for the taste balance of grape juice. In grape juice and SGJ samples, the TA values ranged from 3.58 to 8.11 g/L and 25.22 to 30.75 g/L, respectively. These values are consistent with the results of previous studies (Margraf et al., 2016; Öncül and Karabıyıklı, 2015; Yamamoto et al., 2015; Lima et al., 2014; Tolado et al., 2014; Hayoglu et al., 2009; Nikfardjam, 2008; Soyer et al., 2003).

The color of grape juice is another important parameter, especially for the consumers. In the grape juice and SGJ samples produced, the CI values were measured between 0.17 and 6.75. The highest CI value was observed in red grape juice samples. Lima et al (2014) stated that the CI values of the grape juice samples obtained from six new grape varieties ranged from 2.78 to 11.15. In another study, the CI values of the grape juice samples varied between 10.87 and 16.59 (Yamamoto et al., 2015). Moreover, Margraf et al (2016) found that the CI values of grape juice were between 1.02 and 2.17. The CI values of the current study were lower than previously reported. This is considered to be due to the differences in the species and varieties, as well as the processing method.

In the analyzed samples of grape juice, turbidities were determined between 7.09 and 109.50 NTU. The highest turbidity was observed in the ED unclarified grape juice sample. Kaya & Unluturk (2016) revealed that the turbidity values of grape juice varied between 32.5 and 105 NTU, which is in agreement with our turbidity results. In the SGJ samples, the turbidity values were between 1.59 and 4.01 NTU, which were lower compared to the grape juice samples. This may be due to the processing, in addition to the chemical and physiological differences between grapes berry and sour grapes berry. Hayoglu et al (2009) reported that gelatin applications enhanced the clarity of SGJ samples.

Table 2. The physicochemical, CI and turbidity values of the samples

Samples	pH	SS, °Brix	TA, g/L	CI	Turbidity, NTU
M1	3.74±0.01^{k}	20.90±0.01^{g}	5.06±0.06^{fg}	2.58±0.11^{h}	52.90±0.01^{g}
M2	3.69±0.01^{lm}	18.14±0.05^{m}	3.58±0.05^{l}	3.64±0.11^{f}	39.13±0.17^{i}
M3	3.91±0.01^{f}	19.65±0.07^{j}	4.69±0.15^{gh}	4.93±0.05^{d}	57.15±0.13^{e}
MH1	3.65±0.01^{n}	21.00±0.01^{g}	5.68±0.03^{e}	1.81±0.04^{l}	15.10±0.14^{o}
MH2	3.77±0.01^{j}	19.60±0.01^{j}	4.59±0.05^{ghi}	2.33±0.07^{i}	70.36±0.56^{c}
OKG	3.70±0.01^{l}	19.20±0.01^{k}	5.04±0.09^{fg}	5.03±0.23^{c}	10.48±0.10^{p}
SCGJ	3.97±0.01^{d}	21.20±0.01^{f}	4.48±0.03^{hij}	0.77±0.03^{n}	18.35±0.25^{n}
IT	3.82±0.01^{i}	20.55±0.07^{h}	4.42±0.02^{hijk}	0.59±0.01^{p}	23.23±0.05^{m}
CS	2.81±0.02^{p}	7.15±0.07^{q}	25.22±0.72^{b}	0.23±0.01^{s}	3.05±0.34^{rs}
SC	2.77±0.02^{q}	7.45±0.07^{p}	25.37±0.37^{b}	0.17±0.01^{s}	1.59±0.01^{s}
YD	2.64±0.01^{r}	5.45±0.07^{r}	30.75±0.63^{a}	0.67±0.01^{o}	4.01±0.19^{r}
BL	3.75±0.01^{k}	17.75±0.07^{n}	3.99±0.10^{jkl}	0.49±0.02^{q}	26.50±0.20^{l}
EXL	3.85±0.01^{h}	18.45±0.07^{l}	3.94±0.05^{kl}	0.50±0.01^{q}	48.75±0.13^{h}
KH	3.66±0.01^{n}	16.15±0.01^{o}	4.67±0.05^{gh}	0.32±0.02^{r}	18.68±0.29^{n}
KY	3.83±0.01^{i}	18.25±0.07^{m}	3.64±0.02^{l}	0.22±0.01^{s}	7.09±0.02^{q}
TG	3.68±0.01^{m}	17.85±0.07^{n}	4.16±0.04^{jik}	0.36±0.02^{r}	30.85±0.13^{k}
KO	3.88±0.03^{g}	18.45±0.07^{l}	3.99±0.04^{jk}	2.68±0.06^{g}	26.70±0.01^{l}
CU	3.99±0.05^{d}	20.15±0.07^{i}	4.71±0.04^{gh}	1.01±0.01^{m}	24.80±0.08^{m}
YDM	3.90±0.01^{f}	18.45±0.07^{l}	7.08±0.04^{d}	2.06±0.02^{j}	69.35±0.06^{c}
BK	3.93±0.01^{e}	22.21±0.01^{d}	5.22±0.24^{ef}	1.97±0.03^{k}	55.30±0.50^{f}
ED	3.57±0.10^{o}	24.35±0.07^{b}	8.11±0.04^{c}	4.04±0.03^{e}	109.50±4.80^{a}
KE	4.02±0.07^{c}	25.45±0.07^{a}	5.66±0.07^{e}	5.75±0.08^{b}	66.60±1.10^{d}
DK	4.07±0.01^{b}	21.80±0.01^{e}	4.50±0.10^{hij}	6.75±0.07^{a}	78.70±1.93^{b}
KK	4.19±0.01^{a}	23.90±0.01^{c}	4.92±0.06^{fgh}	2.76±0.03^{g}	33.83±1.33^{j}

Values indicated with different letters within each group and column are significantly different for $p \leq 0.05$

Sugar compositions of the samples

The variations in sugar compositions and total sugar amounts of grape juice and SGJ samples are given in Table 3. The differences between the fructose, glucose and total sugar amounts of the samples were found statistically significant ($p \leq 0.05$). The fructose contents ranged from 15.88 to 48.75 g/100 g, with the highest amount being determined in KE and the lowest in YD. The highest glucose value was 48.00 g/100 g obtained from the KE sample and the lowest was 28.45 g/100 g found in CS. The total sugar amounts were found between 52.16 and 96.75 g/100 g. The highest sugar content was observed in KE and the lowest in YD. The HPLC chromatogram of YD sugars profiles was presented on Figure 1. Expectedly, the sugar contents of the SGJ samples were lower than those of the grape juice samples because they were harvested before maturity according to others.

Eyduran et al (2015) found that the fructose and glucose amounts of some grapes grown in the east of Turkey were 8.03 - 13.47 g/100 g and 9.51-16.47 g/100 g, respectively. Canbaş et al (1996) revealed that the amount of invert sugar varied between 159 and 195 g/L in carbonated grape juice samples. Munoz-Robredo et al (2011) reported that in three table grape varieties (*V. vinifera* L.), the amount of fructose was 7.74-8.74 g/100 g, glucose 8.03-8.70 g/100 g, sucrose 0.73-0.90 g/100 g, and total sugar 16.57-17.74 g/100 g at harvest. In grape juice samples produced from *V. labrusca* L. grapes, the amount of fructose was 72.90 -92.90 g/L, glucose 86.61-108.09 g/L, and total sugar 163.31-200.97 g/L (Coelho et al., 2018). In a similar study, the glucose amount was 39.70-72.16 g/L and fructose was 48.12-80.04 g/L in Concord and Bordo (*Vitis Labrusca* L.) grape juice samples (Barros et al., 2014). Additionally, in their study investigating grape juice concentrations, Piva et al (2008) reported 105 g/L glucose and 98.4 g/L fructose in fresh juice.

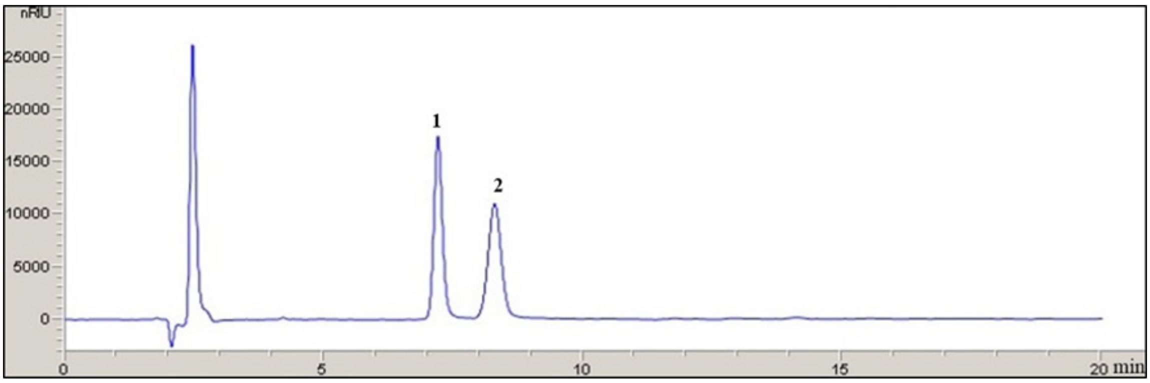

Figure 1. Chromatogram of HPLC sugars profile of YD (1: Fructose; 2: Glucose)

Table 3.Sugar compositions of the samples (g/100 g DW)

Samples	Fructose	Glucose	Total Sugar (Glucose +Fructose)
M1	44.80±0.41^{ghı}	42.68±0.69^{efghı}	87.48±0.95^{efg}
M2	44.83±0.11^{ghı}	43.67±1.38^{cde}	88.05±1.02^{def}
M3	43.86±0.30^{ıj}	41.62±0.10^{hıj}	85.48±0.54^{gh}
MH1	44.97±0.63^{fgh}	41.65±0.35^{ghıj}	86.62±0.85^{fgh}
MH2	45.83±0.13^{def}	42.91±0.44^{defgh}	88.74±0.30^{def}
OKG	45.43±0.90^{efg}	42.09±0.76^{fghıj}	87.52±1.21^{efg}
SCGJ	47.18±0.91^{b}	43.43±0.50^{cdef}	90.61±1.32^{cd}
IT	47.38±0.06^{b}	41.20±1.18^{ıj}	88.58±1.47^{def}
CS	24.54±0.05^{m}	28.45±0.32^{n}	52.99±1.12^{l}
SC	24.56±0.21^{m}	32.26±0.25^{m}	56.82±0.25^{k}
YD	15.88±0.15^{n}	36.28±0.42^{l}	52.16±1.36^{l}
BL	46.07±0.07^{cde}	40.99±0.18^{j}	87.06±0.18^{efgh}
EXL	44.33±0.10^{hıj}	41.63±0.47^{hıj}	85.96±0.61^{gh}
KH	46.80±0.20^{bc}	46.61±1.09^{b}	93.41±1.68^{b}
KY	46.44±0.52^{bcd}	44.60±0.28^{c}	91.04±1.01^{c}
TG	46.52±0.28^{bcd}	42.59±0.35^{efghı}	89.11±0.70^{cde}
KO	44.00±0.35^{hıj}	43.06±0.35^{cdefgh}	87.06±0.17^{efgh}
CU	43.37±0.25^{j}	41.69±0.69^{ghıj}	85.06±0.82^{h}
YDM	40.93±0.17^{l}	37.28±0.45^{k}	78.21±0.72^{j}
BK	41.03±0.39^{l}	40.95±0.65^{j}	81.98±0.70^{ı}
ED	42.43±0.16^{k}	43.22±0.14^{cdefg}	85.65±0.43^{gh}
KE	48.75±0.70^{a}	48.00±0.16^{a}	96.75±0.49^{a}
DK	46.90±0.33^{bc}	44.13±0.18^{cde}	91.03±0.10^{c}
KK	44.06±0.03^{hıj}	44.43±0.01^{cd}	88.49±0.11^{def}

Values indicated with different letters within each group and column are significantly different for $p \leq 0.05$

Ali et al (2010) noted that glucose and fructose were the major grape sugars while sucrose and other sugars were rarely found in *V. vinifera* grapes. The findings of the current study also revealed that fructose and glucose were the major sugars in grape juice. On the other hand, our results were not in agreement to some of the previous studies. The conflicting results concerning sugar compositions can be attributed to the differences in species, variety and maturity of grapes used.

Organic acid compositions of the samples

Table 4 presents the organic acid compositions (tartaric, malic and total acids) of the samples, which statistically significantly differed ($p \leq 0.05$) with respect to tartaric, malic and total acid. The tartaric acid amounts of the samples ranged from 0.53 to 13.16 g/100 g, with the lowest value being determined in ED and the highest in YD. The amounts of malic acid and total acid in grape juice and SGJ samples were 0.45-30.80 g/100 g and 1.21- 43.96 g/100 g, respectively. The highest total amount of acid was found in YD and the lowest in BL. The HPLC chromatogram of YD organic acid profiles was indicated on Figure 2. The organic acid organoleptic properties of grape juice and wine are very important because of their effects on microbiological quality and wine stabilization (Ali et al., 2010). Tartaric acid is the dominant organic acid in grapes and grape products. When the organic acid distribution in grape juice samples was analyzed, tartaric acid was found lower than malic acid in the unclarified red grape juice samples, but this was not observed in the clarified red grape juice samples. These differences might be due to the separation of pulp rich in malic acid during the production of grape juice.

Soyer et al (2003) investigated the organic acid compositions in grape and grape juice of 11 different grape varieties in Turkey and reported tartaric, malic, citric and total acid values as 4.98-7.48 g/L, 1.43-3.40 g/L, 30-164 mg/L and 6.61-10.62 g/L, respectively for grapes, and 4.07-4.92 g/L, 1.36-3.47 g/L, 31-181 mg/L and 6.00-7.83 g L^{-1}, respectively for grape juice. Lima et al (2014) determined that the amounts of tartaric, malic and total acid were 4.60-6.32 g/L, 2.12-4.15 g/L and 8.82-12.04 g/L, respectively in five new Brazilian grape varieties (*V. labrusca* L.). In another study investigating different maceration conditions, tartaric acid ranged from 4.30 to 5.64 g/L, malic acid 3.46 to 3.80 g/L and total acid 9.33 to 10.64 g/L in grape juice samples (Lima et al 2015). Toaldo et al (2015) found the tartaric, malic and total acid amounts as 2.09-3.11 g g/L, 1.29-3.22 g/L and 4.67-8.23 g/L, respectively in grape juice samples produced from organic and conventional grapes (*V. labrusca* L.). In another study on organic acid and sugar methodology in grape juice and wine, tartaric acid was found as 4.02-5.38 g/L, malic acid 1.56-1.92 g/L, and total acid 6.20-7.35 g/L (Coelho et al., 2018). In some commercial table grapes (Red Globe, Thompson Seedless and Crimson Seedless, *V. vinifera* L.), the tartaric, malic and total acid amounts were reported as 7.45-6.55 g/L, 47.78-29.92 g/L and 32.49-36.86 g/L, respectively seven weeks before harvest. In the same study, these values dropped to 1.28-2.05 g/L, 0.39-1.80 g/L and 1.93-3.85 g/L, respectively at harvest (Munoz-Robredo et al., 2011).

The organic acid amounts obtained from this study were generally similar to the previous reports. On the other hand, the tartaric acid amounts in some samples were lower than previously found. This may be due to the grape variety, ecology, harvest time, and grape juice production process. With respect to the differences between the malic acid amounts, species (*vinifera or labrusca*) is another factor that should be take into consideration.

Table 4. Organic acid compositions of the samples (g/100 g DW)

Samples	Tartaric Acid	Malic Acid	Total Acid (tartaric+malic)
M1	1.01±0.01^{i}	1.18±0.10^{g}	2.19±0.10^{f}
M2	1.10±0.01^{h}	0.80±0.01^{jkl}	1.90±0.01^{hij}
M3	1.16±0.01^{f}	1.01±0.07^{ih}	2.17±0.07^{f}
MH1	1.14±0.06^{g}	0.72±0.01^{klmn}	1.86±0.01^{ij}
MH2	1.16±0.10^{f}	0.89±0.17^{j}	2.05±0.17^{gh}
OKG	1.37±0.01^{d}	0.97±0.12^{hi}	2.34±0.11^{e}
SCGJ	0.87±0.12^{e}	0.76±0.01^{jklm}	1.63±0.01^{hi}
IT	0.97±0.04^{j}	0.66±0.01^{mn}	1.63±0.07^{l}
CS	4.98±0.07^{c}	21.36±0.08^{b}	26.34±0.14^{b}
SC	7.52±0.03^{b}	17.16±0.14^{c}	24.68±0.10^{c}
YD	13.16±0.16^{a}	30.80±0.10^{a}	43.96±0.05^{a}
BL	0.76±0.01^{p}	0.45±0.01^{o}	1.21±0.01^{n}
EXL	0.89±0.01^{l}	0.68±0.03^{lmn}	1.57±0.01^{l}
KH	0.93±0.01^{k}	0.89±0.01^{ij}	1.82±0.02^{jk}
KY	0.72±0.01^{q}	0.62±0.01^{n}	1.34±0.11^{m}
TG	0.84±0.01^{m}	1.30±0.01^{fg}	2.13±0.07^{fg}
KO	0.76±0.01^{p}	1.37±0.02^{f}	2.13±0.05^{fg}
CU	0.65±0.01^{s}	1.23±0.01^{g}	1.88±0.02^{hij}
YDM	0.79±0.01^{n}	2.07±0.01^{d}	2.86±0.02^{d}
BK	0.65±0.01^{s}	1.06±0.01^{h}	1.71±0.02^{kl}
ED	0.53±0.01^{u}	1.39±0.01^{f}	1.92±0.02^{hij}
KE	0.63±0.01^{t}	1.57±0.02^{e}	2.21±0.01^{f}
DK	0.77±0.06^{o}	0.89±0.01^{ij}	1.66±0.02^{l}
KK	0.67±0.01^{r}	1.19±0.01^{g}	1.86±0.01^{ij}

Values indicated with different letters within each group and column are significantly different for $p \leq 0.05$

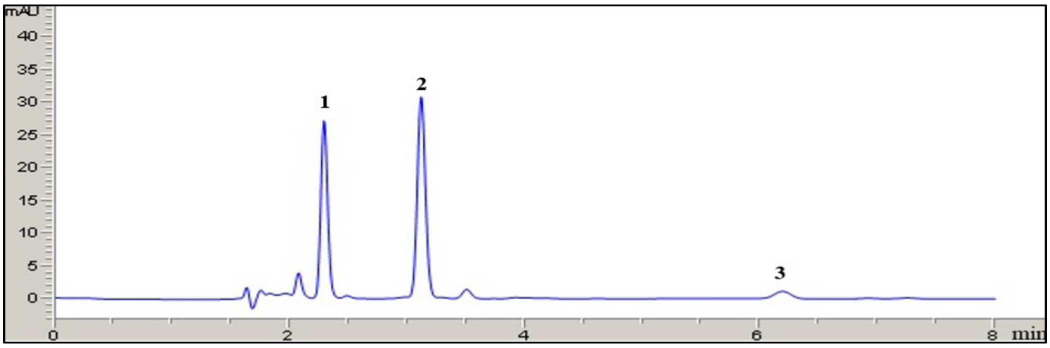

Figure 2. Chromatogram of HPLC organic acids profile of YD (1: Tartaric acid; 2: Malic acid; 3: Citric acid)

The SGJ samples were found to contain much higher amounts of organic acid compare to grape juice samples. These differences resulted from using sour grape samples with a high acid content in the production of SGJ. Besides, in the SGJ samples, the amount of malic acid was higher than tartaric acid due to the higher amounts of malic acid in sour grape samples than tartaric acid. Munoz-Robredo et al (2011) reported significantly higher amounts of malic acid than tartaric acid during the pre-harvest period (seven weeks before harvest) in the Thompson Seedless grape variety. In another study examining the maturation period of different grape varieties, tartaric acid was reported as 10.3-12.3 g/L, malic acid as 9.1-15.1 g/L and total acid as 21.8-30.7 g/L in sour grape samples before veraison (Sabir et al., 2010). Matos et al (2017) investigated the SGJ samples of six grape varieties at three maturation times and found the tartaric and malic acid amounts to range from 5.5 to 10.4 g/L and 10.9 to 30.4 g/L, respectively. In the same study, the total acid amounts were given as 17.4-40.5 g/L. The organic acid compositions of our SGJ samples are consistent with the values determined in previous studies.

Conclusion

In this work, the physicochemical characteristics, sugar and organic acid profiles of SGJ and grape juice samples from Turkey were demonstrated. In particular, the parameters having significant effects on fruit juice quality such as CI and turbidity were determined in detail. The results also revealed the major organic acids were malic acid for SGJ and tartaric acid for grape juice. Additionally, glucose and fructose constituted the majority of total sugar in all investigated samples. The study findings will contribute to the literature and sector regarding Turkish grape juice. On the other hand, more research need to characterize the Turkish grape and grape juice regarding physical, chemical and technological properties.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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The effect of defoliant application on yield and yield components of some cotton (*Gossypium hirsutum* L.) cultivars at timely and late sowing

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Abstract

This study was aimed to assess the effect of defoliant application on yield and yield components of some cotton cultivars at timely and late sowing under Harran Plain conditions in 2017 and 2018 growing seasons. Field trials were arranged employing randomized blocks split-split plots design with 3 replications. In the study, sowing times (i.e.10th of May and 10th of June) consisted the main plots, cultivars (i.e. Candia and Lima) placed in the sub-plots and defoliant applications (i.e. Control and Dropp Ultra (600 ml ha^{-1})) were in the sub-subplots. Each plot was sown with a length of 12 m and 6 rows, with a 70 cm inter-row and 15 cm intra-row spacing. In the trials, the defoliant chemical called Dropp Ultra (i.e.120 g Thidiazuron + 60 g Diuron) was used. The application was practiced when the 60 % of boll opened. It was found that Candia and Lima cotton cultivars sown timely gave seed cotton yields of 5296.7 and 5073.3 kg ha^{-1} respectively, whereas at late sowing gave the seed cotton yields of 4672.5 kg ha^{-1} and 4545.8 kg ha^{-1}in 2017 and 2018**;** Candia gave the higher seed cotton yield (i.e. 5179.2 in 2017 and 5013.3 kg ha^{-1} in 2018) than Lima cultivar (i.e. 4790.0 in 2017 and 4605.8 kg ha^{-1} in 2018) in both years. Results indicated that that the defoliant application increased the seed cotton yield comparing control plots. Defoliant application positively influenced the seed cotton yield (kg ha^{-1}), plant height (cm), number of opened bolls (per $plant^{-1}$), boll weight (g) and boll seed cotton weight (g). However, there were no significant effects on the number of bolls (per $plant^{-1}$) and 100 seed weight (g). It was concluded that defoliant application and timely sowing can be recommended for farmers in the region.

Keywords: Cotton, Defoliant, Yield, Yield components, Timely and late sowing

Introduction

Since cotton has a perennial and indeterminate growth characteristic, it continues to grow vegetative when the environmental conditions are favorable and therefore its maturation is delayed (Stewart et al., 2000; Bondada and Oosterhuis, 2001). Sowing time is a main factor influencing growth and development of cotton as it influences the time of vegetative and reproductive stage of the crop. Moreover, too early and too late sowings resulted in drastic reduction of seed cotton yield (Bange and Milroy, 2010). Cotton plant is very sensitive to temperature fluctuation and cultivated in a wide range of agro-ecological zones. Sowing date is important to explore the potential of cultivars in a region (Ali et al., 2009). Moreover, optimum-sowing time for a cultivar in a region is crucial to be the most significant controllable factor for cotton plant (Bozbek et al., 2006). Cotton cultivars vary for fiber traits (Mohammad, 2001) and may be affected by the environmental condition (Killi and Bolek, 2005). Cotton cultivars exhibited maximum seed cotton yield in early sowing of 15th April as compared to late sowing of 15th June (Siddiqui et al., 2004).

In order to obtain a good and high-quality product, it is extremely important to choose reliable cultivar that will be sown in that region. It is desired that the cultivar to be adopted the region, yield and fiber quality properties are superior. However, sowing time is an important factor in the selection of the cultivars to be sown, as the cultivars with a long vegetation periods

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are sown in late (June), their maturation cannot be completed adequately, it may result in major problems at the harvest and the harvest efficiency could be poor. For this reason, the cultivars to be sown in both optimum and late sowing must be different growing habits.

It is extremely important to harvest cotton timely. Generally, it is desirable the harvest of seed cotton to be clean and the harvest efficiency high. Cotton leaves need to be shed both before hand and machine harvesting. Delay harvest of cotton bear the rain risk, and it may result in quality loss. Besides, non-harvested cotton plant may remain in the field due to the lack of worker and the presence of autumn rains (Mert, 2007). Hence, it was necessary to use defoliant to stimulate the boll aperture before harvesting. Therefore it was possible to increase harvest efficiency, reduce the moisture content of seed cotton, fiber contamination, the negative effect of disease and pest attacks (Oglakci, 1992).

When a defoliant or harvesting chemical is applied to the plant, immature bolls may also be present on the plant. As a general rule, the 4th or 5th node downward from the last maturated boll to be collected is used for the time of defoliant application (Larson et al., 2005; Copur et al., 2010). Optimum dosage for desired outcome of any defoliant depends on defoliant application time rather than which of those chemicals used (Edmisten, 1998). For this reason, optimum defoliant application time should be determined by taking into account the genotype characteristic and the regional conditions (Copur et al., 2010). As a result of leaf shedding in early period, the yield decreased and fiber quality was negatively affected (Snipes and Baskin, 1994). Moreover, in case of leaf shedding in later growing periods, adverse weather conditions were encountered (Kerby et al., 1992) and also due to low temperature conditions, sufficient leaf shedding did not occur. Early or late defoliant applications negatively affected fiber quality (Wright et al., 2014). Early defoliant application was critical for maximum yield. Delaying defoliant applications may increase the risk of yield lose due to rain and early frost in the winter season (Bange and Milory, 2000). In addition, as a result of late defoliant application decreases in ginning outturn, fiber yield and fiber quality were experienced. This might affect textile industry negatively.

Many researches were carried out for the defoliant application and boll openers. Sokat (2008) investigated the effects of various defoliant doses on cotton variety of Stoneville 373 as a second crop. As statistically significant effects of the defoliant application were determined on some fiber quality properties (i.e. fiber strength, short fiber content, fiber reflectance and trash count in fiber). This had no significant effects on boll seed cotton weight, ginning outturn, 100 seed weight, fiber length and fiber maturity. Ataş (2008) applied Dropp Ultra defoliant and used cotton cultivar of Delta Opal at the 2 sowing dates in 5 growing periods (i.e. number of nodes on the cracked boll were 2, 4, 6, 8 and 10) under Diyarbakir conditions. It was found that the seed cotton yields were between 3360-4260 kg ha^{-1}, ginning outturn 39.2-41.00%, and fiber strength 29.5-33.2 g tex^{-1}. Copur et al. (2010) found that the application of dropp ultra 60 days after flowering decreased the seed cotton yield, number of bolls, boll weight and fiber index, and delayed defoliant application increased the number of bolls, seed cotton yield and boll weight. All applications had no effect on the ginning outturn and fiber quality.

Awan et al. (2012) reported that defoliant application resulted in cotton the harvest 25 days earlier than the control, the applications gave high seed cotton yield than control plots. As the application affected significantly fiber fineness and uniformity, had no effect on fiber strength. Tülemen (2016) reported that methods and defoliant doses were not statistically significant for the number of total bolls, number of opened bolls, ratio of opened bolls, boll seed cotton weight, ginning outturn and fiber length. Beyyavaş (2019) stated that Drop Ultra (600 cc ha^{-1}) (5422.7 kg ha^{-1}), Appeal 75 ml ha^{-1} + Efhun 3000 ml ha^{-1} (5382.3 kg ha^{-1}) applications gave the highest seed cotton yield in 2012, Sonround (3000 ml ha^{-1}) (4150.7 kg ha^{-1}) in 2013. The highest earliness ratio was obtained from the application of Drop Ultra (300 cc ha^{-1}) + Efhun (3000 m / ha^{-1}) (96.30% and 96.30 %) in both years.

Harran Plain, where the experiment was established is the most important cotton producing area in Turkey. The most cotton fiber need of the textile sector in Turkey is met from the cotton produced in the GAP region. However, cotton harvest is delayed due to the early autumn rainfall in some years. In GAP region, cotton harvesting is mostly done by combine cotton harvesting machine. In order to increase the efficiency of the combine harvesting and achieving clean seed cotton nowadays, it is compulsory practice to shed the leaves on time and open the bolls. This study was carried out to determine the effects of defoliant application on yield and yield components of some cotton cultivars at timely and late sowing.

Materials and Methods

Field trials were conducted according to randomized blocks split-split plots with 3 replications in Sultantepe village in Harran Plain in 2017 and 2018 growing seasons. In the study, sowing times placed in the main plots, cultivars in sub-plots, and defoliant applications placed in sub-subplots.

Candia and Lima cotton cultivars were used as plant material. In the trial, each plot was arranged with 6 rows of 12 m length, 70 cm inter-row and 15 cm intra-row spacing. Sowing was practiced on May, 10th (timely sowing) and June 10 (late sowing) with a pneumatic drill in both years. Some physical and chemical properties of soil samples taken from the trial sites (0-30 cm) were given in Table 1 and some climate data of Sanliurfa province are given in Table 2.

In the field trial, fertilization was performed to be 160 kg ha^{-1} pure N and 70 kg ha^{-1} P. 70 kg ha^{-1} N and 70 kg P_2O_5 (all of the phosphorus) with 20.20.0 composite fertilizer as a basal, and the remaining 90 kg ha^{-1} of nitrogen as a top (urea 46% N) just before the first irrigation were applied with the lister tool. Defoliant (Dropp Ultra 600 cc ha^{-1}) was applied in both years. The defoliant was mixed with water (300 lt ha^{-1}) and applied with a back pump with the pressure set at 4.22 kg/cm^2. Sprayers were calibrated for 4.80 km h^{-1} walking speed before each application. Only water was sprayed to the control plots (Copur et al., 2010). Defoliant were applied in timely sowing

(May, 10th) on September, 10th in 2017, on September, 12th in 2018; in late sowing time (June 10) on September 25 in 2017 and on September 26 in 2018 when 60% of the bolls opened (Edmisten, 2006).

Harvesting was practiced over the remaining area (10 x1.4 = 14 m^2) by discarding 1 meter from the head and the end of the middle two rows of each plot 15 days after the applications. The harvest was performed by hand on October 27 in 2017, on October 29 in 2018 for timely sowing; on November 9 in 2017, on November 10 in 2018 for late sowing. The evaluation of the data obtained from each parameter was examined by JMP 13.2.0 statistical package program according to the randomized blocks split-split plots and the means were grouped according to the $LSD_{(0.05)}$ test.

Table 1. Some physical and chemical properties of soil (Anonymous, 2018a)

Soil Properties	2017	2018
Structure	Clay	Clay
Clay, %	56.50	59.14
Silt - Loam, %	22.70	22.73
Sandy, %	20.80	19.24
Reaction (pH)	7.76	7.68
Lime ($CaCO_3$), %	24.4	24.7
Total Salt, %	0.062	0.068
Organic Matter, %	1.58	1.47

Table 2. Some meteorological data of Sanliurfa province for 2017 and 2018 (Anonymous, 2018a)

	2017			2018			1929-2018
Months	Montly Avg. Temperature (°C)	Rainfall (kg/m²)	Avg. Relative Humidity (%)	Montly Avg. Temperature (°C)	Rainfall (kg/m²)	Average Relative Humidity (%)	Long Years Avg. Temperature (°C)
April	16.6	79.2	50.2	19.4	38.2	45.4	16.2
May	22.9	7.2	39.0	23.9	112.8	52.6	22.1
June	29.7	0.0	27.0	28.3	6.8	41.4	28.0
July	34.2	0.0	22.9	31.3	0.0	38.7	31.9
August	32.2	0.0	35.7	31.1	0.0	40.9	31.5
September	29.6	0.0	28.8	27.4	0.0	41.6	27.1
October	20.5	17.1	36.9	20.6	28.8	54.3	20.5
November	13.4	17.4	56.0	14.3	30.5	55.5	13.1

Results and Discussion

Seed Cotton Yield (kg ha^{-1})

Candia and Lima cotton cultivars sown timely (May 10) gave the seed cotton yields of 5296.7 and 5073.3 kg ha^{-1} respectively, same cultivars in late sowing (June 10) gave the seed cotton yields of 4672.5 and 4545.8 kg ha^{-1} in 2017 and 2018. It was observed that more yield was obtained from the timely sowing (Table 3). This was due to the fact that genotypes received more vegetation period in timely sowing (Gormus and Yucel 2002; Huang, 2016). The growing season length was important for cotton yield, and selecting the growing season length by the optimal sowing date was of tremendous importance (Huang, 2016). Gormus and Yucel (2002) found that late sowing resulted in the crop late crop flowering and pushed boll development into the cooler weather, resulting in reduced yield. The results obtained in compliance with Kaynak et al. (2003); Killi and Bolek (2005); Ataş (2008); Beyyavaş (2009); Qamar et al. (2016)'s results that indicate the timely sowing was more yielding than late sowing. It has been determined that defoliant application (5074.2 and 4860.8 kg ha^{-1}) increased seed cotton yield compared to control plots (4895.0 and 4758.3 kg ha^{-1}). Awan et al. (2012); Ming-wei et al. (2013); Mrunalini et al. (2018) stated that defoliant application gived more seed cotton yield than control plots were in accordance with our study; Karademir et al. (2007) opposed that by stating that the control plots gave more seed cotton yield than those of defoliant application. This might be due to the differences of the cultivars and trial locations. When the interactions of sowing time*cultivar*defoliant applications were examined, timely sowing (TS)*Candia*defoliant application (DA) interaction (5750.0 kg ha^{-1}) gave the highest seed cotton yield in 2017,

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TS*Candia*DA interaction (5350.0 kg ha^{-1}) and TS*Candia*-Control plot interaction (5283.3 kg ha^{-1}) in 2018.

Plant Height (cm)

It is observed that there was no statistically significant difference between Candia and Lima cotton cultivars in timely sowing (May 10) and late sowing (June 10) in 2017. Same cultivars gave the highest plant height in timely sowing (103.31 cm) than late sowing (97.58 cm) in 2018 (Table 3). Killi and Bolek (2005) stated that late sowing decreased the plant height by 15% compared to timely sowing; Qamar et al. (2016) indicated that early sowing increased the plant height confirming with our findings. Porter et al. (1996) report that plant height increased with delaying of sowing; Beyyavaş (2009) stated that plant height decreased in timely sowing those were contradicting with our results. Ataş (2008) reported that sowing times had no effect on plant height. It was observed that the height of Candia cultivar (95.53 and 91.47 cm) was less than Lima cultivar (113.10 and 109.42 cm). This might be due to the difference of the genotypes of the cultivars used in the trial. Defoliant application was found insignificant in the first year of the trial, the plots defoliant applied (102.04 cm) were higher than that of control plots (98.84 cm) in the second year. Sing et al., (2015) indicated that the defoliant application onto plant height was found to be higher than control confirming our second year results. Interaction of sowing time (ST)*Cultivar*-DA were found to be important and formed different groups. TS*Lima*DA interaction formed the highest plant height in both years.

Table 3. Seed cotton yield (kg ha^{-1}), plant height (cm), number of bolls (per plant^{-1}) related to defoliant application in timely and late sowing, and groups formed according to LSD test.

	Seed Cotton Yield (kg ha^{-1})		Plant Height (cm)		Number of Bolls (per plant^{-1})	
Sowing Time	2017	2018	2017	2018	2017	2018
Timely Sowing (TS)	5296.7 a	5073.3 a	107.29 ns	103.31 a	19.93 a	19.35 a
Late Sowing (LS)	4672.5 b	4545.8 b	101.34	97.58 b	13.52 b	13.90 b
LSD %5	127.3	53.1	7.04	3.55	3.93	0.06
Cultivars						
Candia	5179.2 a	5013.3 a	95.53 b	91.47 b	18.16 a	17.46 a
Lima	4790.0 b	4605.8 b	113.10 a	109.42 a	15.29 b	15.78 b
LSD %5	67.5	83.6	2.86	2.01	2.69	0.80
Defoliant Applications						
Dropp Ultra 600 ccha^{-1}	5074.2 a	4860.8 a	104.68 ns	102.04 a	17.15 ns	16.68 ns
Control	4895.0 b	4758.3 b	103.96	98.84 b	16.30	16.58
LSD %5	95.3	62.7	1.15	1.11	2.35	0.61
Interactions						
TS*Candia*DA	5750.0 a	5350.0 a	98.30 d	94.50 d	19.90 a	19.37 a
TS*Candia*Control	5403.3 b	5283.3 a	95.17 e	91.13 e	20.00 a	19.33 a
TS*Lima*DA	5083.3 c	4866.7 b	119.27 a	116.13 a	19.77 a	19.03 a
TS*Lima*Control	4950.0 cd	4793.3 b	116.43 b	111.47 b	20.03 a	19.67 a
LS*Candia*DA	4846.7 de	4770.0 bc	93.27 e	92.03 e	16.50 ab	15.73 b
LS*Candia*Control	4716.7 ef	4650.0 c	95.40 e	88.20 f	16.23 ab	15.47 b
LS*Lima*DA	4616.7 fg	4456.7 d	107.87 c	105.50 c	9.03 c	12.57 c
LS*Lima*Control	4510.0 g	4306.7 e	108.83 c	104.57 c	12.33 bc	11.83 c
LSD (5%)	190.6	125.4	2.30	2.22	4.71	1.22
CV (%)	2.03	1.38	1.17	1.18	14.97	3.89

* Means in each column followed by the same letter are not significantly different ($p<0.05$) ns: Non-significant TS: Timely Sowing LS: Late Sowing DA: Defoliation Applications ST: Sowing Time

Number of Bolls (per plant^{-1})

Candia and Lima cotton cultivars in the timely sowing (May 10) formed bolls as 19.93 and 19.35 per plant^{-1}, respectively, since the same cultivars were sown in late (June 10), 13.52 and 13.90 per plant^{-1}of bolls were obtained in 2017 and 2018 years. It was observed that timely sowing created more bolls (Table 3). Gür et al. (2001) and Beyyavaş (2009) stated that timely sowing created more bolls than that of late sowing time confirming our results. Cotton had an indeterminate growth habit, which provided more bolls per plant if it was remained longer time in the field/sown earlier (Qamar et al., 2016). It was observed from Table 3 that Candia cultivar (18.16 and 17.46 per plant^{-1}) created more bolls than Lima cultivar (15.29 and 15.78 per plant^{-1}). This might be due to the genotypic differences of the cultivars used as material. It was observed that the defoliant applications on the cultivars were formed in the same group with control plots and were statistically insignificant (Table 3). Copur et al. (2010) and Tülemen (2015) stated that defoliant applications did not affect the number of bolls confirming this study. ST*Cultivar*DA interaction was found important in both years of the experiment and formed different groups. However, when evaluated in general, it can be said that the applications in timely sowing consituted more bolls than late sowing time. This situation can be explained by the fact that plants perform more photosynthesis and form more dry matter in timely sowing.

Boll Weight (g)

Statistically significant differences were found between sowing times in terms of the boll weight. Candia and Lima cotton cultivars sown in timely (May 10) formed boll weights of 6.91 g and 6.59 g respectively, same cultivars being sown in the late (June 10) boll weights of 6.34 g and 6.19 g were obtained, so the heavier bolls weight were obtained from the timely sowing in 2017 and 2018 years (Table 4). It can be said that the prolonged vegetation period contributed positively to the boll weight. Boll weight was an important yield attributes which directly affected the seed cotton yield. Qamar et al. (2016) reported that delays of sowing time gave lower boll weight; Cathey et al. (1988) stated that boll weight decreased as a result of delayed sowing; Killi and Bolek (2005) indicated that the seed cotton weight decreased by 14% in late sowing results were coinciding with our study. As Candia cultivar formed heavier bolls (6.83 g) than Lima cultivar (6.43 g) in 2017, there was no difference between cultivars in 2018. Defoliant application had no significant effect in the first year of the trial, and the plots with defoliant formed heavier bolls (6.44 g) than control plots (6.34 g) in the second year. Awan et al. (2012) stated that defoliant and sulfur dose application plots formed the heavier boll weight than control plots; Gormus et al. (2017) found that the defoliant application was heavier than control parcels in the first year, and this was insignificant. Boll weight results in the second year were compatible with the results obtained from this study. ST*Cultivar*DA interactions were found to be important in both years and formed different groups. However, when evaluated two years together, it can be said that TS*Lima*Control plot interaction gave the heaviest boll weight.

Boll Seed Cotton Weight (g)

No significant differences were found between sowing times and cultivars in terms of the boll seed cotton weight in 2017 and 2018 years (Table 4). Süllü (2001) and Beyyavaş (2009) stated that sowing times had no effect on boll seed cotton weight this was coinciding with this study. Defoliant application made a positive contribution to the boll seed cotton weight (5.08 and 4.95 g) compared to control plots (4.95 and 4.73 g). Awan et al. (2012) stated that the defoliant and sulfur doses increased the boll seed weight compared to control plots. Findings supported the result obtained from this study. Tülemen (2015) stated that defoliant applications had no effect on the boll seed cotton weight contradicts with this study. ST*Cultivar*Defoliation interaction was found significant and formed different groups in both years. TS*Candia*DA and LS*Candia*DA interactions were taken part in the first group in both years. It can be said that the application of defoliant to Candia cultivar increaseed the boll seed cotton weight.

Number of Opened Bolls (per plant^{-1})

Candia and Lima cotton cultivars sown in timely (May 10) formed opened bolls as 16.88 and 16.20 per plant^{-1}, respectively in 2017 and 2018 years, since the same cultivars sown in late (June 10) formed 12.97 and 12.41 per plant^{-1}. It can be observed that timely sowing forms more opened bolls than late sowing (Table 4). The Candia cultivar used in the study created more opened bolls (16.20 and 15.79 per plant^{-1}) than Lima cultivar (13.65 and 12.83 per plant^{-1}). Defoliant applications have created more opened bolls (15.96 and 15.13 per plant^{-1}) than control plots (13.89 and 13.48 per plant^{-1}). Ming-wei et al. (2013) stated that all applications contributed to more opened bolls than control plots; Beyyavas (2019) indicated that defoliant applications formed more opened bolls than control plot in the first year of the study this confirmed our results in this study. ST*Cultivar*DA interaction was found significant in both years of experiment and formed different groups. The application of TS*Candia*DA interaction formed the highest opened bolls (18.43 and 18.13 per plant^{-1}) in both years.

Number of Unopened Bolls (perplant^{-1})

Statistically no significant differences were found in terms of the number of un-opened bolls in both timely sowing (10 May) and late sowing (10 June) in 2017 and 2018 (Table 5). Lima cultivar has created more unopened bolls (2.79 and 2.97 per plant^{-1}) than Candia cultivar (1.97 and 2.33 per plant^{-1}) which was not desired. In cultivation, the goal is to achieve the higher number of opened bolls. Defoliant application (1.63 and 2.02 per plant^{-1}) caused more opened bolls than control plots (3.13 and 3.28 perplant^{-1}). These results revealed that defoliant application caused more opened bolls. Ming-wei et al. (2013) stated that defoliant applications contributed to opening more bolls than control plots; Beyyavas (2019) reported that defoliant applications created more opened bolls than control plots in the first year of this study, which were consistent with our results. Tülemen (2015) found that the number of opened bolls between all defoliant applications and control plots were insignificant and was incompatible with this study. ST*Cultivar*DA interaction was found significant in both years of experiment and formed different groups. The least number of unopened bolls were obtained from the TS*Candia*DA interaction. The fact that the same interaction gave the highest number of opened bolls confirms this result.

Ginning Outturn (%)

Sowing times, cultivars used as material and defoliant applications in the first year of the experiment had no effects on the ginning outturn in 2017 and 2018 (Table 5). Süllü (2001), Gormus and Yucel (2002) and Beyyavaş (2009) stated that sowing times had no effect on ginning outturn; Denizdurduran and Efe (2009), Copur et al. (2010), Ming-wei et al. (2013), Tülemen (2015); Gormus et al. (2017) and Beyyavas (2019) stated that the defoliant application had no effect on ginning outturn which were coinciding with results of 2017. ST*Cultivar*DA interactions were found important and formed differ-

Table 4. Boll weight (g), boll seed cotton weight (g), number opened bolls (per plant^{-1}) related to defoliant application in timely and late sowing, and groups formed according to LSD test

	Boll Weight (g)		Boll Seed Cotton Weight (g)		Number of Opened Bolls (per plant^{-1})	
Sowing Time	2017	2018	2017	2018	2017	2018
Timely Sowing (TS)	6.91 a	6.59 a	5.05 ns	4.87 ns	16.88 a	16.20 a
Late Sowing (LS)	6.34 b	6.19 b	4.98	4.80	12.97 b	12.41 b
LSD %5	0.35	0.32	0.51	0.27	1.71	1.18
Cultivars						
Candia	6.83 a	6.48 ns	5.10 ns	4.85 ns	16.20 a	15.79 a
Lima	6.43 b	6.30	4.93	4.83	13.65 b	12.83 b
LSD %5	0.20	0.21	0.19	0.11	0.45	0.81
Defoliant Applications						
Dropp Ultra 600 cc ha^{-1}	6.71 ns	6.44 a	5.08 a	4.95 a	15.96 a	15.13 a
Control	6.54	6.34 b	4.95 b	4.73 b	13.89 b	13.48 b
LSD %5	0.17	0.07	0.09	0.18	0.68	0.89
Interactions						
TS*Candia*DA	7.10 a	6.63 b	5.27 a	4.93 ab	18.43 a	18.13 a
TS*Candia*Control	6.90 a	6.57 bc	4.90 cd	4.77 ab	16.40 bc	15.73 b
TS*Lima*DA	6.83 ab	6.37 de	5.03 bc	5.10 a	17.67 ab	16.23 b
TS*Lima*Control	6.80 ab	6.80 a	5.00 bc	4.70 b	15.03 cd	14.70 bc
LS*Candia*DA	6.80 ab	6.47 cd	5.13 ab	5.07 a	16.07 c	15.67 b
LS*Candia*Control	6.50 b	6.27 e	5.10 ab	4.63 b	13.90 d	13.63 c
LS*Lima*DA	6.10 c	6.30 e	4.90 cd	4.70 b	11.67 e	10.50 d
LS*Lima*Control	5.97 c	5.73 f	4.80 d	4.80 ab	10.23 f	9.87 d
LSD (5%)	0.35	0.15	0.19	0.36	1.37	1.34
CV (%)	2.81	1.24	1.94	3.91	4.87	6.59

*Means in each column followed by the same letter are not significantly different ($p<0.05$) ns: Non-significant TS: Timely Sowing LS: Late Sowing DA: Defoliation Applications ST: Sowing Time

Table 5.Number of unopened bolls (per plant^{-1}), ginning outturn (%)and 100 seed weight (g)related to defoliant application in timely and late sowing, and groups formed according to LSD test.

	Number of Unopened Bolls (per plant^{-1})		Ginning Outturn (%)		100 Seed Weight (g)	
Sowing Time	2017	2018	2017	2018	2017	2018
Timely Sowing (TS)	2.39 ns	2.64 ns	42.85 ns	41.91 ns	10.04 ns	10.63 ns
Late Sowing (LS)	2.37	2.66	43.17	42.29	9.82	10.33
LSD %5	0.87	0.91	1.93	0.90	0.27	0.32
Cultivars						
Candia	1.97 b	2.33 b	42.70 ns	42.03 ns	9.91 ns	10.48 ns
Lima	2.79 a	2.97 a	43.32	42.17	9.95	10.48
LSD %5	0.69	0.33	0.69	1.21	0.10	0.12
Defoliant Applications						
Dropp Ultra 600 cc ha^{-1}	1.63 b	2.02 b	43.09 ns	42.43 a	9.93 ns	10.45 ns
Control	3.13 a	3.28 a	42.93	41.78 b	9.93	10.50
LSD %5	0.53	0.29	0.64	0.50	0.17	0.12
Interactions						
TS*Candia*DA	0.43 c	0.77 e	42.87 b	42.77 ab	10.13 ab	10.43 cde
TS*Candia*Control	3.47 a	3.50 a	42.93 b	41.60 c	9.90 abc	10.63 bc
TS*Lima*DA	2.50 ab	2.90 bc	42.83 b	41.83 bc	9.97 abc	10.93 a
TS*Lima*Control	3.17 ab	3.40 ab	42.77 b	41.43 c	10.17 a	10.50 bcd
LS*Candia*DA	1.20 c	1.63 d	42.33 b	41.77 bc	9.77 c	10.17 f
LS*Candia*Control	2.77 ab	3.43 ab	42.67 b	42.00 bc	9.83 abc	10.70 ab
LS*Lima*DA	2.37 b	2.77 c	44.33 a	43.33 a	9.87 abc	10.27 def
LS*Lima*Control	3.13 ab	2.80 c	43.33 ab	42.07 bc	9.80 bc	10.20 ef
LSD (5%)	1.05	0.57	1.28	1.01	0.34	0.24
CV (%)	23.46	11.60	1.58	1.27	1.82	1.22

*Means in each column followed by the same letter are not significantly different ($p<0.05$) ns: Non-significant TS: Timely Sowing LS: Late Sowing DA: Defoliation Application ST: Sowing Time ent groups in both years. The highest ginning outturn was obtained from the LS*Lima*DA interaction (44.33 and 43.33%).

100 Seed Weight (g)

Statistically no significant differences were found on 100 seed weight in terms of the sowing times, cultivars and defoliant applications in the 2017 and 2018 (Table 5). Seed index (100 seed weight) was a major yield-contributing component that was affected by soil nutrients status, irrigation availability and the rapid environmental changes (Qamar, 2016). Abd-El Gawad et al. (1986) and Beyyavaş (2009) indicated that sowing times had no effect on 100 seed weight; Karademir et al. (2007) and Sokat (2008) stated that defoliant application had no effect on 100 seed weight support the results obtained from this study. Statistically significant differences were found between ST*Cultivar*DA interactions in both years, but this difference varied over the years.

Conclusion

Candia and Lima cotton cultivars sown timely (May 10) gave 5296.7 and 5073.3 kg ha^{-1} of seed cotton yield respectively when 4672.5 kg ha^{-1} and 4545.8 kg ha^{-1} of seed cotton yield were obtained from the same cultivars sown in late (June 10). High yields were obtained from late sowing time in 2017 and 2018.Candia cultivar (5179.2 and 5013.3 kg ha^{-1}) gave the higher seed cotton yield than Lima cultivar (4790.0 and 4605.8 kgha^{-1}) in both years. It was thought that this difference between cultivars caused from the genotypic structure of cultivars. While the defoliant application affected positively the properties of examined traits such as seed cotton yield, plant height, number of opened bolls, number of unopened bolls, boll weight and boll seed cotton weight, not effected the number of bolls and 100 seed weight. In addition, it was determined that the seed cotton yield decreased with the delaying of sowing time and negatively affected by early autumn rains. Candia cultivar performed better in defoliant application. According to the results of this study it can be concluded that defoliant application and timely sowing provided higher yield.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Mercury intake via consumption of imported Atlantic mackerel (*Scomber scombrus*) in Istanbul

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Abstract

The aims of this study were to determine the concentrations of mercury (Hg) in frozen imported Atlantic mackerel consumed in Istanbul and to predict their potential health consequences. In this study, the concentration of Hg was determined following US EPA Method 7473 (2007) using a direct mercury analyzer (DMA-1). Mercury level of the Atlantic mackerel ranged between 0.045 to 0.065 mg/kg. The mercury levels were well below the limit value of 1.00 mg/kg wet weight (EC, 2006; Turkish Food Codex, 2011) for fish such as mackerel. The potential human health risks of Atlantic mackerel sold in Istanbul were also assessed in terms of Hg levels. The estimated weekly intakes (EWI) of the mercury were lower than established provisional tolerable weekly intakes (PTWI). Target hazard quotient (THQ) values were below 1, indicating that Atlantic mackerel consumption is not a potential health risk in adults and children. According to the amount of Hg, this fish can be consumed safely 3 times a week. Furthermore, it is determined that consumption of fish from the IV. Region 4 times a week will not be a problem because of the low amount of Hg. Our results provide a good tool to determine the Hg exposure of Turkish consumers (adult and children) via Atlantic mackerel consumption in terms of food monitoring and food safety.

Keywords: Mercury, *Scomber scombrus*, Health risk, PTWI, THQ

Introduction

Fish is an important source of proteins, aminoacids, fatty acids, vitamins and minerals, which are necessary elements for human diet (FAO, 2020). In addition, Omega-3 fatty acids in fish have been reported to reduce the incidence of heart disease and stroke (Ababneh, 2013). In addition to the good benefits of fish, fish may contain some toxic contaminants, which are of significant concern because of their potential adverse effects on human health (Visciano et al., 2014). The heavy metals released into the environment have created an environmental problem in the world. Toxic metals are important water pollutants due to their toxicity, long-term environmental stability and bioaccumulation properties (Guerin et al., 2011). Mercury is classified as a toxic heavy metal and its presence in food is limited by law, considering human health (Visciano et al., 2014). The environmental risk of Hg is very high since it can be volatile and transported over long distances in the atmosphere (Ordiano-Flores et al., 2012). Once Hg entered the marine environment as a result of environmental pollution such as transportation, agriculture, industry and urbanization, it can accumulate in trophic level (Gorur et al., 2012). Its organic compounds are the most toxic forms, particularly methylmercury (Ikem and Egiebo, 2005). Organic mercury is absorbed more easily by fish, so that methyl mercury enters the food chain through fish consumption (Agusa et al., 2005; Kibria, 2016). Fish has good health effects due to PUFAs, but methyl mercury may inhibit their efficiency. It causes significant behavioral disorders in children, damaging the developing fetus and young children (Gualllar et al., 2002; JECFA, 2007). Fish is the main way of human exposure to Hg. Therefore, the

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maximum mercury concentration that can be found in fish in terms of human health is limited by law. It was generally concluded that if the maximum Hg level in fish muscle exceed the permitted limit value, consuming contaminated fish may lead to adverse health effects on human. However, as much as the amount of Hg in fish, the amount of fish consumed by human is also important in terms of potential health risk. The risk assessment based on the target hazard coefficient (THQ) indicates the potential health risks from dietary metal intake (Burger, 2009; Kral et al., 2017).

Atlantic mackerel is a great source of omega-3 polyunsaturated fatty acids (PUFAs), which makes it an excellent food for nutrition (Romotowska et al., 2016). Frozen imported Atlantic mackerel (*Scomber scombrus*) is an important food fish in the diet of Turkish consumers. In recent years, consumption of imported mackerel from Norway has increased considerably in Turkey. According to the Norwegian Seafood Council, Turkey is the sixth country that imports most frozen mackerel from Norway in 2016 (Statista, 2018). Imported mackerel can be consumed over the year, independently from the closed fishing season implemented in Turkey. Besides, its desirable taste appreciated by Turkish people, its price also is affordable. So, consumption of this fish has increased in this country. Unfortunately, there is limited information on mercury level in imported frozen Atlantic mackerel sold in our country. According to the table of mercury levels in commercial fish and shellfish (1990-2012), prepared by FDA (2017), Northern Atlantic mackerel contains low levels of Hg. However, this list was prepared according to the data of NMFS REPORT 1978. Since it is known that mercury levels in fish can vary significantly over the years due to pollution and environmental factors; monitoring the current status of commercial Atlantic mackerel in terms of mercury content and potential risks is important for international trade and human health.

Atlantic mackerel is a pelagic carnivorous fish (Luna, 2019). Mercury distribution in the fish depends on the age, maturation status and habitat of the fish. It is stated that the main way of mercury intake of fish is the diet, and differences in the feeding ecology of such fish affects the accumulation of Hg (Bae et al., 2011; Barone et al., 2015). Daily industrial, agricultural and domestic human activities contribute to accumulation of mercury in the ecosystem (Abubabakar et al., 2015), and there may be potential risks due to heavy metal uptake changes. Although there are few studies on Hg concentrations in Atlantic mackerel, no study focused to the Estimated Weekly Intakes (EWI), Provisional Tolerable Weekly Intakes (PTWI), Hazard Index (HI) and the Target Hazard Quotient (THQ). Thus, it is very important to determine of potential risks related to Hg concentrations in Atlantic mackerel which constitutes a large percentage of imported fish consumed in the world.

In this study, Hg levels in the edible tissues of frozen Atlantic mackerel (*Scomber scombrus*) sold in the local fish markets in Istanbul were determined and dietary intakes of Hg were assessed. The health risk of Atlantic mackerel was evaluated by calculating EWI, PTWI, HI and THQ values according to Turkish consumers. The aims of this work were to determine the concentrations of Hg in frozen imported Atlantic mackerel consumed in Istanbul and to predict their potential health consequences.

Materials and Methods

Samples

Atlantic mackerel samples were obtained from local fish markets located in five main regions (Region I= Eminonu, Region II=Uskudar, Region III= Kadıkoy, Region IV= Karakoy and Region V=Beyoglu) of Istanbul (Fig 1). Sampling (n=15) was carried out in these markets during spring season, 2018. According to the label information of the batches, Atlantic mackerels were caught from the Atlantic, Northeast (FAO 27). These samples were produced in Norway and the production time of them varied between 20.10.2018-05.09.2018.

Figure 1. The locations of sampled local fish markets.

Norwegian mackerels can be caught from different catching areas in different seasons; stored as frozen, then can be exported. They can also be marketed in Istanbul at different times. This is the reason of our sampling from various regions of Istanbul. Istanbul, with a population of 15.067 724, represents a significant part of the Turkish population, since it is an immigration megapol of Turkey (Istanbul Governorship, 2018). Density of the population and the performance of trade are the reasons for choosing this city as the study area. The average lengths of samples were 32 ±1.32; 31.43 ±0.12; 31.50 ±0.50; 31.00 ±1.00 and 31.67 ±1.53 while the average weights were 422.59 ±77.41; 408.68 ±10.64; 412.63 ±4.65; 385.17 ±31.00 and 381.67 ±24.01 for Region I, II, III, IV and V, respectively. Each individual of fish was analyzed for Hg concentrations.

Mercury analysis

The concentration of Hg was determined following US EPA Method 7473 (2007) using a direct mercury analyzer (DMA-1; Milestone SRL, Sarisole BG, Italy). The method is based on the thermal decomposition of sample, mercury amalgamation and atomic absorption detection. Each fish sample was weighed (0.1 g) into a quartz tube, then, the tubes were placed into the auto injector. The samples were heated with oxygen stream passing over them at 650 °C. The mercury vapor was mounted on a gold coupling trap and then quantitatively decoded. The Hg content was determined using atomic absorption spectrometry at 254 nm, and the results were calculated using DMA-1 PC software. The operating times in the study were 1, 2 and 1 min, respectively for drying, combustion, and post-combustion flushing periods. Total analysis time per sample was less than 10 minutes. The DMA-1 was calibrated using the certified reference material (fish muscle (Catalogue No. ERM-BB422, the Joint Research Centre (JRC), Italy). The results were determined in mg/kg wet weight. All analyses were in triplicate, the mean values and standard deviations were calculated.

Health risk assessment

The estimated weekly intake (EWI) value in µg/kg body weight was determined by multiplying the mean concentration of Hg (µg/g) with the amount of weekly consumed fish, and divided by the average value of children and adult body weights (14.5 and 70 kg) (Hajeb et al., 2009; US EPA, 2000).

EWI= [WFC X C] / BW

In Turkey, weekly fish consumption (WFC) amounts for adults and children are 106 g and 50 g, respectively (Fisheries Statistics, 2018). C is the mean concentration of Hg (µg/g) and BW is the average body weight. The provisional tolerable weekly intake (PTWI) values were multiplied by the average children and adult body weights. Then, the percent PTWI was calculated (EFSA, 2012).

PTWI=PTWI (supplied for each metal) x BW

Target hazard quotient (THQ) values indicate health risks to humans via dietary intake of fish because of heavy metal exposure. The THQ, expressing the risk of noncarcinogenic effects, is the ratio between exposure and the oral reference dose (RfD). If the ratio is less than 1, therefore, it seems to be carry no obvious health risk. Conversely, if the dose is equal to or greater than 1, the RfD (Yi et al., 2011), an exposed population will experience health risks (Pazi et al., 2017). In this study, health risks through Atlantic mackerel consumption in Turkey were examined based on THQ values. The dose calculations in the method of determining the THQ value were provided in the US EPA Regional Screening Levels Generic Tables (US EPA, 2018) and THQ values were calculated according to the following formula (Ihedioha and Okoye, 2013):

$(E_{Fr} \times E_D \times F_{IR} \times C) / (R_{FD} \times W_{AB} \times T_A)] \times 10^{-3}$,

where EFr is the exposure frequency (350 days/year); ED is exposure duration, total (70 years for adults; 6 years for children); F_{IR} is the food ingestion rate (15.07 and 7.14 g/person/day for adult and children Turkish consumers, respectively; C is metal concentration (µg/g); R_{FD} is the oral reference dose (mg/kg/day); W_{AB} is the average body weight (70 kg for adults, 14.5 kg for children), T_A is averaging time for non-carcinogens (365 days/year x ED).

An allowable fish consumption (CR_{lim}) rate for a noncarcinogen can be calculated with the following formula and is expressed in kilograms of fish per day (kg/d) (US EPA, 2000):

$CR_{lim} = R_{FD} \times BW / C_m$

where R_{FD} is for methylmercury 1.0×10^{-4} mg/kg/day (ATSDR, 2009); BW (consumer body weight) is 70 and 14.5 kg for adults and children, respectively; C_m is measured concentration of chemical contaminant in a given species of fish (mg/kg).

Statistical analysis

Statistical analysis was performed with SPSS 21.0 (SPSS Inc. Chicago, IL). Data were analyzed by one-way ANOVA, and Tukey test were applied for multiple comparison. Statistical significance was expressed at $p < 0.05$.

Results and Discussion

Observed and certified values (mg/kg) were shown in Table 1. The mean concentrations of Hg were 0.056 ±0.01, 0.063 ±0.02, 0.057 ±0.00, 0.045 ±0.01 and 0.059 ±0.01 mg/kg for Region I, II, III, IV, V, respectively (Fig 2). EWI and PTWI values for adults and children were presented in Table 2 while THQ and CR_{lim} values for adults and children were given in Table 3.

Table 1. Observed and certified values (mg/kg) of mercury concentrations in standard reference material (dry weight) (n=3)

	Certified value	Uncertainty	Observed value	Recovery (%)
Hg	0.601	0.030	0.645 ±0.040	107.32

In our study, there were no significant differences ($p > 0.05$) between Hg concentrations of fish, obtained from different regions of Istanbul. Alcala-Orozco et al. (2018) also showed similar finding to our study. In general, the permitted limit for Hg in fish is 0.50 mg/kg, but this limit is 1.00 mg/kg for the species such as mackerel (EC, 2006; Turkish Food Codex, 2011). The highest mean concentration of Hg was found as 0.063 mg/kg for Region II (Figure 2), and the mercury concentrations of all samples were well below the limit value of 1.00 mg/kg wet weight. Tuzen (2009) reported Hg concentration in Atlantic mackerel (*Scomber scombrus*) as 0.06 mg/kg. Likewise, Visciano et al. (2014) reported the concentrations of Hg for Atlantic mackerel samples below 1 mg/kg. Łuczyńska and Krupowski (2001) stated that the contents of Hg in *Scomber scombrus* bought from supermarkets of Olsztyn, Poland ranged between 0.039–0.068 mg/kg (mean 0.052 mg/kg). In addition, Kral et al. (2017) and Mol (2011) found that average concentrations of total mercury (mg/kg) lower than the legal limit (1.00 mg/kg) for canned mackerel. The value of Hg in frozen Atlantic mackerel purchased from the Jagalchi fish market of Korea was found to be 0.08 mg/kg (Bae et al., 2011). Likewise, total Hg content in mackerel from Malaysia (Hajeb et al. 2010), Italy (Plessi et al., 2001; Storelli et al., 1998), and Croatia (Juresa and Blanusa, 2003) reported below 1 mg/kg. These results are similar to our findings. However, Abubakar ve et al. (2015) reported Hg concentrations in frozen *Scomber scombrus* above the permitted limits and suggested periodical monitoring regarding human health risks.

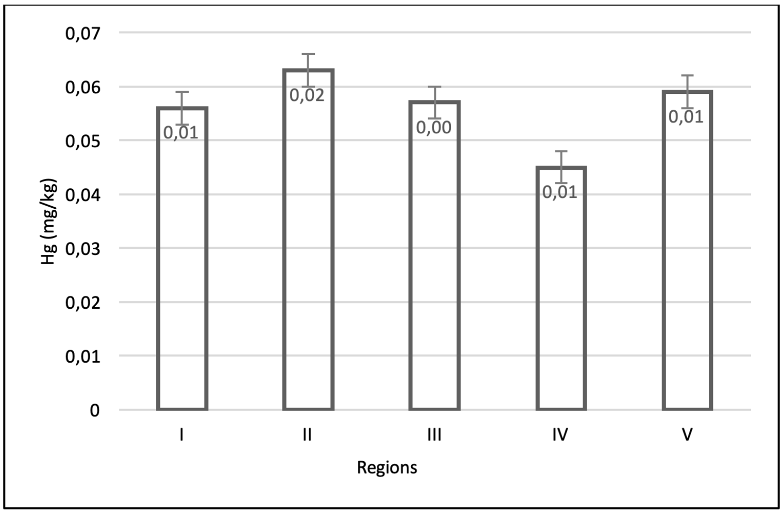

Figure 2. Mean concentrations of Hg (mg/kg wet weight) in Atlantic mackerel samples of different regions.

Table 2. Estimated weekly intakes (EWI) and percent PTWI's for Atlantic mackerel, consumed by children and adults

	PTWI (µg Hg /week/kg body weight)	PTWI* (%) Adult	PTWI** (%) Child	Adult		Children	
Regions				EWI	PTWI (%)	EWI	PTWI (%)
	1.6[a]	112	23.2				
I				0.08	5.29	0.19	12.05
II				0.10	5.99	0.22	13.64
III				0.09	5.41	0.20	12.33
IV				0.07	4.26	0.16	9.71
V				0.09	5.59	0.20	12.72

*PTWI for a 70 kg adult (µg/week body weight)

** PTWI for a 14.5 kg child (µg/week body weight)

a EFSA, 2012

The PTWI values for adults and children were calculated and compared with EWI values (Table 3).

The estimated weekly intake (EWI) values of Hg in our study were ranged between 0.07- 0.10 µg/kg bw/week for adults, and 0.16- 0.22 µg/kg bw/week for the children. Even though EWI of young's were higher than that of adults; it was still very far from indicating a potential risk, due to consumption of Atlantic mackerel. The established PTWI values (µg/week/kg) were proposed as 1.6 for Hg (EFSA, 2012). In this study, the average adult and child body weight were considered as 70 and 14.5 kg, respectively. The results indicated that the EWIs of total mercury were below the respective PTWI (Provisional tolerable weekly_intakes) (µg/kg/week) recommended by EFSA (2012). A potential risk has been notified when the percent PTWI is above 100 % (Mol et al., 2019). So, average Turkish adult and young population, consuming frozen Atlantic mackerel, does not have a health risk in terms of mercury. Likewise, Llull et al. (2017) reported the estimated weekly intakes (EWI) in children (7–12 years of age, 34.48 µg/kg body weight) and adults (>17 years of age, 68.48 µg/kg body weight) below the provisional tolerable weekly intake (PTWI) of Hg established by EFSA in 32 fish species from the Balearic Islands._Similarly, the EWI of total mercury in grey mullet from The Caspian Sea was below the respective PTWI for an adult (70 kg) (Hosseini et al., 2013). However, the absence of risk for the average consumer does not mean that there is no risk for the heavy consumers (Guerin et al., 2011). Olmedo et al. (2013) reported that there is no risk of heavy metal in some shark species. However, they emphasized the possibility of health risk in over-consumption of these species. But at the same time they highlighted the possibility of health risk for heavy consumers on account of excessive shark consumption. Akhbarizadeh et al. (2018) found that Hg weekly intakes for adults below the reference values in fish species caught from the northeast Persian Gulf. But, they stated that children under 16 kg should have less than 50 g of meal size. In addition, Hajeb et al. (2009) found that the EWI values were below the respective PTWI for in mackerel from fish markets in Malaysian, and also underlined that the large consumption pattern for mackerel may increase health risks in terms of especially pregnant women and children._

Table 3. Estimated Target Hazard Quotients (THQs) and cancer risk (CR_{lim}) for Hg caused by consuming Atlantic mackerel for children and adults.

CR_{lim} (g/day) / CR_{lim} (meals per week)		THQ	
Children	Adults	Children	Adults
26 / 3	125 / 3	0.34	0.12
23 / 3	111 / 3	0.39	0.14
25 /3	122 / 3	0.35	0.12
32 /4	155 / 4	0.28	0.10
25 /3	119 / 3	0.36	0.13

The THQ was used to express the potential health risks of adult and young Turkish people, consuming Atlantic mackerel. If the ratio is less than 1, potential risk is not concerned. This level of exposure is thought to be insignificant enough to have no negative effect during a person's lifetime (Yi et al., 2011). In the present study, the THQs values for Hg via Atlantic mackerel consumption were determined well below 1 for adults and children (Table 3). It shows that exposure to Hg via imported Atlantic mackerel consumption do not pose a significant health risk for children and adults. Yi et al. (2011) studied the health risks of heavy metals to the general population and fisherman in Yangtze River, China. Likewise, they reported THQ values for all species below 1, but emphasized health risks associated with fish consumption should be controlled regularly.

CR_{lim} is the maximum allowable consumption rate without adverse health effects. Therefore, to determine the allowable fish consumption (daily or weekly) is very important for human health (Hosseini et al., 2013). In this study, the allowable fish consumption rates were determined according to adults and children for all regions (Table 3). The standard portion amount of raw fish consumed by an average adult and child are 227 g and 48 g, respectively according to US EPA (2000). The average body weights of Turkish adults and children assumed to be 70 and 14.5 kg, respectively. According to the amount of Hg, this fish can be consumed safely 3 times a week. Furthermore, it is determined that consumption of fish from the IV. Region 4 times a week will not be a problem because of the low amount of Hg. According to weight of adult and children, the consumption rates and the number of meals can be proportionally higher or lower, respectively. Hosseini et al. (2013) calculated CR_{lim} and the permissible amount of grey mullet from the Caspian Sea in terms of mercury intake, using the same method. They reported allowable consumption rate as 51 g, and concluded that is not a serious threat for Iranian consumers. Asare-Donkor and Adimado (2016) evaluated the concentrations of the total mercury in fish from the Ankobra and Tano River basins in South Western Ghana, and estimated the THQs, allowable consumption rate and EDI values with regard to human health. They emphasized that these values should be carefully monitored and controlled to reduce the potential health risks of Hg levels. Alcala-Orozco et al. (2017) studied Hg concentrations in canned tuna sold in Colombia and estimated maximum allowable tuna consumption rate in meals/week (CR_{mw}) regarding human exposure. They resulted that the consumption of canned tuna may pose a high risk to the people of Colombia. Moreover, Olivero-Verbel et al. (2016) assessed the levels of Hg in fish in the Caqueta River, at the Colombian Amazon, as well as to determine fish consumption-based risks (CR_{mw}, THQ), but THQS values were so high than our results, and CR_{mw} indicated that Hg concentrations are limited to two meals a week, recommending these fish species may be risky for consumer health revealed. Pal and Maiti (2017) evaluated health risks of the heavy metal pollution in cultured fish from Urban Aquaculture Pond, India in case of children and adults, and THQs values were higher than our results.

Conclusions

The frozen Atlantic mackerel is one of the most commercially valuable fish species in European countries. Also, it is considerably important food source, commonly consumed all over the world. In the present study, it was determined that estimated weekly intakes (EWI) of mercury via consumption of Atlantic mackerel were far below the established provisional tolerable weekly intake (PTWI) values recommended by EU (2006) in case of adult and children. The THQ values were below 1, indicated no potential health risk in adult and children with consumption of Atlantic mackerel. Considering the Hg concentrations in Atlantic mackerel the allowable fish consumption rate for adults and children is recommended as 3 meals per week. More than this, 4 meals of fish per week may be allowable for this species having low Hg content, obtained from some regions. Therefore, it is concluded that the consumption of Atlantic mackerel, which sold in Istanbul local fish markets, posed no health risk to the consumer on the basis of adult and children in our society. Our results provide a good tool to determine the Hg exposure of adult and children via Atlantic mackerel consumption in terms of food monitoring and food safety. At the same time, these results showed useful data for the unfounded news about the mercury content of frozen Atlantic mackerel in Turkey.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Research Article

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Comparison of fluoride contents in terms of teeth health and water quality in drinking water at the northern and southern regions of Meriç River Basin (Edirne/Turkey)

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Abstract

This research was carried out to investigate and compare the fluoride accumulations and some physical and chemical properties (dissolved oxygen, oxygen saturation, pH, oxidation – reduction potential, electrical conductivity, total dissolved solid, salinity, nitrate nitrogen) in the drinking water of Enez and Süloğlu Districts, which are located in the northern and southern parts of the watershed of Meriç River. Water samples used for drinking were taken from tap waters in a total of 22 residential areas in the Enez and Süloğlu Districts at the winter season of 2019. Fluoride levels of water samples were determined by using a spectrophotometer and Principle Component Analysis (PCA) was applied to the results. The detected fluoride amounts and physical/chemical data were also evaluated in terms of teeth health of humans. According to the results obtained, although the detected fluoride accumulations both in the northern and southern part of the basin are slightly below the optimum levels for teeth health, it has been found that fluoride concentrations did not exceed the permitted values for drinking water. The minimum and maximum fluoride levels recorded as min. 0.159 ppm (Süloğlu District) – max. 0.475 ppm (Küküler Village) in the northern part of the basin and recorded as min. 0.068 ppm (Hasköy Village) – max. 0.603 ppm (Karaincirli Village) in the southern part of the basin. As a result of PCA, 2 factors named as “Agricultural Factor” and “Fluoride Factor” explained 79% of the total variance. It was also determined that contamination rates in terms of physicochemical variables of investigated regions were found as South Region of the Basin > North Region of the Basin in general.

Keywords: Drinking water quality, Enez and Süloğlu Districts, Fluoride, Teeth health

Introduction

Fluorine is an essential element that is easily soluble in water, soil, and air. It is one of the most reactive chemical element which do not exist on its own in the natural environment but rather in the form of fluoride. This element may enter the body through food, water, industrial exposure, drugs and drinking water which is the main source of fluoride intake (75–90% of daily intake) (Güner et al., 2016). Surface waters have fluoride levels less than 0,5 mg/l, while groundwater can contain higher concentrations of fluoride depending on geological conditions (Day and Giri, 2016). There are many countries around the world where fluoride naturally occurs in groundwater. India and China are endemic areas where fluorosis is severe and widespread. Turkey is also in this geographical fluoride belt and there are endemic regions with different fluoride concentrations in drinking water. Fluoride ion is highly electronegative and it can easily be attracted by positively charged calcium ions in teeth and bones. Major health concerns by fluoride include dental and skeletal fluorosis as well as other non-skeletal manifestations (Andezhath et al., 1993). Excess fluoride intake

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through drinking water for a prolonged period may result in dental fluorosis. Children are more susceptible to dental fluorosis during tooth development and drinking water with excess fluoride levels may result in different degrees of tooth mottling since pathological changes in dental fluorosis are time and dose-dependent (Tokatlı and Güner, 2018; Onur et al., 2019).

Another important element is nitrogen compounds in drinking water resources. Nitrate is a compound of nitrogen that is found naturally in moderate concentrations in many environments. The nitrate amount in groundwaters and surface waters is mostly in low concentration but nitrate levels may reach higher concentrations due to agricultural runoff or contamination with human or animal wastes (Rodvang and Simpkins, 2001; Tokatlı et al., 2013; Ustaoğlu et al., 2017; Tokatlı, 2018). Along with fluoride, nitrate may pose a major environmental health hazard if the optimal levels are exceeded. Nitrates in groundwater represent a widely distributed pollution concern. The key concern regarding the usage of groundwater with excessive concentrations of nitrates is related to human health effects, particularly concerning infants. The major effects are associated with losses in oxygen transport/transfer capabilities in the blood. The toxicity of nitrate to humans is due to the body's reduction of nitrate to nitrite. This reaction takes place in the saliva of humans of all ages and the gastrointestinal tract of infants during their first 3 months of life (WHO, 2011a; Tokatlı, 2014).

Considering the adverse effects of these chemicals, World Health Organization (WHO, 2011b) has set some guidelines for the permissible concentrations of these elements in waters used for drinking (11.3 ppm for nitrate and 1.5 ppm fluoride).

Enez District, which is the south studied area in this study, is located on the south west corner of Edirne City. The eastern half of the district is on the Pelin Plateau extending north of the Saroz Gulf in the Aegean Sea. The main livelihood of the people of Enez is agriculture and animal husbandry. Fishing is also carried out in our central and coastal villages (Anonymous, 2016). Süloğlu, which is the north studied area in this study is located on the northern half of Edirne City and on the Lalapaşa plateau. The economy of the district is predominantly agricultural. The drinking water of the villages in the investigated regions is being provided by the drilling and spring waters, in general (Anonymous, 2016).

This study aimed to determine the drinking water qualities of north and south region in the Meriç River Basin and compare the fluoride accumulations in the water used as drinking water resources of these districts in terms of teeth health of humans.

Material and Method

Investigated region and sample collection

In this study, sampling was performed in winter season of 2019 from a total of 22 stations from the settlement areas located in the Enez (south part of the study area) and Süloğlu Districts (north part of the study area). The samples were taken from the tap water used in the houses of the villages and the obtained water materials put into polyethylene bottles. The sampling localities of villages are presented in Table 1 and map of the watershed of Meriç River with the investigated areas are presented in Figure 1.

Physical, chemical and statistical analysis

Measurements of dissolved oxygen, oxygen saturation, pH, oxidation – reduction potential (ORP), electrical conductivity (EC), total dissolved solid (TDS) and salinity were measured by using Hach branded (HQ40D) Portable Multi – Parameter Measurement Device and turbidity parameter was measured by using Hach branded (2100Q) Portable Turbidimeter Device during the field studies. The water samples were transported to the laboratory to determine the other chemical features. Nitrate nitrogen (NO3-N) values were determined by using Hach branded (DR890) Colorimeter Device; Fluoride rations were determined by using classical spectrophotometric method and "Hach Lange DR 3900 Spectrophotometer" device (wavelength range 320 – 1100 nm). Cuvette Test LCK 323 was used in spectral photometer. This method provides fluoride ions react with zirconium to form a colourless zirconium fluoride complex.

Table 1. Location properties of villages

Enez District			Süloğlu District		
Location	Coordinate		Location	Coordinate	
	North	East		North	East
Yenice	40.700	26.149	Domurcalı	41.816	26.819
Çavuşköy	40.688	26.171	Sülecik	41.814	26.850
Büyükevren	40.651	26.228	Tatarlar	41.835	26.886
Abdürrahim	40.641	26.258	Taşlısekban	41.802	26.877
Hasköy	40.665	26.317	Yağcılı	41.784	26.829
Kocaali	40.666	26.345	Geçkinli	41.731	26.848
Şehitler	40.692	26.293	Küküler	41.715	26.896
Işıklı	40.719	26.309	Akardere	41.681	26.930
Çeribaşı	40.664	26.256	Büyükgerdelli	41.735	26.951
Karaincirli	40.627	26.297	Keramettin	41.787	26.977
Vakıf	40.616	26.258	Süloğlu	41.767	26.911

This causes the red zirconium lake which is present to lose colour (https://tr.hach.com/). Principle Component Analysis (PCA) is a powerful multivariate statistical method that facilitates the interpretation of large data. It is widely used in ground and surface water quality evaluation studies on all over the globe (Liu et al. 2003, Kazi et al. 2009, Tokatlı et al. 2014, Tokatlı 2017). Principle Component Analysis (PCA) was made by using the "SPSS 17" package program.

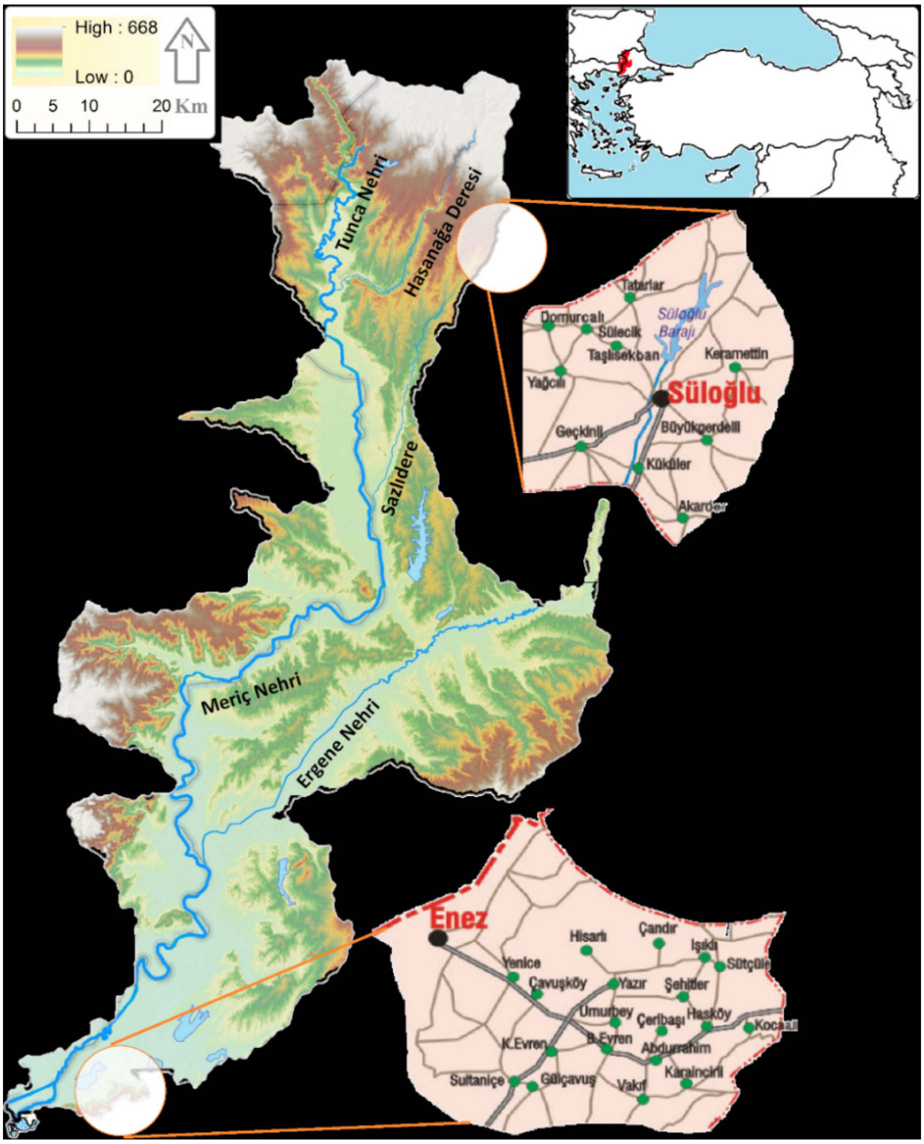

Figure 1. Map of Meriç River Basin and investigated residential areas

Results

Physical and chemical data

Results of the physical and chemical data detected in the drinking water of Enez and Süloğlu Districts and some water quality and drinking water standards are presented in Table 2. Fluoride distribution map of investigated villages is presented in Figure 2. Correlation graphics between fluoride and the other investigated parameters are presented in Figure 3.

According to the results of Pearson Correlation Index, no statistically significant relationship was determined between the fluoride and any physical and chemical parameters (Figure 3).

Principle Component Analysis (PCA)

Principle Component Analysis (PCA) is a powerful multivariate statistical method that facilitates the interpretation of large data. It is widely used in ground and surface water quality evaluation studies on all over the globe (Liu et al. 2003, Kazi et al. 2009, Tokatlı et al. 2014, Tokatlı 2017).

In this research, PCA was used to detect the effective varifactors. Low and non – correlation variables were removed from present application to improve the reliability of the PCA and a total of 6 variables were used to detect the effective varifactors (n = 22 for all parameters).

Kaiser Meyer Olkin (KMO) test result, which means the measure of sampling competence, was recorded as 0.727 in this application. Eigenvalues over 1 were taken as criterion assessing the principal components in this application (Figure 4). Component plot is given in Fig. 4 and factor loadings before and after rotation is presented in Figure 5.

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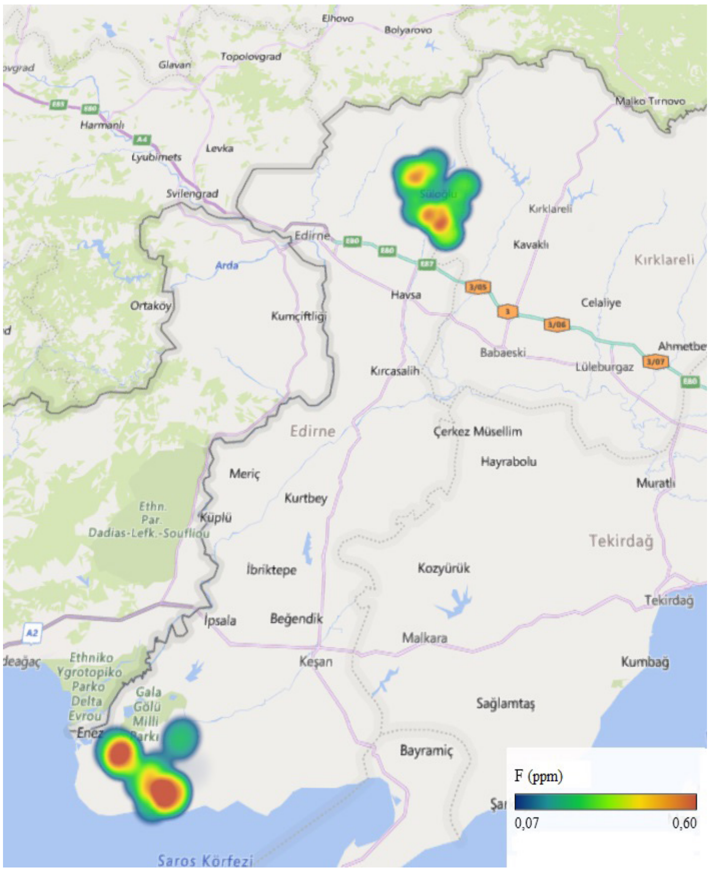

Figure 2. Fluoride distribution map in the studied area

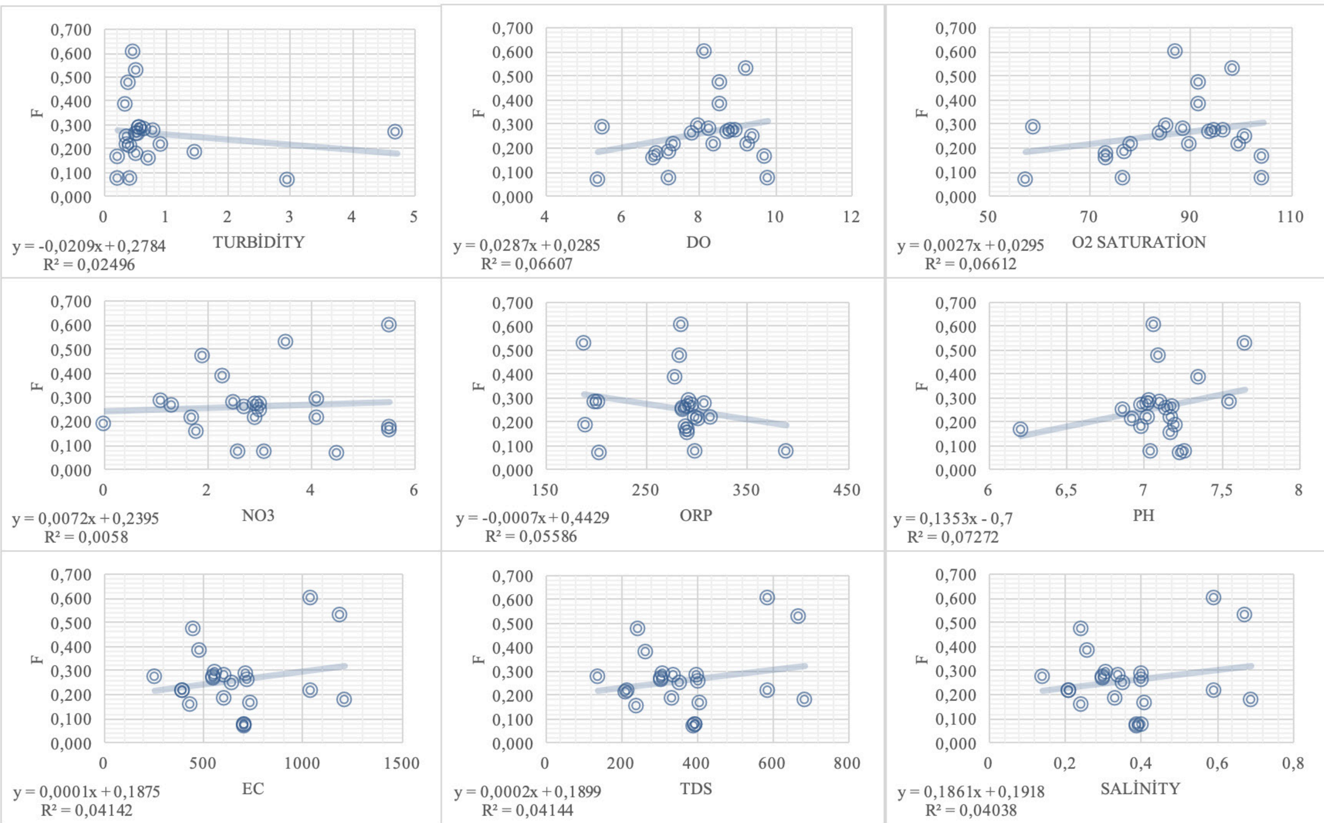

Figure 3. Correlation data between fluoride and the other physical and chemical variables

Table 2. Results of investigated parameters and limit values

Some Limits and the Data of Present Study DO (ppm)		Parameters									
		O_2 Sat. (%)	pH	ORP	EC (mS/cm)	[a]TDS (ppm)	Sal. ($\%_0$)	Tur. (NTU)	NO_3 (ppm)	F (ppm)	
Turkish Regulations Water Quality Classes (2015)	**I. Class** (Very Clean)	>8	>90	6.5-8.5	-	400	500	-	-	5	1
	II. Class (Less Polluted)	6	70	6.5-8.5	-	1000	1500	-	-	10	1.5
	III. Class (Much Polluted)	3	40	6.0-9.0	-	3000	5000	-	-	20	2
	IV. Class (Extremely Polluted)	3>	40>	Out of 6.0-9.0	-	>3000	>5000	-	-	>20	>2
Drinking Water Standards	**TS266** (2005)	-	-	6.5-9.5	-	2500	-	-	5	50	1.5
	EC (2007)	-	-	6.5-9.5	-	2500	-	-	-	50	1.5
	WHO (2011)	-	-	-	-	-	-	-	-	50	1.5
Results of South region in the Basin	**Yenice**	8.27	88.5	7.55	198	605	336	0.34	0.64	2.5	0.281
	Çavuşköy	9.21	98.4	7.65	188.7	**1184**	670	0.67	0.5	3.5	0.529
	Büyükevren	**5.51**	**59**	7.1	201.8	714	398	0.4	0.56	1.1	0.285
	Abdürrahim	7.21	77.1	7.2	189.4	603	335	0.33	1.46	0	0.187
	Hasköy	**5.36**	**57.2**	7.23	203.4	704	393	0.39	2.94	4.5	0.068
	Kocaali	7.19	76.8	7.04	389.1	708	396	0.4	0.22	3.1	0.075
	Şehitler	9.78	104.4	7.26	298.4	704	394	0.39	0.42	2.6	0.076
	Işıklı	7.31	78.1	7.17	298.2	**1037**	586	0.59	0.91	4.1	0.220
	Çeribaşı	6.87	73.4	6.99	288	**1209**	686	0.69	0.51	5.5	0.178
	Karaincirli	8.13	86.9	7.06	284.4	**1041**	588	0.59	0.46	5.5	0.603
	Vakıf	7.84	83.7	7.14	286	718	402	0.4	0.55	2.7	0.258
	Min	5.36	57.2	6.99	188.7	603	335	0.33	0.22	0	0.068
	Max	9.78	104.4	7.65	389.1	1209	686	0.69	2.94	5.5	0.603
	Mean	7.5164	80.318	7.2173	256.85	838.82	471.27	0.4718	0.8336	3.1909	0.2509
	SD	1.3556	14.467	0.2074	64.846	230.27	133.11	0.1346	0.7701	1.7009	0.1757
Results of North region in the Basin	**Domurcalı**	8.97	96.6	7.02	306.9	255	137	0.14	0.79	3	0.275
	Sülecik	8.39	90	7.02	312.5	395	215	0.21	0.37	1.7	0.220
	Tatarlar	9.28	99.5	6.92	300.8	393	215	0.21	0.4	2.9	0.216
	Taşlısekban	9.7	104.1	6.21	291.2	735	409	0.41	0.21	5.5	0.168
	Yağcılı	9.4	100.8	6.86	286.3	638	353	0.35	0.36	3	0.248
	Geçkinli	8.82	94.6	6.98	294.4	550	304	0.3	4.71	2.9	0.273
	Küküler	8.55	91.7	7.09	282.4	446	245	0.24	0.39	1.9	0.475
	Akardere	8.54	91.6	7.35	278	482	265	0.26	0.34	2.3	0.384
	Büyükgerdelli	8.74	93.7	7.18	291.3	549	303	0.3	0.5	1.3	0.267
	Keramettin	7.95	85.3	7.03	292.2	560	309	0.31	0.57	4.1	0.293
	Süloğlu	6.81	73.2	7.17	290.8	436	239	0.24	0.73	1.8	0.159
	Min	6.81	73.2	6.21	278	255	137.5	0.14	0.21	1.3	0.159
	Max	9.7	104.1	7.35	312.5	735	409	0.41	4.71	5.5	0.475
	Mean	8.65	92.827	6.9845	293.35	494.45	272.31	0.27	0.8518	2.7636	0.2707
	SD	0.7853	8.4022	0.2901	10.143	131.25	74.187	0.0744	1.2912	1.2077	0.0918

[a]Turkish Regulations 2004; [b]Uslu and Türkman 1987; Sal. Salinity; Tur. Turbidity; Sat. Saturation
EC – European Communities; WHO – World Health Organization; TS266 – Turkish Standards Institute
Class III – IV water quality given in bold

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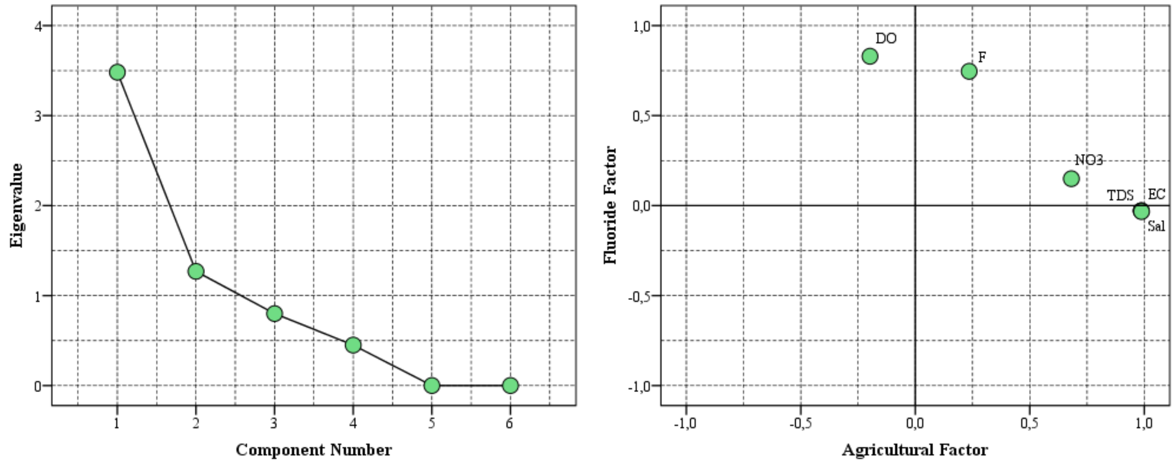

Figure 4. Eigenvalues (left) and component plot (right)

As a result of PCA, 2 factors explained 79% of the total variance (Table 3). First factor (F1) named as "Agricultural Factor" and it was explained 58% of total variance, which was related to the variables of salinity, TDS, EC and nitrate parameters (Figure 5). Second factor (F2) named as "Fluoride Factor" and it was explained 21% of total variance, which was related to the variables of dissolved oxygen and fluorine parameters (Figure 5).

Table 3. Total variances explained in FA

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.481	58.022	58.022	3.481	58.022	58.022	3.481	58.018	58.018
2	1.269	21.147	79.170	1.269	21.147	79.170	1.269	21.151	79.170

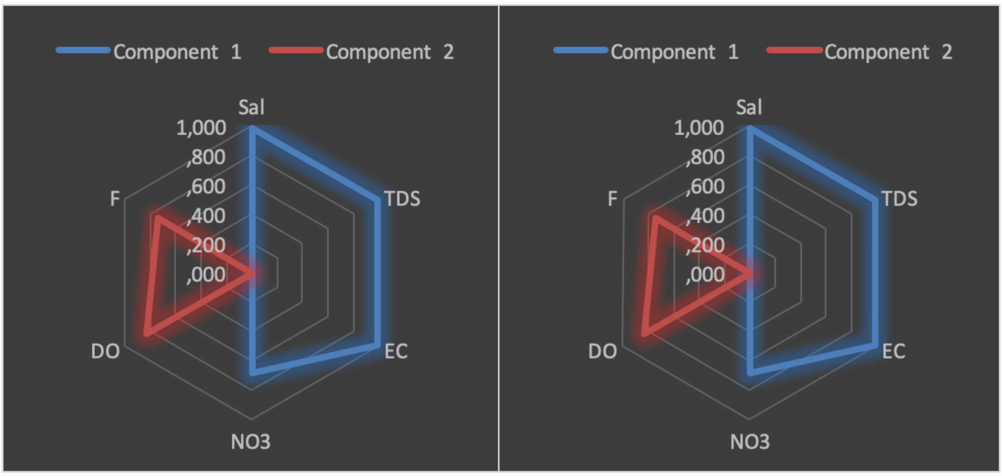

Figure 5. Component matrix before (left) and after (right) rotation

Discussion

According to the Water Pollution Control Regulation criteria in Turkey (2015), all the investigated villages both in northern and southern regions of Meriç River Basin ranges between Class I and Class II water quality in terms of pH, TDS, nitrate and fluoride rations. It was also determined that while the Süloğlu District (northern part) has Class I – II water quality in terms of dissolved oxygen, oxygen saturation and electrical conductivity, the Enez District (southern part) has Class II – III water quality in terms of these parameters. In addition, all the investigated physical and chemical parameters were recorded under the drinking water limit values specified by WHO (2011b), EC (2007) and TS266 (2005).

Groundwater plays such an essential role for the human population therefore its quality and safety has become issue of concern across the world. It is the main source of drinking water and contaminants found in groundwater may possess health hazards for humans (Howard et al., 2006). Among these contaminants nitrate and fluoride are the more widespread. Drinking water is a direct source of fluoride exposure and depending on the concentration and daily consumption, it can have adverse health effects on human health (Ayoob and Gupta, 2006).

Drinking water with optimal fluoride levels has preventive effect on dental caries. However, health may be adversely affected if excessive amounts are ingested through drinking water. Exposure to fluoride concentrations more than optimal level may result in fluorosis (Güner et al., 2016).

In the present study area, the concentrations of fluoride range from 0.068 to 0.603 ppm. It was observed that 22 samples have fluoride concentrations within the acceptable limits for drinking water specified by WHO (2011), EC (2007) and TS266 (2005). NO_3–N levels ranged from 0–5.5 ppm in the study region, which were also found in the limits of Turkish Regulations Water Quality Classes (2015).

Chen et al. (2017) conducted a study in the Semiarid Region of Northwest China and reported NO_3–N and F^- concentrations in groundwater were in the ranges of 2.66–103 and 0.11–6.33 ppm, respectively. Of the 50 samples in their study, 30 and four samples have high NO_3–N and F^- levels exceeding the acceptable limits for drinking purpose recommended by the WHO (10 and 1.5 ppm), respectively.

Adimalla et al. (2019) assessed the drinking water quality and risks of fluoride and nitrate contamination in terms of human health in Nirmal Province of South India. The levels of fluoride in the drinking water ranged from 0.06 to 4.33 ppm (with a mean of 1.13 ppm). 20.59% of the total drinking water samples, the content of fluoride was more than its permissible limit of 1.5 ppm and the very high fluoride concentration was noticed in the villages of Karegaon (4.33 ppm), Mudhol (2.56 ppm), and Bamangaon (2.44 ppm).

In a study performed in the Meriç River Basin (Tokalı and Güner, 2018), fluorine accumulations in groundwater of Havsa District, which is located in the Edirne Province of Turkey in Thrace Region were investigated. Drinking water samples were collected from 15 stations including almost all the residential areas of the Havsa District and the fluoride levels were found between 0.006 ppm (Bakışlar Village) – 0.567 ppm (Hasköy Village), which were within the limits recommended by WHO (2011b).

In another research performed in the same region (Güner et al., 2017), tap water qualities of Havsa and Suloglu regions were investigated in terms of oral health and the prevalence of dental caries and dental fluorosis in children were evaluated. According to the results of this study, as similar to the results of the present research, it was found that dental caries levels were lower in the optimal fluoride area (0.703 ppm in Havsa District) than below-optimal fluoride area (0.357 ppm in Süloğlu District). It was also reported that optimal fluoride concentrations may have a positive effect on reducing dental caries among children in their study population.

Onur et al. (2019) investigated fluoride levels in drinking water in 3 districts of Edirne and evaluated children in terms of dental caries and dental fluorosis. According to the fluoride levels in drinking water, the region was divided into 3 groups; group 1: <0.5 ppm, group 2: 0.5-1.2 ppm and group 3: 2.39 ppm. It was reported that increase in fluoride concentrations in drinking water increased the severity of dental fluorosis. Children living in the area with high fluoride level in drinking had less dental caries on their permanent teeth and high dental fluorosis scores compared with children living in areas with low and optimal fluoride levels in drinking water.

Conclusions

In this study, drinking water quality and fluoride accumulations in drinking water of northern and southern regions on the Meriç River Basin were investigated. Also Principle Component Analysis were applied to detected data in order to evaluate the data properly and according to the results of this application, "Agricultural Factor" and "Fluoride Factor" were determined as effective factors on drinking water quality. The detected fluoride levels in the northern and southern part of the basin are recorded as slightly below the optimum levels for teeth health and any investigated parameter in any investigated location did not exceed the permitted values for drinking water. As a result of this study, it was also determined that contamination rates of drinking water were found as southern region (Enez District) > northern region (Süloğlu District) in general.

In conclusion, the findings in the present study can provide a real insight into drinking water safety at Meriç River Basin. Since fluoride concentrations detected in drinking waters are below optimal level it is beneficial to evaluate the children residing in this area in terms of dental health.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Investigation of opportunities the addition of canned watermelon pomace and watermelon juice produced from unmarketable watermelon in broiler quail ration

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Abstract

This study was conducted to evaluate the use of watermelon pomace and watermelon juice obtained from unmarketable watermelon in the diets of fattening quails. A total of 90 Japanese quails (*Coturnix coturnix japonica*) were assigned to three groups (30 quails in each group), each comprising of 5 replicates each having 6 quails. One group was designated as control groups whereas other groups fed 5% watermelon pomace or watermelon juice on dry matter basis. All the diets were isonitrogenous and isocaloric formulated in order to meet the nutrient requirements of quails outlined in NRC standards. Live weight was greater in quails fed watermelon pomace compared with control group in first week only (P<0.05). Average daily gain of the quails was not different among the groups (P>0.05). In first week and overall period (1 to 4 wk), average daily feed intake (ADFI) was higher in control group than quails fed watermelon juice which, in turn, was higher in comparison with those fed watermelon pomace (P<0.001). In second week, ADFI was greater in control group than other dietary treatments (P<0.01). The ADFI was higher in quails fed diet with watermelon juice than those fed diets containing watermelon pomace (P<0.05). Feed conversion ratio (FCR) in the first week was lower quails fed watermelon pomace than control group (P<0.01). The FCR in overall period improved in quails receiving diets with watermelon pomace compared with other dietary treatments (P<0.001). Nutrients digestibility and mortality rates almost were similar across the groups (P>0.05). In conclusion, it was observed that watermelon pomace can be used in quail diets having additional growth enhancer properties.

Keywords: Juice, Pomace, Quail, Watermelon

Introduction

Turkey annually produces 4 million tons of watermelon (Turkish Statistical Institute, 2019) and ranks second worldwide (Özbay and Çelik, 2016). Depending on the temperature and humidity of the warehouse, the storage time of the watermelon is short (Turkey Standards İnstitute, 2019). It has been reported that approximately 20% of watermelon produced remains unmarketable attributed to storage conitions (temperature and humidity) and shorter storage time (Fish et al., 2009). The watermelon flesh (Anguelova and Warthsen, 2000; Edwards et al., 2003; Scott, 2012; Johnson et al., 2013; Sa'id, 2014; USDA, 2019), rind (Rimandoa and Perkins-Veazie, 2005; Rasheed, 2008; Erukainure et al., 2010; Choudhary, 2014), and seed (Sabahelkhier et al., 2011; Acar et al., 2012) contain water, carbohydrates, fat, protein, vitamins, minerals, citrulline, pectin, and lycopene. Recent studies have reported the use of lemon wastes (Chaudry et al., 2004), orange and banana shells (Siyal et al., 2016), cashew meal (Fernandes et al., 2016), and apple by-products (Zafar and Idrees, 2005) in poultry diets. Other study reported that corn can be replaced by 20% fruit juice mixture consisting of carrot, apple, mango, avocado, orange, melon, and tree tomato (Rizal et al., 2010).

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It is well known that the addition of vitamin C (ascorbic acid) into poultry diet enhances growth performance of poultry at high ambient temperature (Konca and Yazgan, 2002). In addition, β-carotene protects from oxidative stress and stimulates the immune system of birds (Ayaşan and Karakozak, 2010). Moreover, supplemental electrolytes in poultry diets increase egg yield, body weight and breeding performance, and decrease mortality rate (Register, 2019).

Recently, Shazali et al. (2013) reported an increase in live weight, protein consumption, protein efficiency rate, and feed utilization rate in response to addition of 20% full-fat watermelon seeds in broiler diets (Shazali et al., 2013).

The instability of unmarketable watermelon at room temperature and humidity poses considerable challenge as it yields waste at large. Terlemez (2017) concluded that unmarketable watermelon can be converted to durable canned watermelon pomace and watermelon juice by processing at an elevated temperature with the addition of acid. These by-products can be used as new feedstuffs in poultry production. No previous study has reported the use of watermelon pomace and watermelon juice in poultry diet. Therefore, unmarketable watermelons were converted into stable watermelon pomace and juice that were added to quail diets in order to evaluate their effect on growth performance and nutrient digestibility.

Material and Methods

Animals

A total of 90 male Japanese quails (*Coturnix coturnix japonica*) were used in the study. The animals were divided randomly into three experimental groups. These groups were further divided into 5 replicates each consisting of six animals. Experimental procedures were approved by Animal Experiments Local Ethics Committee of Fırat University (Decision / Protocol no: 157 / 10.05.2016 / 103).

Isocaloric and isonitrogenous diets were prepared for each group according to the nutrient requirements of quails recommended by NRC (1994). The trial groups were assigned as follows: 1) control group (Group C) without canned watermelon pomace and watermelon juice, 2) diet with 5% watermelon pomace (Group P), and 3) diet with 5% watermelon juice.

The composition and nutrient content of experimental diets are given in Table 1.

Table 1. The composition and nutrient content of diets

	Group C	Group J	Group P
Feed materials (%)			
Corn	47.62	42.50	39.75
Wheat bran	7.88	10	10
Soybean meal (48% CP)	39.40	38	39
Vegetable oil	2.60	2	3.75
Watermelon juice (on DM basis)	-	5	-
Watermelon pomace (on DM basis)	-	-	5
Dicalcium phosphate	0.99	1	1
Limestone	1.15	1.15	1.14
Sodium bicarbonate	0.10	0.10	0.10
Salt	0.26	0.26	0.26
Nutrient content (%)			
Dry matter	93	93	94
Crude protein	24	23.9	23.9
Crude fiber	8	8	10
Crude fat	3	2	4
Crude ash	0.30	0.30	0.30
Metabolizable energy (kcal/kg)*	2900	2898	2899

*: has been determined through the calculation. ME (kcal / kg) = 53 + 38 B formula was used where B = (crude protein%) + (2.25 x crude fat%) + (1.1 x starch%) + (sugar %)

The experiment was carried out in fattening quail cages at poultry research unit, Faculty of Veterinary Medicine, Fırat University. The experiment lasted for 37 days (7 days preparation and 30 days trial). Then, digestion experiment was carried out in individual cages for 7 days. Animals were given feed and water ad libitum.

The production of watermelon pomace and watermelon juice

Unmarketable watermelons were washed, and watermelon pomace was obtained by blending the chopped rind, seed, and edible parts. Citric acid was added to the watermelon pomace and juice to adjust the pH between 3.5-4. Then the watermelon pomace and juice were transferred to cauldron and boiled.

Meanwhile the lid of the cauldron was closed and left at the boiling point for 10 minutes on low heating rate. The product in the boiler was then allowed to cool to 60 °C and the canned product was filled into 5-liter plastic bottles and the bottles were capped.

Live weight and average daily gain determination

The quails were weighed before the experiments. The weighing process was repeated weekly. Average daily gain (ADG) was calculated by subtracting the live weight between the consecutive weeks and dividing by the number of days.

Determination of average daily feed intake

Feeds were weighed daily and then was given to the quails. After one week, all the residual feed was weighed again. Average daily feed intake (ADFI) was calculated by subtracting residual feed amount from feed given for one week. The ADFI per animal was calculated by dividing the amount of feed consumed by the group by the number of days and the number of animals belonging to that group.

Determination of feed efficiency

The amount of feed consumed by the quails in the two weighing periods was divided by the total live weight gain between those two weighing periods and the feed efficiency rates were calculated.

Mortality and vitality

The number of animals that died during the trial was recorded and then the mortality calculated using the following formula at the end of the trial.

$$Mortality\ (\%) = \frac{No.of\ birds\ befor\ experiment - No.of\ birds\ at\ the\ end\ of\ experiment}{Initial\ No.of\ animals} \times 100$$

Determination of nutrient digestibility

The degree of nutrient digestibility was determined by using the indicator method. Natural lignin was used as an indicator. For this purpose, at the end of the experiment, 10 animals were taken from each group and fed in individual cages. The fecal samples of the animals were collected once a day during the 7 days, dried at 60°C for 36-48 hours. Nutrient digestibility was calculated according to the following formula.

$$DDNF\ (\%) = 100 - \left[100 \times \frac{Indicator\ in\ feed\ (\%)}{Nutrient\ in\ feed\ (\%)} \times \frac{Nutrient\ in\ feces\ (\%)}{Indicator\ in\ feces\ (\%)}\right]$$

Laboratory analysis

Dry matter, crude protein, and crude fat content of feed and feces were determined according to the methods specified in AOAC. (1980). Crude fiber and lignin levels were determined in Fiber Analyzer Ankom 220 according to the method reported by Van Soest et al. (1991). Crude protein content of poultry excreta samples was corrected for uric acid (Rotter et al., 1989).

Statistical analysis

Analysis of variance was applied for comparison among the groups using Duncan's test as post-hoc test. Chi square analysis was used for mortality and vitality data. These analyses were performed in a computer software package SPSS.

Results and Discussion

No systematic studies are available for the evaluation of unmarketable watermelons in the feed industry and animal feeding areas. To fill this gap, unmarketable watermelons were converted into durable watermelon pomace and watermelon juice that were added to male quail rations as concentrate feed (Table 1).

Table 2. Effect of canned watermelon juice and pomace on live weight in quails (g)

Parameter	Groups			P
	Group C	Group J	Group P	
Beginning	84.1±4.12	84.39±2.66	84.86±1.38	NS
1. Week	122.84±4.09[b]	129.35±1.67[ab]	137.31±3.99[a]	*
2. Week	156.84±4.01	156.24±1.99	162.16±3.66	NS
3. Week	174.61±3.31	174.00±4.78	180.42±5.71	NS
4. Week	182.31±2.51	182.68±4.81	189.01±5.72	NS

±: Standard error value of means

*:P<0.05, NS: not significant p>0.05

a, b, c: The difference between values expressed in different letters on the same line is important.

Table 3. Effect of canned watermelon juice and pomace on average daily gain in quails (g/quail/day)

Parameter	Groups			P
	Group C	Group J	Group P	
1. Week	5.30±0.58	6.08±0.52	7.35±0.67	NS
2. Week	4.64±0.47	3.75±0.32	3.45±0.34	NS
3. Week	2.61±0.28	2.53±0.48	2.66±0.42	NS
4. Week	1.17±0.12	1.40±0.14	1.25±0.11	NS
1-4. Week	3.43±0.25	3.44±0.22	3.68±0.23	NS

±: Standard error value of means

NS: not significant p>0.05

Table 4. Effect of canned watermelon juice and pomace on average daily feed intake in quails (g/quail/day)

Parameter	Groups			P
	Group C	Group J	Group P	
1. Week	10.35±0.13[a]	8.23±0.17[b]	7.04±0.45[c]	***
2. Week	13.23±0.56[a]	11.41±0.30[b]	9.90±0.44[b]	**
3. Week	11.56±1.03	8.89±0.70	9.01±0.65	NS
4. Week	6.54±0.30[ab]	7.34±0.63[a]	5.19±0.24[b]	*
1-4. Week	10.42±0.37[a]	8.97±0.17[b]	7.79±0.23[c]	***

±: Standard error value of means
*: P<0.05, **:P<0.01, ***:P<0.001, NS: not significant p>0.05
[a, b, c:] The difference between values expressed in different letters on the same line is important.

Table 5. Effect of canned watermelon juice and pomace on feed conversion ratio in quails (g/quail/day)

Parameter	Groups			P
	Group C	Group J	Group P	
1. Week	2.06±0.26[a]	1.39±0.11[ab]	1.00±0.12[b]	**
2. Week	2.94±0.24	3.10±0.18	2.97±0.32	NS
3. Week	4.60±0.60	4.03±0.87	3.62±0.44	NS
4. Week	5.80±0.59	5.42±0.67	4.29±0.43	NS
1-4. Week	3.85±0.18[a]	3.48±0.13[a]	2.97±0.08[b]	***

±: Standard error value of means
**:P<0.01, ***:P<0.001, NS: not significant p>0.05
[a, b, c:] The difference between values expressed in different letters on the same line is important.

Although isocaloric and isonitrogenous rations were used in this study, live weight development value was found higher in watermelon group P (Table 2). The results of ADG in the first week and in the average of four weeks were found to be higher in Group P than in other groups, and lower in the other three weeks than in other groups. However, these differences between the groups were not found to be statistically significant (Table 3). The ADFI was higher in the control group than in group P and group J. The lowest value was also determined in Group P (Table 4). The FCR was statistically significantly higher in Group P than in other groups in the average value of four weeks of the research process (Table 5).

As mentioned in the materials and methods section, watermelon pomace contains the rind, edible parts and seeds whereas watermelon juice contains water and water-soluble substances and does not contain pulp. The pulp found in watermelon pomace, but not in watermelon juice, consists of the fleshy, core and rind part of the watermelon. The pulp contains carbohydrate, crude protein, crude fat, crude fiber as well as lycopene, beta carotene and ascorbic acid (Terlemez, 2017). Studies have reported that lycopene is higher in the fleshy part of watermelon whereas beta carotene and citrulline in the rind of watermelon. It was reported that watermelon seed contains 16.06-18.13% crude protein, 23.31-26.83% crude fat, 44.70-45.72% crude fiber, 2.31-2.59% crude ash, and 0.28-0.30 mgGAE / g total phenol (Acar et al., 2012). It has also been reported that its protein contains a lot of glutamic acid, aspartic acid, arginine and leucine (Khalil, 1998; Mello et al., 2001; El-Adawy and Taha, 2001). Also, its fat contains 63.19-72.03% linoleic acid, 17.55% (feed watermelon) – 24.65% oleic acid and 6.41-9.73% stearic acid (Acar et al., 2012). According to the information given here, watermelon pomace is more advantageous in terms of amino acids, fatty acids, vitamins and antioxidants than both watermelon juice and control group. However, it is disadvantageous in terms of crude fiber structure due to the watermelon seed peel found in watermelon pomace. Watermelon pomace has been more effective than the control group and watermelon juice on the live weight development, daily weight gain and feed efficiency in quails due to this advantageous structure. The reason that feed consumption in watermelon pomace group was lower than in other groups may be the crude fiber content and structure incoming from watermelon seeds, rind, and pulp. Although there are not many studies on the effect of the addition of canned watermelon pomace and juice to poultry rations on fattening performance, we believe that the examination of the studies with other fruit and vegetable waste products will contribute to the understanding of the research results. In this context, a previous study revealed that addition of 20 g and 30 g watermelon seed meal significantly improved the growth performance and carcass yield of broiler chicken compared to the control group (Ukpanukpong et al., 2018). On the other hand, the watermelon peel powder crush diet supplementation increases plasma L-citrulline concentration in chicks. The increase in plasma L-citrulline concentrations suggest that watermelon rind could be used as a natural source of L-citrulline in poultry to ameliorate the adverse effects of heat stress (Nguyen et al., 2019). Studies conducted with other fruit by-products reported that feed consumption was lower and live weight and feed efficiency were higher in the banana peel group than orange peel group although the crude fiber content of banana peel is higher than orange peel (Siyal et al., 2016). Again, in another study, it has been reported that the addition of apple juice instead of corn to the broiler ration increases the live weight gain (Zafar and Idrees, 2005). All these arguments and literature data support the results obtained in this study.

Digestibility of dry matter, crude protein and crude fat were determined in control group, canned watermelon pomace and juice groups as 62.50, 62.64 and 62.56; 67.42, 67.50 and 67.56; 69.12, 69.14 and 69.18, respectively. The digestibility of dry matter, crude protein and crude fat were not statistically significant among the groups (Table 6). These results show that watermelon juice and pomace can be used in poultry rations instead of corn and soybean meal in terms of digestibility.

Although there was a slightly higher mortality rate in the Group-J, the difference between the groups was not statistically significant (Table 7).

Table 6. Effect of canned watermelon juice and pomace on nutrient digestibility in quails (%)

Parameter	Groups			SEM	P
	Group C	Group J	Group P		
Dry matter	62.50	62.64	62.56	0.28	NS
Crude protein	67.42	67.50	67.56	0.13	NS
Crude fat	69.12	69.14	69.18	0.27	NS

SEM: Standard error value of means

Table 7. Mortality and vitality values in research groups

Days	Group C	Group J	Group P	---P--- X^2
1-8	-	-	-	0.856 X^2=0.310
8-15	-	-	1	
15-22	-	1	-	
22-29	2	1	1	
29-36	-	1	-	
Total number of dead	2	3	2	
Death rate, %	6.7 (2/30)	10 (3/30)	6.7 (2/30)	
Vitality rate, %	94.59	91.89	94.59	

Conclusion

In conclusion, the study suggest that canned watermelon pomace and canned watermelon juice can be added to quail diets without any negative effect on the growth performance of quails. In addition, quails fed diet with canned watermelon pomace had better growth performance than those fed control or canned watermelon juice added diets. Accordingly, it can be said that watermelons that cannot be marketed can be added to the poultry rations as canned watermelon pomace and watermelon juice. It is a preliminary study that requires further studies in this area to support this idea.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Experimental procedures were approved by Animal Experiments Local Ethics Committee of Fırat University (Decision / Protocol no: 157 / 10.05.2016 / 103).

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Research Article

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Analysis of cocoa farmer's poverty status in Abia State, Nigeria: The Foster, Greer and Thorbeck (FGT) decomposable poverty measure

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Abstract

A poverty measure is said to be decomposable if the poverty quota or size of a group is a weighted average of the poverty measures of the individuals in the group. This study analyzed Cocoa farmer's poverty status in Abia State, Nigeria with the application of Foster, Greer and Thorbeck (FGT) decomposable poverty measure. A total of 90 farm households found in Ikwuano, Umuahia North and Bende Local Government Areas (LGAs) of Abia State who were the major Cocoa producing LGAs of the State were chosen for the study. A Multi-stage purposive sampling technique was adopted in selecting the respondents. Descriptive statistics and Foster-Greer-Thorbeck (FGT) decomposable poverty measure was employed in analyzing the research objectives. Result from the socio economic characteristics shows that majority (86.67%) were males while 13.33% were females. Analysis from the FGT showed that 36.67% of Cocoa farmers in Ikwuano LGA fell below the estimated poverty line while the other 63.33% were classified as non-poor, whereas 40% of Cocoa farmers in Umuahia North LGA were moderately poor while the other 60% were classified as non-poor. In Bende LGA, 10% of the Cocoa farmers were extremely poor, 36.67% were moderately poor while the remaining 53.33% were classified as non-poor. The result implies that the poverty status in the three Cocoa producing LGAs of the State varies; with greater percentage of the Cocoa farmers classified as non-poor while the others were categorized as poor. It is therefore recommended that greater equality in income distribution should achieved by improving the productivity of the poor Cocoa farmers, especially through increasing their credit facilities, basic education, health and technical skills.

Keywords: Cocoa Farmers, Poverty Status, Foster-Greer-Thorbeck (FGT) decomposable poverty measure, Abia State, Nigeria

Introduction

About 2.8 billion persons of the World's population live on less than \$2 a day, and 1.4 billion on less than \$1 a day (World Bank, 2013). Poverty is a major limitation of economic development and the dearth of economic opportunity is seen to increase the poverty level of an individual or household.

Research has shown that majority (> 70 %) of the Cocoa (*Theobroma cacao*) farmers are smallholders who live in the rural areas faced with extreme inequality and poverty coupled with the use of obsolete tools and technology; devoid of social amenities (such as electricity, pipe borne water, hospitals and schools); with their income very low (Agwu *et al*., 2014). The International Bank for Reconstruction and Development – IBRD (2008) also observes that high level of income inequality and poverty exists in most subsistence farming households in Nigeria. Canagarajah *et al*., (1997) posits that most of the Cocoa farmers are at the bottom of income distribution chart, and are living in abject poverty. Since the source of livelihood

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and income generation of majority of the poor is agriculture, alleviating poverty entails boosting agricultural production.

The production of this very important cash crop called Cocoa has been experiencing a downward trend which has resulted in poverty and food insecurity to the cash crop farmers. Food insecurity ranks the topmost among the developmental problems facing Nigeria as a whole (Okezie *et al*., 2011). The already fragile food security situation in Sub Saharan Africa and Nigeria in particular is at risk from emerging stress factors. To reduce poverty and hunger in the region, there is an urgent need for global, national, and local actors to pursue innovative approaches to improve agricultural productivity (Iheke and Nwaru, 2013). Moreover, Nigeria which used to be second largest country in Cocoa production in the world after Ghana is presently in the fourth position after Cote d'ivoire, Indonesia and Ghana with production of 210 thousand metric tons representing 5.9% of the world production. This gloomy situation has generated some unpleasant concern to the Nigerian economy and therefore calls for an immediate attention of government, individuals and researchers.

Cocoa remains the most important cash crop in terms of foreign exchange earnings. Nigeria is one of the principal producers of Cocoa and has risen as a major exporter of the product over the last century. In terms of foreign exchange earnings, no single agricultural export commodity has earned more than Cocoa. Nigeria is the fourth leading exporter of Cocoa in the world. Cocoa is mainly exported as beans, processing activities being limited within the country. Cocoa is the main agricultural export in Nigeria (FAO - Food and Agricultural Organization, 2013). Agwu *et al*., (2014) asserts that enterprises that promote income growth and distribution; and enhance revenue of poor households are most likely to lead to poverty reduction; and one of such enterprises is Cocoa production. The export of Cocoa beans accounts for the largest single non-oil foreign earning commodity and contributes significantly to Nigeria's (GDP) Gross Domestic Product (Ogunniyi, 2015).

Foster, Greer and Thorbeck - FGT (1984) proposed a family of poverty indices based on a single formula capable of incorporating any degree of concern about poverty through the poverty aversion parameter α. A poverty measure is said to be decomposable if the poverty measure of a group is a weighted average of the poverty measures of the individuals in the group. An important property of decomposable poverty measures is that a *ceteris paribus* reduction in the poverty measure of a subgroup always decreases poverty of the population as a whole. Decomposable poverty measures are particularly useful in poverty studies where a population is broken down into subgroups defined along ethnic, geographical or other lines. We can use these measures to obtain the contribution of each subgroup to total poverty and to estimate the effect of a change in subgroup poverty on total poverty (Todaro and Smith, 2009).

This study therefore provides a deeper understanding of the poverty status among Cocoa farmers in Ikwuano, Bende and Umuahia North LGA of Abia state, Nigeria. The research findings will provide a quantitative policy framework to tackle the poverty problems among Cocoa farmers. Consequently, for the Cocoa farmers to increase their income, earn foreign exchange and reduce food insecurity; their poverty situation has to be reviewed and abridged. The research is therefore aimed at exploring the poverty status of Cocoa farmers in Abia State, Nigeria.

Methodology

Description of the Study Area

This study was carried out in three major Cocoa producing Local Government Area of Abia State, Nigeria. Abia State is situated in the South-East geo-political zone of Nigeria. Abia State lies between longitudes 7° 23'E and 8° 2'E East of the equator and latitudes 4° 47'N and 6° 12'N North of the Greenwich Meridian. The State is located East of Imo State and shares common boundaries with Anambra, Enugu and Ebonyi States in the North West and North East respectively. On the East and South East, it is bounded by Cross River and Akwa Ibom States and by Rivers State on the South. Abia State is made up of 17 local government areas and most of the people especially the rural dwellers are engaged mainly in subsistence farming.

Abia State is one of the Cocoa producing states in Nigeria. The State is divided into three agricultural zones namely; Umuahia, Ohafia and Aba Agricultural Zones. Umuahia and Ohafia Agricultural Zones are the two major zones of Cocoa production in the state. According to Abia State Government (2012) Cocoa is majorly produced in Bende, Ikwuano, Umuahia North and some parts of Ukwa East and West.

According to Nwaru (2005) most families in Abia state are involved in one farming activity or the other as a primary or secondary occupation, over 70% of the population is involved in agriculture as an occupation. The state is blessed with favourable warm climate and sufficient moisture ideal for the growing of tree crops, root and tuber crops, cereals, vegetables, nuts and food crops including rice, while a good number of the people engage in trading on various agricultural produce, either on retail or wholesale basis. Some of the people engage in non-farm economic activities, like craft making, carpentry, and bricklaying. Livestock are also kept especially on a small-holder basis (Nwaru and Iheke, 2010).

Sampling Technique and Size

A Multi-stage Purposive Sampling Technique was adopted in selecting the respondents. Data were collected in stages. In the first stage, Umuahia Agricultural zone and Ohafia Agricultural zone were purposively selected from the three agricultural zones in Abia state; because the zones are the major areas of Cocoa production coupled with the presence of higher number of Cocoa farming households. In the second stage, three (3) Local Government Areas (LGAs) were purposively selected from the two agric. zones; which were Ikwuano, Umuahia North and Bende LGAs of Abia State. The selection was based on the fact that the LGAs were the major Cocoa producing LGAs in the State. In the third stage, (3) three Autonomous Communities were purposively selected from each of the Local Government Areas; making a total of (9) nine Autonomous Communities. In the fourth stage, 10 (ten) Cocoa farming households were purposively selected from the nine (9) Autonomous Communities. In all, a total of 90 Cocoa farming households were enlisted for the study.

Analytical Technique

ι. Socio-economic characteristics of the Cocoa farmers were analyzed with the application of descriptive statistics, such as mean, percentages and frequency counts.

ιι. Assessment of the poverty status of the Cocoa farming households was realized with Foster-Greer-Thorbeck (FGT) decomposable poverty measure. The major reason for this choice is due to its decomposability and usage by IBRD, FAO and other agencies.

The general Foster, Greer and Thorbecke (FGT) poverty measure (Pαi) is expressed as

$$P_a = \frac{1}{n}\sum_{i=1}^{q}\{\frac{z-Yi}{z}\}^{\alpha} \quad - \quad - \quad - \quad - \quad - \quad - \quad - \quad 1$$

When a = 0, i.e. Poverty Incidence or Head count $P_0 = \frac{1}{n}\sum_{i=1}^{q}\{\frac{z-Yi}{z}\}^{0} = \frac{q}{n}$ 2

When a = 1, i.e. Poverty Incidence or Head count $P_1 = \frac{1}{n}\sum_{i=1}^{q}\{\frac{z-Yi}{z}\}^{1}$ - 3

When a = 2, i.e. Poverty Incidence or Head count $P_2 = \frac{1}{n}\sum_{i=1}^{q}\{\frac{z-Yi}{z}\}^{2}$ - 4

The FGT index of the Cocoa Farmers will be estimated as:

$$Pa = \frac{1}{N}\sum_{j=1}^{q}\{\frac{z-Yi}{z}\}^{\alpha} \quad - \quad - \quad - \quad - \quad - \quad 5$$

Where Pa = Weighted FGT Poverty Index

q = Number of Cocoa farmers below the Poverty line/number of poor Cocoa farmers

Yi = Per capita Expenditure of the Cocoa farmers

α = Degree of Concern for the depth of poverty, and takes the values 1, 2, 3…

Z = Poverty Line (two-third of Mean Per Capita Household Expenditure (MPCHE) of the farmers); and n = total number of Cocoa farmers in the study area

Po (Head Count) measures prevalence of Poverty

P_1 (Poverty Gap Index) measures the depth of poverty, while

P_2 (Squared Poverty Gap) measures Poverty severity

$$\text{Per Capita Household Expenditure} = \frac{\text{Total Household Monthly Expenditure}}{\text{Household size}} \quad - \quad - \quad 6$$

The poverty line that was used in the study was based on the Cocoa farmers' monthly consumption expenditure. The classification of household poverty status was based on Mean Per Capita Household Expenditure (MPCHE).

$$\text{MPCHE} = \frac{\text{Total Per Capita Household Expenditure}}{\text{Total Number of Cocoa Farming Households}} \quad - \quad - \quad 7$$

Two – thirds (2/3) of the Mean Per Capita Household Expenditure (MPCHE) was used as the moderate poverty line, while one – third (1/3) of MPCHE was used as the line for extreme poverty, i.e. extreme poverty was defined as 1/3 of the mean per capita total household expenditure. Cocoa farmers with MPCHE less than this would be considered extremely poor, (following Iheke and Nwaru, 2013) while those spending > 2/3 of MPCHE are considered to be non-poor Cocoa farmers.

Results and Discussion

Socio – Economic Characteristics of Cocoa Farmers in Abia State, Nigeria

Table 1 is the socio economic characteristics of the Cocoa farmers in Abia State.

From Table 1, it was observed that a greater percentage of the respondents (86.67%) were males and only 13.33% were females. This could be attributed to the high intensive labour requirement for Cocoa farming which the male gender could afford. Ebewore and Emuh (2013) observed that females were mostly involved as helpers and suppliers of labour in some meager aspect of the business, such as weeding, processing and some marketing operations.

To a large extent, age of an individual dictates his availability as a member of the workforce. Greater percentage of the farmer's age ranged from 59 – 68 years (34.44%) and 49 – 58 years (24.44%). This implied that there were older Cocoa farmers compared to their younger counterparts and this could have a negative impact on Cocoa production since young people are economically active, stronger and are expected to cultivate larger farm size compared to the older farmers.

Table 1. Summary of Socio Economic Profile of Cocoa Farmers

		Frequency	Percentage (%)
Gender	Male	78	86.67
	Female	12	13.33
Total		**90**	**100**
Age (Years)	18-28	4	4.44
	29-38	7	7.78
Minimum (18)	39-48	15	16.67
Maximum (78)	49-58	22	24.44
Mean (55)	59-68	31	34.44
	69-78	11	12.22
Total		**90**	**100**
Membership of Cooperative Society	Yes	14	15.56
	No	76	84.44
Total		**90**	**100**
Sources of Credit	Bank Loan	4	4.44
	Equity/Personal Savings	69	76.67
	Relatives/Friends	12	13.33
	Cooperative Organizations	1	1.11
	Others	4	4.44
Total		**90**	**100**
Farm Size (Hectare)	1-4	12	13.33
	5-8	19	21.11
Minimum (1)	9-12	15	16.67
Maximum (20)	13-16	28	31.11
Mean (14)	17-20	16	17.78
Total		**90**	**100**
Household Size (Person)	1-3	2	2.22
	4-6	16	17.78
Minimum (1)	7-9	42	46.67
Maximum (15)	10-12	24	26.67
Mean (7)	13-15	6	6.67
Total		**90**	**100**

Source: Field Survey Data, 2016

The implication of the foregoing is the decreasing availability of an energetic population who could cope with the task of farm operations. Alternatively, this could mean that the older Cocoa farmers might be experienced enough and could afford the huge financial requirements in the establishment and maintenance of Cocoa farms. Also from the age distribution of the respondents in Table 1; 29 – 38 years (signifying 7.78%) and 18 – 28 years (4.44%) represents the least percentage of age ranges. This has a lot of negative implications as the able bodied middle aged farmers (youths) who can cope better with the daily challenges of the enterprise and readily accept new innovations are not enough.

Cooperative is defined as a registered voluntary association of persons, with a common interest formed and operated along democratic principles, for the purpose of economic and social interest. Majority of the Cocoa farmers (84.44%) do not belong to any cooperative society.

Credit helps farm firms to meet seasonal and annual fluctuation in income and expenditure and also for the adoption and acquisition of new technologies. A total of 76.67% of the respondents opined that they use equity capital, while 13.33% got their financial assistance from friends and relatives. The low bank loan (4.44%) can be as a result of unavailability of collaterals or credit unworthiness of the farmers. Lack of credit is generally recognized as one of the major constraints not only in expanding production but also in modernizing agriculture. Kanu (2012) observed that high frequency of personal savings implies that the institutional sources of finance were not well developed and advanced. Also, institutional agencies may not be eager to give loans to farmers due to the inherent risk associated with agriculture.

Majority of the Cocoa farmers (31.11%) cultivated 13-16 hectares of Cocoa, while 21.11% had 5-8 hectares. About 17.78% of the respondents had farm sizes between 17-20 hectares of Cocoa. Only 13.33% cultivated 1-4 hectares. Cocoa farming involves large expanse of farm land. This result implied that majority of the Cocoa farmers has enormous areas of farm land, but were limited due to inaccessible roads, unskilled and semi-skilled labourers (most especially the abled bodied youths) and absence of credit facilities for innovation adoption; hence, they produce below their optimal levels. This result is contrary with Ebewore *et al*., (2013) that majority of the Cocoa farmers were small scale who operated near subsistence level of productivity.

Household size is defined as the total number of individuals headed by a family head who resides in a given apartment. A total of 46.67% of the Cocoa farmers have between 7 and 9 household members, 26.67% have between 10 and 12 members, while 6.67% had 13-15 members in their household. Only 2.22% of the respondents have 1-3 persons. The mean household size was 7 persons. The larger household size connotes that marriage is highly cherished by majority of the respondents which has implication on family labour supply. Similarly, having a larger household size may provide most of the labour needed for Cocoa production, thus, reducing the cost expanded in hiring labourers, thereby leading to increased productivity. On the other hand, Akin-Olagunju and Omonona (2014) observed that Cocoa farmers with larger household size are usually associated with low per capita income especially in resource-constrained economies. In other words, large household size is associated with poverty.

Figure 1 is Pie Chart Showing Sources of Income among Cocoa Farmers in Abia State. The major source of income is equity/personal savings (77%) followed by income from relatives/friends (13%).

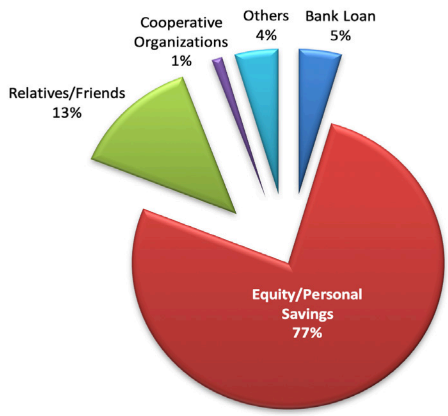

Figure 1. Explosive Pie chart showing sources of income among Cocoa farmers in Abia State, Nigeria (Field Survey Data, 2016)

Poverty Status of Cocoa Farmers in Abia State, Nigeria

The fundamental principle in the determination of the FGT decomposable poverty measure is the estimation of the poverty line. The estimation of the poverty line involves the valuation of the Mean Per Capita Household Expenditure (MPCHE) of Cocoa farmers based on their basic consumption expenditure. The MPCHE was derived for Cocoa farmers in Ikwuano, Umuahia North and Bende L.G.A., and as a group. This result further assisted in the determination of the poverty status of the Cocoa farmers.

Table 2. Mean Per Capita Expenditure (MPCHE) of Cocoa Farmers in Ikwuano LGA; based on their Basic Needs

Basic Needs (Consumption Expenditure)	Amount/Month (₦) MPCHE	Amount/Annum (₦) MPCHE	Percentage (%) of Total Expenditure
3 square meal (food)/drinks	11200.00	134400.00	30.12
Clothing	6050.00	72600.00	16.25
Health/Medication	2766.67	33200.04	7.43
Education	1600.00	19200.00	4.29
Rental Value of Residence/shelter	4833.33	57999.96	12.98
Transportation Cost	7883.33	94599.96	21.18
Miscellaneous	2886.67	34640.04	7.75
TOTAL MPCHE	**37,220.00**	**446,640.00**	**100**
2/3 of MPCHE	24,813.33	297,760.00	
1/3 of MPCHE	12,406.67	148,880.00	

Source: Field Survey Data, 2016

Table 2 shows the average monthly and yearly expenses on basic needs of Cocoa farmers in Ikwuano LGA of Abia State. Food, which is a very basic necessity accounted for about 30.1% of the total MPCHE (Mean per Capita Household Expenditure). The cost of transportation (21.18%) was second highest in terms of the MPCHE of Cocoa farmers in the study area. The amount spent on clothing, shelter, miscellaneous and health care constituted 16.25%, 12.98%, 7.75% and 7.43% respectively. Education (4.29%) has the least percentage of MPCHE. Literacy rate determines the levels of poverty and the distribution of income in an economy. Improving access to education, for example, can reduce inequality (and hence poverty). World-Bank Nigeria Country brief in 2012 declared that the Nigerian economy has realized rapid and impressive economic growth in the last few years, currently estimated at 7.9 percent per annum. Iheke and Nwaru, (2013) observed that as households acquire more education, their rise out of poverty increases. Therefore, increased agricultural productivity depends primarily on the education of the rural farmers to understand and accept the complex scientific changes which are difficult for the illiterate rural farmer to understand.

Greater percentage of MPCHE was spent on food items. The findings corroborated with that of Okezie *et al*., (2011) and Adekemi *et al*., (2012) that food is the most fundamental human need. A study conducted in Akwa Ibom State in 2010 by Etim *et al*., confirms that food constituted the highest Mean Per Capita Expenditure of rural farm households.

Two – thirds (2/3) of the Mean Per Capita Household Expenditure was taken for the moderate poverty line for Cocoa farmers in Ikwuano LGA, while one – third (1/3) was taken as the core/extreme poverty threshold. The value of the moderate poverty line was define as ₦24,813.33 while the extreme poverty line was put at ₦12,406.67. Based on these estimated poverty thresholds, Cocoa farmers in Ikwuano LGA were classified into mutually exclusive groups as presented in Table 3

Table 3. Classification of Cocoa Farmers in Ikwuano LGA, According to Poverty Status

Poverty Status	MPCHE Amount (₦)	Frequency	Percentage (%)
Extremely/Core Poverty (1/3 of MPCHE)	< 12,406.67	2	6.67
Moderately Poor (2/3 of MPCHE)	12406.67 ≤ Z < 24813.33	9	30.00
Non Poor (> 2/3 of MPCHE)	> 24813.33	19	63.33
		30	**100**

Source: Field Survey Data, 2016

Table 3 shows that only 36.67% of Cocoa farmers in Ikwuano LGA fell below the estimated poverty line while the other 63.33% were classified as non-poor. Non-poor Cocoa farmers in Ikwuano LGA were those farmers whose per capita household expenditure (MPCHE) was above or was equal to two-third (2/3) of the mean per capita expenditure of all the Cocoa farmers in Ikwuano, while those whose per capita expenditure was below two-third of the mean per capita expenditure were classified as poor. Based on this development, poverty line was constructed as two-third of the mean per-capita expenditure of all the Cocoa farmers in Ikwuano LGA; which was ₦24,813.33. This implies that Cocoa farmers in Ikwuano LGA whose monthly per capita expenditure fell below ₦24,813.33 were classified as poor while Cocoa farmers in Ikwuano whose per capita expenditure equaled or was above the poverty line were classified as non- poor.

The implication of this result is that majority of the Cocoa farmers in the study area were non-poor. Lawal *et al*., 2011 and Adepoju, 2012 opined that the number of those in poverty has continued to increase In Nigeria, despite the various efforts of government to reduce the incidence through different poverty alleviation programmes and strategies. In a contrasting view, Osayande and Osabuohien (2016) stated that the number of poor Nigerians is put as 58 million or 33.1 percent of the population. This represents an improvement from the previous study conducted in 2009/2010 which put the poverty level at 61% of Nigeria's population. The promotion of Cocoa farming can stimulate linkages between farm and other non-farm activities, which are important for poverty reduction. Iheke *et al*., (2013) suggested that to reduce poverty and hunger in the region, there is an urgent need for global, national, and local actors to pursue innovative approaches to improve agricultural productivity.

Table 4. Mean Per Capita Expenditure (MPCHE) of Cocoa Farmers in Umuahia North LGA; based on their Basic Needs

Basic Needs (Consumption Expenditure)	Amount/Month (₦) MPCHE	Amount/Annum (₦) MPCHE	Percentage (%) of Total Expenditure
3 square meal (food)/drinks	11830.00	141960.00	25.70
Clothing	7850.00	94200.00	17.05
Health/Medication	2730.00	32760.00	5.93
Education	2399.33	28791.96	5.21
Rental Value of Residence/shelter	13833.33	165999.96	30.05
Transportation Cost	3606.67	43280.04	7.83
Miscellaneous	3773.33	45279.96	8.19
TOTAL MPCHE	**46,022.67**	**552,271.92**	**100**
2/3 of MPCHE	30,681.78	368,181.28	
1/3 of MPCHE	15,340.89	184,090.64	

Source: Field Survey Data, 2016

Table 4 shows the mean per capita household expenditure of Cocoa farmers in Umuahia North LGA of Abia State. From the table it is observed that rental value of residence/shelter accounted for a whopping 30.05% of the MPCHE of the farmers. This could be as a result of high cost of living experienced in Umuahia Metropolis as compared to other rural areas of the state. People in towns and cities may have more wealth and resources due to higher infrastructural facilities and employment than those in villages or hamlets, their standard of living is also generally higher.

Food, clothing, miscellaneous expenses and transportation cost constituted 25.7%, 17.05%, 8.19% and 7.83% respectively. A total of 5.93% was spent on medication and health care while 5.21% was spent on education. Greater percentage of MPCHE was spent on food items. The findings also agreed with that of Okezie *et al*., (2011) that food is the most fundamental human need. Asogwa *et al*., (2012) observed that a 1% increase in household income, farm size, economic efficiency and formal education would reduce the intensity of household poverty by 2.69%, 2.28%, 2.21% and 1.02% respectively, and vice versa. On the other hand, a 1% increase in the total value of household assets and the extent of agricultural product commercialization would reduce the intensity of household poverty by 0.15% and 0.06% respectively, and vice versa.

The implication of this result is that fewer amounts were spent on transportation, medication and education respectively. In regards to transportation cost, Umuahia North LGA of Abia state has more efficient road network compared to Ikwuano LGA. This justified the results of Cocoa farmers' MPCHE on transportation in Ikwuano LGA; which accounted for 21.18%, while that of Umuahia North LGA accounted for only 7.83%.

Okpachu *et al*., (2014) posits the major problems facing Agricultural productivity in Nigeria is illiteracy. This has over the years posed great challenges to Agricultural development as well as productivity. The level of literacy of farmers in Nigeria generally affects agricultural practices.

Table 5. Classification of Cocoa Farmers in Umuahia North LGA, Abia State According to their Poverty Status

Poverty Status	MPCHE Amount (₦)	Frequency	Percentage (%)
Extremely/Core Poverty (1/3 of MPCHE)	< 15340.89	0	0.00
Moderately Poor (2/3 of MPCHE)	15340.89 ≤ Z < 30681.78	12	40.00
Non Poor (> 2/3 of MPCHE)	> 30681.78	18	60.00
		30	**100**

Source: Field Survey Data, 2016

Table 5 shows the classification of Cocoa farmers in Umuahia North LGA, based on their poverty status; two – thirds (2/3) of the Mean Per Capita Household Expenditure was taken for the moderate poverty line, while one – third (1/3) was taken as the core/extreme poverty threshold. The value of the moderate poverty line was defined as ₦30,681.78 while the extreme poverty line was put at ₦15,340.89. Based on these estimated poverty thresholds, Cocoa farmers in Umuahia North LGA were classified into mutually exclusive groups as seen in Table 5 above.

The analysis shows that 40% of Cocoa farmers in Umuahia North LGA were moderately poor while the other 60% were classified as non-poor. The implication of this result is that majority of the Cocoa farmers in the study area were non-poor. Nkang *et al*., (2009) observes that in terms of foreign exchange earnings, no single agricultural export commodity has earned more than Cocoa. Osayande and Osabuohien (2016) stated that the number of poor Nigerians is put at 33.1 percent of the population. This represents an improvement from the previous study conducted which put the poverty level at 61 percent of Nigeria's population.

Evidence has however shown that the growth rate of Cocoa production has been declining, which has given rise to a fall in the fortunes of the sub-sector among other reasons (Nkang *et al*., 2009). However, the problem is that most individual investors and even governments have only vague ideas, of the potential of the industry and as such are sometimes slow in committing investment funds into the sub-sector.

Table 6. Mean Per Capita Expenditure (MPCHE) of Cocoa Farmers in Bende LGA; based on their Basic Needs

Basic Needs (Consumption Expenditure)	Amount/Month (₦) MPCHE	Amount/Annum (₦) MPCHE	Percentage (%) of Total Expenditure
3 square meal (food)/drinks	14066.67	168800.04	29.38
Clothing	6833.33	81999.96	14.27
Health/Medication	3316.67	39800.04	6.92
Education	1682.67	20192.04	3.51
Rental Value of Residence/shelter	12446.67	149360.04	25.99
Transportation Cost	6153.33	73839.96	12.85
Miscellaneous	3376.33	40515.96	7.05
TOTAL MPCHE	**47,875.67**	**574,508.04**	**100**
2/3 of MPCHE	31,917.11	383,005.36	
1/3 of MPCHE	15,958.55	191,502.68	

Source: Field Survey Data, 2016

Table 6 shows the average monthly and yearly expenses on Mean Per Capita Household Expenditure (MPCHE) of Cocoa farmers in Bende LGA of Abia State. From the table, it is observed that food constitute 29.38% of the MPCHE of the farmers. Other non-food items such as clothing, health/medication, education, rental value of residence, transportation cost and miscellaneous; and other unlisted commodities accounted for the remaining 70.62%. The implication of the result is that food is very necessary for individuals, firms and organization. Etim *et al*., (2010) posits that food constitute the highest Mean Per Capita Expenditure of farm households in Nigeria. Education (3.51%) has the least percentage of MPCHE. The higher the education of the Cocoa farmers, *Ceteris paribus*, the higher the increased agricultural productivity.

Two – thirds (2/3) of the Mean Per Capita Household Expenditure was taken for the moderate poverty line for Cocoa farmers in Bende LGA, while one – third (1/3) was taken as the core/extreme poverty threshold. The value of the moderate poverty line was delineated as ₦31,917.11 while the extreme poverty line was put at ₦15,958.55. Based on these estimated poverty thresholds, Cocoa farmers in Bende LGA; Abia State were classified into mutually exclusive groups as presented in Table 7

Table 7. Classification of Cocoa Farmers in Bende LGA, Abia State According to Poverty their Status

Poverty Status	MPCHE Amount (₦)	Frequency	Percentage (%)
Extremely/Core Poverty (1/3 of MPCHE)	< 15958.55	3	10.00
Moderately Poor (2/3 of MPCHE)	15958.55 ≤ Z < 31917.11	11	36.67
Non Poor (> 2/3 of MPCHE)	> 31917.11	16	53.33
		30	**100**

Source: Field Survey Data, 2016

Table 7 shows that only 10% of Cocoa farmers were extremely poor in the study area; while 36.67% of Cocoa farmers fell below the estimated poverty line or were moderately poor while the remaining 53.33% were classified as non-poor. The implication of this result is that majority of the Cocoa farmers in the study area were non-poor. Adepoju, (2012) opined that the number of those in poverty has continued to increase. The World Bank in 2009 stated that about 2.8 billion persons of the World's population live on less than $2 a day, and 1.4 billion on less than $1 a day. In a contrasting view, Osayande and Osabuohien (2016) stated that the number of poor Nigerians is put as 58 million or 33.1 percent of the population. This represents an improvement from the previous studies which put the poverty level at 61% of Nigeria's population.

Table 8. Mean Per Capita Household Expenditure (MPCHE) of Cocoa Farmers in (Ikwuano, Umuahia North and Bende LGA) Abia State; based on their Basic Needs

Basic Needs (Consumption Expenditure)	Amount/Month (₦) MPCHE	Amount/Annum (₦) MPCHE	Percentage (%) of Total Expenditure
3 square meal (food)/drinks	12365.56	148386.72	28.30
Clothing	6911.11	82933.32	15.81
Health/Medication	2937.78	35253.36	6.72
Education	1894.00	22728.00	4.33
Rental Value of Residence/shelter	10371.11	124453.32	23.73
Transportation Cost	5881.11	70573.32	13.45
Miscellaneous	3345.44	40145.28	7.65
TOTAL MPCHE	**43,706.11**	**524,473.32**	**100**
2/3 of MPCHE	29,137.41	349,648.88	
1/3 of MPCHE	14,568.70	174,824.44	

Source: Field Survey Data, 2016

Table 8 represents the cumulative mean per capita household expenditure of Cocoa farmers in Abia State. This comprises the Cocoa farmers located in the three Local Government Areas of the State, where Cocoa was mainly produced. From Table 8, it is observed that food items accounted for 28.3% of the MPCHE of the farmers in the State. The highest percentage of food items could be as a result of the necessity food have for individuals, firms and organization. Etim *et al*., (2010) posits that food constitute the highest Mean Per Capita Expenditure of farm households in Nigeria.

Rental value of land, clothing, transportation, miscellaneous expenses, health and education constituted 23.73%, 15.81%, 13.45%, 7.65%, 6.72% and 4.33% respectively. Greater percentage of MPCHE was spent on accommodation or rental value of residence. Also fewer amounts were spent on transportation, health care and education expenses respectively. In regards to low amount of MPCHE on education, Okpachu *et al*., (2014) posits that the major problems facing Agricultural productivity in Nigeria is illiteracy. This has over the years posed great challenges to Agricultural development as well as productivity. In this regards, farm firms should inculcate the habits of acquiring formal and informal knowledge; especially formal knowledge backed up with scientific approach on agricultural production, sustainability and development.

Table 9. Distribution of all the Cocoa Farmers in (Ikwuano, Umuahia North and Bende LGA) Abia State, According to their Poverty Status

Poverty Status	MPCHE Amount (₦)	Frequency	Percentage (%)
Extremely/Core Poverty (1/3 of MPCHE)	< 14568.70	5	5.56
Moderately Poor (2/3 of MPCHE)	14568.70 ≤ Z < 29137.41	32	35.56
Non Poor (> 2/3 of MPCHE)	> 29137.41	53	58.89
		90	**100**

Source: Field Survey Data, 2016

Two – thirds (2/3) of the Mean Per Capita Household Expenditure was taken for the moderate poverty line for Cocoa farmers in Abia State, while one – third (1/3) was taken as the core/extreme poverty threshold. The value of the moderate poverty line was defined as ₦29,137.41 while the extreme poverty line was put at ₦14,568.70. Based on these estimated poverty thresholds, Cocoa farmers in Abia State were classified into mutually exclusive groups as presented in Table 9. The non-poor Cocoa farmers in Abia State were classified as those farmers whose per capita expenditure was above or was equal to two-third (2/3) of the mean per capita household expenditure (MPCHE) of all the farmers, while those whose per capita expenditure was below two-third of the mean per capita expenditure were classified as poor. Based on this status quo, the poverty line constructed as two-third of the mean per-capita expenditure of all the Cocoa farmers found in Abia State was ₦29,137.41. This implies that all the Cocoa farmers in Abia State whose monthly per capita expenditure fell below ₦29,137.41 were classified as poor while the Cocoa farmers whose per capita expenditure equaled or was above the poverty line were classified as non- poor.

Table 10 shows that only 41.12% of Cocoa farmers in Abia State fell below the estimated poverty line while the other 58.89% were classified as non-poor. The implication of this result is that majority of the Cocoa farmers in Abia State were non-poor. Osayande and Osabuohien (2016) stated that the number of poor Nigerians is put as 58 million; this represents an improvement from previous study which put the poverty level at 61% of Nigeria's population.

The ultimate goal of agricultural production plans in national development is to raise the standard of living and one of the important yardsticks for measuring standard of living is the average distribution income. Iheke *et al*., (2013) suggested that to reduce poverty and hunger, improve the standard of living and increase farm income there is an urgent need for global, national, and local actors to pursue innovative approaches to improve agricultural productivity.

Conclusion and Recommendation

Majority of the Cocoa farmers (86.67%) were males and only 13.33% were females. This can be attributed to the high intensive labour requirement for Cocoa farming which the male gender could afford. Analysis from poverty status of the Cocoa farmers showed that 36.67% of Cocoa farmers in Ikwuano LGA fell below the estimated poverty line while the other 63.33% were classified as non-poor, while 40% of Cocoa farmers in Umuahia North LGA were moderately poor while the other 60% were classified as non-poor. In Bende LGA, 10% of the Cocoa farmers are extremely poor, 36.67% are moderately poor while the remaining 53.33% were classified as non-poor. It is therefore recommended that greater equality of income is achieved by improving the productivity of the poor, especially through improving credit facilities, basic education, health and the skills of the farmers. Improvement in basic education of the farmers will lead to increased income and enhancement in the quality of life which invariably reduces poverty.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Visible evidence of climate change and its impact on fruit production in Nepal

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Abstract

In recent years, Nepal is experiencing visible impact of climate change in agriculture. This review paper describes the recent climate change pattern and phenomenon with its impact on fruit production in Nepal. Various researches suggest that change in temperature has played vital to reduce the level of soil organic carbon, and soil micronutrients. With exposure to extreme temperatures, insects may produce heat shock proteins, cryoprotectants and osmolyte compounds within their bodies to survive, so these insects can exist in such environment. Most of the researches resulted that higher temperature induces early flowering under the subtropics may result in low fruit-set because of abnormalities arising from prevailing low night temperatures. Similarly, insufficient chilling greatly influences flower initiation and fruit coloration along with deterioration in fruit texture and taste. Besides the effect of high and low temperature, papers also claimed that decline in rainfall from November to April adversely affects the winter and spring crops that ultimately reduces food production. Hence, organization related to fruit research, education and development in Nepal have to run effective program to bring new genetic sources that can resist the adverse effect of climate and may bring some positive vibe to tackle the climate change.

Keywords: Climate change, Global warming, Fruit species, Sustainable agriculture

Introduction

Agriculture is a vital part of the Nepalese economy. It contributes to about a third of the country's GDP and involves about two-thirds of its population (SANDEE, 2014). Horticulture sector contribute 23 % in AGDP out of which fruit sector contribute half (FDD, 2015).An average size of ownership of agricultural land in Nepal is 0.85 ha per household, but majority (45%) owns less than 0.5 ha (Timsina, 2011a). Horticultural crops mainly vegetable, spices, fruit and flower are prime sector and ranked third after agronomical and livestock resources in farmer's preferences (MoAD, 2015). The unique agro-ecological zones favored by altitudes, topography, and aspect with in the country offer an immense opportunity for growing different types of fruit crops (Atreya and Manandhar, 2016).The current total area, productive area, production and productivity of fruits are are 1,50,387 ha, 1,10,802 ha, 9,92,703 mt and 8.96 mt/ha respectively in FY 2014/015 (FDD, 2016). Out of total cultivated area fruit covered 4.79% (MoAD, 2015).

In recent years, Nepal is experiencing visible impact of climate change on agriculture, biodiversity, health, tourism, infrastructure and water resources (Timsina, 2011b).The established commercial varieties of fruits, vegetables and flowers performed poorly in an unpredictable manner due to aberration of climate (Datta, 2013). Problem of frequent drought, severe floods, landslides and mixed type of effects in agricultural crops (Malla, 2008) and Increased risk of hailstorm, flooding, pest and diseases will also adversely affect farming because of climate change (Adhikari, 2014). It is likely that climate change and increasing variability will have both negative and positive impacts on production systems (LI-Bird, 2009). Effects of climate change on agriculture are particularly high as the agriculture produces food and provides the primary source of livelihood for large chunks of weaker sections of the society (Pant, 2012). Many studies so far have focused on the impact

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of climate change on cereals and major crops only despite the importance of fruits in terms of nutrition and food security (Sthapit and Scherr, 2012b), very little or no work has been done. As woody perennials, fruits trees are perceived to be less susceptible to the changing climate. The changes in climate in the form of erratic precipitation, increase in temperature, lesser days serving as the chilling period have started affecting the mountain agricultural production systems and ultimately the food security of the people (Datta, 2013).

Methodology

This paper was prepared based on review from different published and unpublished printed materials, books, research papers, reports of different organizations. Discussion was made with horticulturists, farmers, lecturers/professors of universities and indigenous communities involved in fruit production. Related different web sites also visit during reviewed period.

Result and Discussion

Fruit production status and climate change vulnerability

Status of fruit production in Nepal (2015/16)

Table 1. Area, production and productivity of fruits in Nepal

Fruits	Area (ha)	Productive area (ha)	Production (mt)	Production rate(mt/ha)
Summer fruits	84227	68691	641759	9.34
Citrus fruits	39035	25261	222789	8.82
Winter fruits	27125	16849	128155	7.61
Total	150387	110802	992703	8.96

Source: FDD, 2016

Major fruits grown in Nepal

Table 2. List of major fruits with scientific name and family which are grown in Nepal

English name	Scientific name	Family	Nepali Name
1. Tropical Fruits			
Mango	*Mangifera indica*	Anacardiaceae	Aanp
Litchi	*Litchi chinensis*	Sapindaceae	Litchi
Banana	*Musa paradisiaca*	Musaceae	Kera
Plantain	*Musa sapientum*	Musaceae	Tarkari Kera
Coconut	*Cocos nucifera*	Anecaceae	Nariwal
Pineapple	*Ananas comosus*	Bromeliaceae	Bhuin katahar
Mountain Papaya	*Carica candomarcensis*	Caricaceae	Pahari mewa
Papaya	*Carica papaya*	Caricaceae	Mewa
Jackfruit	*Artocarpus heterophyllus*	Moraceae	Rukh katahar
Date	*Phoenix dactylifera*	Palmae	Chhohara
Bael	*Aegle marmelos*	Rutaceae	Bel
Sapota	*Achras zapota*	Sapotaceae	Sapota
Cashew nut	*Anacardium occidentale*	Anacardiaceae	Kaju
Guava	*Psidium guajava*	Myrtaceae	Amba, Belauti
Avocado	*Persea americana*	Lauraceae	Ghew phal
Jujube	*Zizyphus mauritiana*	Rhamnaceae	Ber
B. Sub Tropical Fruit			
Lime	*Citrus aurantifolia*	Rutaceae	Kagati
Sour orange	*Citrus arantium*	Rutaceae	-
Pummelo	*Citrus grandis, C. Maxima*	Rutaceae	Bhogate
Rough lemon	*Citrus jambhiri*	Rutaceae	Kathe Jyamir
Sweet lime	*Citrus limettioides*	Rutaceae	Chaksi
Lemon	*Citrus limon*	Rutaceae	Nibuwa
Citron	*Citrus medica*	Rutaceae	Bimiro
Hill lemon	*Citrus psuedolimon*	Rutaceae	Pahari nibua
Mandarin	*Citrus reticulata*	Rutaceae	Suntala
Sweet orange	*Citrus sinensis*	Rutaceae	Mausam
Kumquat	*Citrus japonica*	Rutaceae	Muntala
Trifoliate orange	*Citrus trifoliata*	Rutaceae	Tinpate suntala
Nepalese Hog plum	*Choerospondias axillaris*	Anacardiaceae	Lapsi

English name	Scientific name	Family	Nepali Name
Pistachio nut	*Pastacia vera*	Anacardiaceae	Pesta
Indian hog plum	*Spondias pinnata*	Anacardiaceae	Amaro
Tea	*Thea sinensis*	Combretaceae	Chiya
Aonla	*Embilica officinalis*	Euphorbiaceae	Amala
Chestnut	*Castanea crenata*	Fagaceae	Katus
Tamarind	*Tamarindus indica*	Leguminosae	Imali
Grapes	*Vitis vinifera*	Vitaceae	Angoor
Fig	*Ficus carica*	Moraceae	Anjir
Mulberry	*Morus alba*	Moraceae	Seto Kimbu
Mulberry	*Morus Indica*	Moraceae	Kimbu
Pomegranate	*Punica granatum*	Punicaceae	Aanar
Coffee	*Coffea arabica*	Rubiaceae	Coffee
Coffee	*Coffea robusta*	Rubiaceae	Coffee
C. Warm Temperate Fruit			
Persimmon	*Diospyros kaki*	Ebenaceae	Haluwabed
Olive	*Olea europaea*	Oleaceae	Jaitun
Olive	*Olea cuspidata*	Oleaceae	Jangali Jaitun
Apricot	*Prunus armeniaca*	Rosaceae	Khurpani
Plum	*Prunus domestica*	Rosaceae	Aalu bakhara
Peach	*Prunus persica*	Rosaceae	Aaru
Pecan nut	*Carya illinoensis*	Juglandaceae	Chuche Okhar
Japanese plum	*Prunus salicina*	Rosaceae	Aalucha
Sand pear	*Pyrus communis*	Rosaceae	Naspati
European pear	*Pyrus pyrifolia*	Rosaceae	Bedeshi naspati
D. Temperate Fruits			
Apple	*Malus pumila, M. domestica*	Rosaceae	Syayu
Walnut	*Juglans regia*	Juglandaceae	Okhar
Crab apple	*Malus baccata*	Rosaceae	Mayel
Almond	*Prunus amygadalus*	Rosaceae	Badam

Source: Shrestha, 1998; Singh, 1969; Shrestha et.al., 2001; Regmi and Shrestha, 2005; Tomiyashu et.al.,1999

Vulnerable district with respect to climate change

Table 3. Vulnerability ranking of fruit growing districts of Nepal

Vulnerability ranking	Districts
Very high (0.787-1.000)	Kathmandu, Ramechhap, Udayapur, Lamjung, Mugu, Bhaktapur, Dolakha, Saptari, Jajarkot,
High (0.61-0.786)	Mahottarai, Dhading, Taplejung, Siraha, Gorkha, Solukhumbu, Chitwan, Okhaldhunga, Achham, Manang, Dolpa, Kalikot, Khotamg, Dhanusha, Dailekh, Parsa, Salyan,
Moderate (0.356-0.600)	Sankhuwasabha, Baglung, Sindhuli, Bhojpur, Jumla, Mustang, Rolpa, Bajhang, Rukum, Rauthat, Panchthar, Parbat, Dadeldhura, Sunsari, Doti, Tanahu, Makawanpur, Myagdi, Humla, Bajura, Baitadi, Bara, Rasuwa, Nawalparasi, Sarlahi, Sindhuplachok, Darchula, Kaski
Low (0.181-0.355)	Nuwakot, Dhankuta, Kanchanpur, Bardiya, Kapilbastu, Terathum, Gulmi, Pyuthan, Surkhet, Argakhachi, Morang, Dang, Lalitpur, Kailali, Syanja, Kavrepalanchok
Very low (0.000-0.180)	Ilam, Jhapa, Banke, Palpa, Rupandehi

Source: NAPA,2010

The ranking of the district shows that majority of the districts are vulnerable to climate change. The analysis suggests that 1.9 million people are highly climate vulnerable and 10 million are increasingly at risk, with climate change likely to increase this number significantly in the future. Figure inside parenthesis indicate climate change vulnerability index.

It is not always possible to determine which changes are due to environmental degradation and which are due to climate change. The important thing is to understand what is changing and to plan an appropriate response. Despite bestowedfavorable geographic and climatic factors for growing varieties of fruit, fruit industry in Nepal is constrained by a number of factors such as monsoon rains during the period of fruit development and maturation, uneven distribution of rainfall, dry springs and winters, spring frosts and winds, hailstones during fruit development and ripening are major (Acharya and Atreya, 2013).

Drivers of climate change and its impact

Impact of temperature

Increasing temperatures will directly impact crops by affecting their physiology. Higher temperatures will also indirectly affect crops through changes in the water regime and due to the increase in the intensity of pests and diseases, they will produce. The warming, moreover, is projected to be strongest in the Himalayan Highlands (including the Tibetan Plateau) and in the arid regions of Asia (Karn, 2014). Early greening of vegetation and early breeding of organism in response to warming (Adhikari, 2014). Higher temperature speed plant growth and development in annual crops (Bakshi, 2015). Mean annual temperature to increase by 1.4 degree Celsius by 2030, 2.8 degree Celsius by 2060 and 4.7 degree Celsius by 2090 (NCVST, 2009). The temperature is increasing in faster rate than the previous decades. Same studies show higher temperature increment for winter compared to the summer and monsoon seasons. In terms of spatial distribution, the NCVST (2009) study shows a higher increment in temperature over western and central region as compared to eastern Nepal for the year 2030, 2060 and 2090. As temperature increases, cropping pattern as well as disease of human and livestock's can be expected to shift in higher ecological zones. Similarly, increase in temperature may lead to reduce the level of soil organic carbon, soil micronutrients and accelerate decomposition by activating the microbial population in the soil (Malla, 2003). These findings are also supported by Timsina as he stated that increases in temperature may have impact on the changes in the timing, intensity and volume of rainfall and rising carbon dioxide levels (Timisina, 2011). In another hand, other researchers identified some positive vibe of higher temperature on fruit development considering the quantity and quality of fruits. Higher temperature at the fruit development stage speeds up maturity, fruit size and quality, Temperature also determines the number and quality of flowers, and thus directly influences the fruiting potential for the season (Dinesh and Reddy, 2012).

Impact of rainfall variation

Nepal is already a country vulnerable to natural disasters, particularly floods and landslides. Much of the population is relay on rain fed agriculture to feed their family which is vulnerable to drought and more variable precipitation. With increased intensity of summer monsoon, the risk of flash flooding, erosion and landslides are supposed to be increased (FAO, 2010). There has already been an increase in the frequency and intensity of rainfall events in many parts of Asia, which has been largely attributed to increasing temperature. Such changing climate changes have caused severe floods, landslides and mudflows (Karn, 2014). Which has supported by Shrestha et al. (2003) who suggested that the number of flood days and consecutive days of flood events have been increasing in Nepal where changes in amount and seasonality of precipitation have affected soil moisture and groundwater reserves. Furthermore, Dahal and Khanal have provided an evidence that high increases in summer river flow are witness of leading to faster glacial melt (Dahal and Khanal 2010). There have been frequent droughts, soil erosion and land degradation through landslides, river cutting and floods, all resulting in a decline in production of crops. Unpredictable rainfall pattern resulting in more in some place and less rain in others. Moreover, IPCC (2007) projected that there will be a general increase in the intensity of heavy rainfall and an overall decrease by up to 15 days in the annual number of rainy days over a large part of the South Asia. Crops are also affected by more intense rainfall and other extreme weather events (Karn, 2014). Decline in rainfall from November to April adversely affect the winter and spring crops that ultimately reduces food production and threatened to the food security of people .Koshi, Narayani, Karnali and Mahakali are four major rivers of Nepal flowing from the Himalayas, which are the main source of water of the whole country. Agricultural sectors: mainly crops, livestock's and horticulture largely depend on the surface water sources in the country (Malla, 2008), however, floods from these rivers have increased in each year and have taken lives, destroyed physical assets, displaced people and inundated and deposited sediments on agricultural land. Terai is the most prone area of the flooding (Timisina, 2011).

Impact of drought

During the summer and normally drier months frequency and intensity of droughts seems also to have increased (Karn, 2014). This will affect agricultural production with water and sanitation, leading to increase the levels of malnutrition and incidence of water-borne disease (FAO, 2010).Such drought has been another phenomenon of climate change and has affected both winter and summer crops (Timisina, 2011).

Impact of wind and hot/cold wave

Winter drought along with cold wave is responsible to cause widespread damage to agriculture in Terai and Mid-hills of Nepal (FAO, 2010).

Impact of greenhouse gases

Greenhouse gases (GHGs) like Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are the three main responsible for climate change. Among all greenhouse gases, CO_2 can significantly stimulate the growth, development and reproduction of plants including fruits. (Sthapit and Scherr, 2012 a). C3 plants benefit much more from increase in CO2 than C4 which has beneficial effect on plants to increase productivity. Atmospheric CO2 found more hazardous which is increasing exponentially and will likely to double i.e. about 700 ppm within the next century (Bakshi, 2015).

Impact of air pollution

The findings from Datta, air pollution has significantly reduced the yield of several horticultural crops and increases the intensity of certain physiological disorders like black tip of mango, which is induced by coal fume gases, sulphur dioxide, ethylene, carbon monoxide and fluoride (Datta, 2013). 3.2.7. Severity of fruit insect and pest in changing climate It was observed that warmer temperatures generally lead to rapid development and survival of insects in mid to high latitudes, which can account for detectable and unambiguous shifts in a range of insect species over the past half century (Matthew P Ayres, 2010). Insect crop pests and their natural enemies may also have modified their physiological system to cope with warmer temperatures. With exposure to extreme temperatures (both minimum and maximum temperatures), insects may produce heat shock proteins, cryoprotectants and osmolyte compounds within their bodies to survive shortterm exposure to high and low temperatures (Colinet et al. 2015; Ghaedi and Andrew 2016).

Consequences of climate change

The rising temperature and emission of CO2 to some extent is helpful in production of major crops. For example, increase in agricultural production by enhancing photosynthetic processes, water use efficiency, shortening physiological period and soil microbial activities. Increase in respiration process, fertilizer use efficiencies, shift in agricultural zone, increase in insect pest population, desertification, increase in soil erosion, evapo-transpiration and cause malnutrition in a world overflowing with food due to reducing protein and decrease in mineral nutrients content in different crops are negative effects (Malla, 2008).In perennial crop, being grown in a climate near its optimum, a temperature increase of several degrees could reduce photosynthesis and shorten the growing period affecting the productivity e.g. banana (Bakshi, 2015). The positive effects of climate change such as longer growing seasons, lower natural winter mortality, and faster growth rates at higher altitudes may be offset by negative factors such as changes in established reproductive patterns, migration routes, and ecosystem relationships (Adhikari, 2014). As in many developing countries, concerns are increasing about the negative impact of climate change on agricultural yields and food security (SANDEE, 2014). Decline in biodiversity and bio-cultural practices one of the adverse impacts of modern agriculture and a likely outcome of climate change have also caused many problems, especially the food crisis (Adhikari, 2014). Increase in temperature and CO2 will lead to an increase in population of pests and severity of diseases in presence of host plant. It increases the rate of reproductive cycle of insect and pest. The increase in insect population leads to demand for more use of pesticide, which unknowingly causes lots of harm to ecosystem as well as human society (Malla, 2008). Severe droughts and intense flooding create problems for field operation, more compaction of soil, and possible crop losses due to lack of oxygen for roots and disease problems associated with wet condition and flooding (Bakshi, 2015). Insufficient chilling greatly influences flower initiation and fruit coloration along with deterioration in fruit texture and taste. Further, the lack of proper chilling is also posing serious problems like scab disease, premature leaf fall and infestation of red spider mite in apple.

Implication of climate change in fruit production

A large variety of fruits are grown in Nepal, of which Summer fruits: mango, litchi, banana, guava, papaya, jackfruit, pineapple, arecanut, coconut; Citrus fruits: mandarin orange, sweet orange, lime, lemon, ; Winter fruits: apple, pear, walnut, peach, plum, pomegranate, apricot,

persimmon, hog plum and kiwi fruit are widely cultivated (Atreya, 2014). As fruit crops are perennial moving production area is difficult. In areas where current temperatures are below optimal for specific crops, there will be a benefit, while in areas where plants are near the top of their optimal range, yields will decrease (Bakshi, 2015). A significant change in climate at global and national level is certainly impacting our fruit production and quality. Climate change is one major factor affecting the fruit crop. Fruit yield is a function of light interception, variety's photosynthetic efficiency and cost of respiration (Dinesh and Reddy, 2012). Various quality traits such as fruit coloration, spottiness, fruit texture and taste can be altered by change in temperature, humidity and rainfall (Dinesh and Reddy, 2012).Climate change is likely to increase the occurrence of pests and diseases affecting humans, crops, animals and forests (Shrestha et al., 2003). The development and spread of crop diseases, pests and weeds will also have an adverse impact on agriculture, human health, and the environment (Malla 2008).Overall, it is clear that, when all factors are taken into account, climate change is likely to have a negative impact on agricultural yields in South Asia (Karn, 2014).Climate change effects on horticultural crops are speedily becoming issues in the present situation. Tropical fruits (banana, mango, papaya) and other crop like (croton) has been adopted in mid hills and observed off-season flowering in high altitudes crops like peach, pear and apple (Malla, 2008).

Tropical fruits

Incidence of pest and diseases would be most severe in tropical region due to climate change (Malla, 2008). Even a minor climate shift of 1-2°C could have a substantial impact on the geographic range of these crops. High night temperature reduces anthocyanin accumulation, increased humidity due to prolonged rainfall makes fruits tasteless and skin cracking, high temperature above 115°F causes thick skin similarly rainfall during flowering and fruiting is detrimental in grape (Bakshi, 2015).Early flowering under the subtropics may result in low fruit-set because of abnormalities arising from prevailing low night temperatures. Mango phenology is highly influenced by variations in temperature. Water stress is known to increase the fruit drop in mango. High humidity, rainfall and frost during flowering are harmful. High temperature by itself is not so injurious to mango, but in combination with low humidity and high winds affects the growth of the mango trees adversely (Bakshi, 2015). Rains during fruiting periods may blacken fruits in mango or prevent desirable fruit coloration in guava, making the produce less appealing for the consumers (Dinesh and Reddy, 2012). The unusual impact of climate change has been witnessed in litchi production system as noted in flowering pattern (shifted early), fruit growth and harvesting periods. The young plants of litchi require protection from frost and hot desiccating winds otherwise their growth and survival is affected (Bakshi, 2015).Due to high temperature physiological disorder of horticultural crops will be more pronounced eg. Spongy tissue of mango, fruit cracking of litchi (Datta, 2013). Increase in temperature at maturity will lead to fruit cracking and burning in litchi (Kumar and Kumar 2007). The climate change increases the atmospheric temperature and change of rainfall pattern, as a result, banana cultivation may suffer from high temperature, soil moisture stress or water logging (Datta, 2013).In papaya, higher temperatures have resulted in flower drops in female and hermaphrodite plants as well sex changes in hermaphrodite and male plants. The promotion of stigma and stamen sterility in papaya is mainly because of higher temperatures (Bakshi, 2015).

Sub tropical fruits

Pest and disease of plain ecosystem may gradually shift to hills and mountains (Malla, 2008).Some pathogens of important crops from Terai zones has adapted in hills and mid-hills that may adversely affect the fruit production for example Citrus psylla that once only populated below 1000 masl are now able to survive in mid hill up to 1500 masl. that is due to increase in temperature. Seventy-five percent increase in air CO_2 content increased sourness in orange . Vitamin C was increased approximately by 5 percent due to increase in carbon dioxide gas (Kimball and Mitchell, 1981) in citrus. High temperature and high evaporation during flowering and fruit set result in low yield due to flower and fruit drop. The fruits have poor colour if the temperature during fruit maturation is high. In Navel oranges the content of acidity was affected by low temperature leading to low TSS content (Bakshi, 2015). Low chilling fruits being cultivated in sub-tropics are also under the threat due to non-availability of required chilling hours which has adverse effect on their flowering and the abrupt rise in temperature after fruit set is causing excessive fruit drop as well as there is poor sugar accumulation in the fruit due to steep rise in temperature during fruit development. Fruit that are exposed to high sunlight and high temperature conditions near harvest may experience sunburn to the skin that can cause cracking. Severely stressed, because of high temperature and insufficient rainfall, premature fruit drop may occur in peach(Bakshi, 2015). Specific chilling requirements of pome and stone fruits will be affected hence dormancy breaking will be earlier. Delay in monsoon, dry spells of rains, and untimely rains during water stress period, supra-optimal temperatures during flowering and fruit growth, hailstorms are some of the most commonly encountered climatic conditions experienced by the citrus growers over the past decade (Datta, 2013). The stability of the genotype to perform under different environment is the ultimate deciding factor in the expression of any trait act fruit industry and region (Bakshi, 2015).

Temperate fruits

Climate change is likely to affect chilling requirement of temperate fruit crops significantly and therefore, the opportunity to meet this requirement will be reduced as the climate becomes warmer. Increase in average global temperature would move the existing plant species and varieties to new latitudinal belts with favorable climates. It is, therefore, possible that crops that are used to be productive in one area may no longer be so or the other way round. The resultant of these climate changes are clearly apparent in the shifting of apple cultivation from lower elevations to higher altitudes in India (Rai et. al., 2015). On set of early snow in December and January had occurred more infrequently overtime and extended through the months of Feb. and March. Early snow contributes nitrogen for plant use, replenish soil moisture and prevent humidity build up. Amount of snow determines the number of chilling hours and thereby the times of bud break (Bakshi, 2015).Cool temperatures in the winter are essential for fulfilling their chilling requirements to ensure homogeneous flowering and fruit set and generate economically sufficient yields. In order to escape the damage of sensitive tissues from winters, trees from temperate or cold climates have evolved the mechanism of dormancy.The climate change affects not only the winter chilling of fruit crops but it also affects the other aspects like increase in the incidence of physiological disorders, pollination failure and phenology. As global warming is considered inevitable, endeavor should thus be undertaken to manipulate the chilling requirements of the temperate fruit crops by various means, so that the effect of changing climate could be mitigated more efficiently (Rai et. al., 2015). High temperature and moisture stress also increase sunburn and cracking in apples, apricot and cherries (Kumar and Kumar 2007). Melting of ice cap in the Himalayan regions will reduce chilling effect required for the flowering of many of the horticultural crops like Apple (Datta, 2013). With warmer winters, particularly at higher altitudes, less precipitation will fall as snow, further accelerating glacial retreat but also reducing soil moisture and accelerating erosion and therefore impacting on winter crops (FAO, 2010). Vulnerability, rarity and rapid extinction of many species of temperate fruits will be among the other consequences of climate change . High temperature and moisture stress is increasing sunburn and cracking in apple, apricots and cherries in the higher altitudes . Insufficient chilling reduces pollination, fruit set and ultimately the yield in walnuts, pistachio and peaches. Advanced flowering has been found in olive, apple and pear. Reduced flower size and pedicel lengths were observed in cherry due to less chilling (Rai et. al., 2015).

Strategies for improving the fruit production

I. Assessment of the vulnerability and climate risks associated with fruit production in all agro ecological region

II. Conduct seminar, workshops, training and general awareness program regarding climate change and its effect in fruit production.

III. Active participation of Nepalese researcher and development workers in the international seminar/conference/dialogue about greenhouse gas emissions management and global warming with respect to fruit species.

IV. Identification of present issues of climate change in fruit sector via research.
V. Strengthen Horticulture Research Station and Commodity Program to run effective researches.
VI. Develop smart Information Communication and Technology (ICT) system to transfer climate adaptation methods and technologies at farmer's field.
VII. Preservation of genetic materials to reduce extinction of biodiversity due to climate change
VIII. Develop genotypes having resistance to heat and drought. In vitro conservation of rare and useful fruit species for future use.

IX. Promotion of fruit crop insurances program for social security's and food securities targeting commercial fruit growers. • Change in national policies that emphasizes incentives to the farmers for agricultural inputs in fruit production.
X. Evaluate varieties and rootstocks to minimize of climate change hazard.
XI. Phenotyping of allimportant fruits genetic wealth to make tolerable to temperature and moisture stressWork on genetic enhancement for tolerance to biotic and abiotic stress.
XII. Integrated nutrient management, Integrated pest management, Integrated weed management, development of water harvesting techniques to cope climate change in fruit species.
XIII. Agronomic management strategies such as Agri-silvicultural system, Agri-horticulture system, Agri-horti-silvicultural system, Horti-pastoral system, Inter cropping annual crops under fruit trees, Integrated Farming system should be adopted.
XIV. Diversification of high value fruit crops like peach, apricot, walnut, kiwi and olive.
XV. Introduction of low chilling cultivars of temperate fruits like apple, walnut, peach and plum etc.
XVI. Crop phenology of all major fruit crops under changing climate will be monitored.

Recommendations

I. Proper management of irrigation infrastructure in both rural and urban area of fruit pocket to reduce drought hazards is essential
II. Develop and introduce heat and drought resistant verities/breeds.
II. Introduce insect and pest resistant varieties in coordination with national agriculture research center
IV. Focus on application of IPM (integrated pest management) in fruit production.
V. Train producer to use safe agrochemicals to minimize pest and disease damage in the fruit crops.
VI. Sharing workshop on good practices of adaptation and mitigation with neighboring country to cope with vulnerabilities would bring new result.
VII. Develop climate-forecasting system for reducing hazards.
VIII. Need to build capacity of government officials at central ministry, provincial ministry and local government levels to communicate climate concerns.
IX. It would be better to use machines operated from renewable energy during intercultural operation

Conclusion

Nepal with diverse soil and climate comprising several agro ecological regions provides ample opportunity to grow a variety of horticultural crops comprising of fruits, vegetables plantation crops, spices and tuber crops and flowers. Among all fruits play a unique role in Nepalese economy by improving the income of the rural people. Fruits are also rich source of vitamins, minerals, proteins, and carbohydrates etc. which are essential in human nutrition and assumed great importance in nutritional security of the people. Cultivation of fruits plays a vital role in the prosperity of the nation and is directly linked with the health and happiness of the people. The knowledge about the impact of climate change on fruit production is limited. Addressing problems of climate change are more challenging in fruit crops as compared to other cereals. Consequences of climate change are global warming, change of seasonal pattern, excessive rain, melting of ice cap, flood, rising sea level, drought etc leading to extremity to fruit production. The issue of climate change and solution to the problems arising out of it requires thorough analysis, advance planning and improved management. Fruits are grown from tropical to temperate region of Nepal. Climate change poses serious challenges to human and places unprecedented pressure on the sustainability of fruit industry. The most effective way is to adopt conservation agriculture; using renewable energy, forest and water conservation, reforestation etc. to sustain the productivity modification of present horticultural practices and greater use of greenhouse technology are some of the solutions to minimize the effect of climate change. Climate change will impact fruit production through; Changes in the distribution of existing pests, diseases and weeds and an increased threat of new incursions, Increased incidence of physiological disorders such as tip burn and blossom end rot, Greater potential for downgrading

product quality, Increases in pollination failures if heat stress days occur during flowering, Increased risk of spread and proliferation of soil borne diseases as a result of more intense rainfall events along with warmer temperature, Increased irrigation demand especially during dry periods of the year, Increased atmospheric CO_2 concentrations will benefit productivity of most fruit crops, although the extent of this benefit is unknown, Increased risk of soil erosion and off farm effects of nutrients and pesticides, from extreme rainfall events, Increased input cost especially fuel, fertilizers and pesticides. Experiments on varietal evaluation will also be conducted under natural conditions at different altitudes/conditions with natural variations in temperature and moisture falling under various agro-climatic zones of the countries. Hence there is a need to protect these valuable crops for sustainability against the climate change scenario. To sustain the productivity, modification of present horticultural practices and greater use of greenhouse technology are some of the solutions to minimize the effect of climate change. Development of new cultivars of horticultural crops tolerant to high temperature, resistant to pests and diseases, short duration and producing good yield under stress conditions, as well as adoption of hi–tech horticulture and judicious management of natural resources will be the main strategies to meet this challenge.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Research Article

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Effect of different fertilizer forms on yield and yield components of chickpea varieties

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Abstract

This research was conducted to determine the effects of different fertilizer forms (control, diammonium phosphate, urea, phosphorus and bacteria) on yield and yield components of some chickpea varieties (Gokce, Diyar 95, Aziziye 94 and Taek-Sagel) in Diyarbakir, Turkey, during 2018 and 2019 growing seasons. In the study, plant height, plant biomass, pod weight, seed yield per plant, number of pods and number of seeds per pod, biological yield, grain yield, 100-seed weight and harvest index were evaluated. The effect of fertilizer treatments on chickpea varieties for all traits were significant, except 100-seed weight, and harvest index. Grain yield ranged from 1274 kg ha^{-1} to 1479 kg ha^{-1} among treatments. The control group (1479 kg ha^{-1}), urea (1478 kg ha^{-1}) and diammonium phosphate (1449 kg ha^{-1}) fertilizer treatments had produced more grain yield than bacteria inoculation (1274 kg ha^{-1}) and phosphorus (1332 kg ha^{-1}) treatments.

Keywords: Chickpea, *Cicer arietinum*, Nitrogen, Phosphorus, Bacteria

Introduction

Chickpea is produced in over 40 countries from all continents at the present. However, the most important chickpea producing countries are India, Australia, Myanmar, Egypt, Turkey, Pakistan, Iran, Mexico, Ethiopia and Canada. Chickpea is currently grown on about 14 million hectares worldwide. India and Australia rank the first and second with the shares of 65% and 7.3%, respectively. Average annual production of chickpea is about 14 million tons with 95% of chickpea cultivation and consumption occurring in the developing countries (FAO, 2018). The chickpea area in Turkey is about 514 000 ha with a production of 630 000 tons and an average yield of 1230 kg ha^{-1} (TUIK, 2018). Chickpea acreage and productivity in Turkey increased in last decades. The use of improved cultivars and agronomic techniques by the farmers and government supports had an increasing effect on total chickpea production. Even last four-year data shows that harvested area and production in Turkey increased by 31 and 40%, respectively. Chickpea is less labor-intensive and requires fewer external inputs as compared to cereals. Chickpea is played a significant role in improving soil fertility by fixing the atmospheric nitrogen up to 140 kg ha^{-1} meeting most of the requirement. After harvest, it leaves substantial amount of residual nitrogen for subsequent crops and adds some amount of organic matter to maintain and improve soil health and fertility. Farmers can save fertilizer cost not only for chickpea but also for the subsequent crops.

The total amount of N that was fixed by the plant for growth of chickpea varies between 19 to 24 kg N per hectare in drought season (Carranca et al., 1999) and average estimate is 70 kg per hectare in Australia (Drew et al., 2012). The excess amount of nitrogen given as inorganic nitrogen or fertilizer in the soil causes the amount of biologically fixed nitrogen to decrease (Doughton et al., 1995).

It has been suggested that low levels of fertilizer N (i.e., “starter N”) may increase yield. For example, recommendations for Southeast Anatolia region of Turkey indicates “if nitrogen fixation is not optimized due to unfavorable growing conditions (e.g., relatively dry seed bed), chickpea may benefit from low rates of starter N in some years. The generally recommended fertilizer rates for chickpea include 20-30 kg nitro-

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gen (N) and 40–60 kg phosphorus (P) per hectare. Total quantities of N and P should be given as a basal dose in absence of bacterial inoculation. Also, farmers have recently begun to apply excessive fertilization in chickpea and lentil crops mostly at sowing time and rarely during flowering period by spraying. Some researchers reported that foliar spray of 2% urea at flowering has been found beneficial in rainfed crops (Das and Jana, 2015). Although many studies are examining the effect of fertilization on yield in chickpea, there are still doubts about chickpea fertilization among chickpea producers. In this study, we tried to determine the effect of different fertilizer applications on yield and some agronomic traits.

Materials and Methods

This research was conducted in Dicle University Agricultural Faculty, Diyarbakir, Turkey during 2018 and 2019 growing seasons.

Experimental Area

Diyarbakir is located on grid 37.91 °N and 40.2 °E, at an altitude of 640 m above sea level. The general climatic conditions of experimental area were characterized as hot and drought during crop growing season. Average long term precipitation is a little bit lower than 500 mm, and it differently fluctuates between the years. The seasonal rainfall distribution is mainly between November and June. Although unusual, irregular precipitation in June is recorded in recent years. Mean temperature is about 16-20 ºC between January and June. May has an irregular precipitation distribution, but June is usually dry and hot. The relative humidity varies between 60 % and 75% from January to April but decreases to about 20-30% after May. In the first experiment year (2018), total precipitation from February to April was 146.8 mm, and weather was dry, but in May, rainfall was 157.8 mm. Mean temperature (February to May) was 11.9 ºC. Precipitation from February to April was 365.2 mm in the second year (2019), but May was considerably dry with 45.8 mm, and mean temperature (February to May) was 11.35 ºC. Growing season in 2019 was quite cool compared to the preceding growing season (Table 1). The soil analysis indicated that soils were neutral (pH: 7.24), insufficient in organic matter (0.79%) and phosphorus content (13.2 kg ha $^{-1}$) with clay texture.

Table 1. Meteorological data of Diyarbakir for experiment years

Month	Mean temperature (ºC)		Total precipitation (mm)		Moisture (%)	
	2018	2019	2018	2019	2018	2019
January	5.2	3.8	86.6	67.6	77.3	81.7
February	7.6	5.4	86.4	77.4	74.5	77.0
March	12.3	8.2	11.6	135.2	63.2	74.9
April	15.9	11.8	48.8	152.6	53.0	78.4
May	19.4	20.1	157.8	45.8	67.5	58.5
June	26.5	28.3	14.4	1.0	37.9	32.5
July	31.2	30.3	0.0	0.07	24.2	24.8

Experimental Design and Agricultural Practices

The experiment was laid out following a split-plot in completely randomized block design with three replications. Fertilization treatments and cultivars were designed as main and sub factors, respectively. Fertilization treatments included the applications of control, diammonium phosphate, urea, phosphorus and bacteria. Four chickpea varieties (Gokce, Diyar 95, Aziziye 94 and Taek-Sagel) were used. The seeds were sown with inter and intra row space of 40x10 cm, respectively, on 7 February 2018 and 11 February 2019. Before sowing, seeds were inoculated with specific strain of Rhizobium at a rate of 10 g /kg seed as bacteria treatment. Inorganic fertilizers were applied at a rate of 40 kg ha^{-1} nitrogen and 80 kg ha^{-1} phosphorus during sowing in the forms of diammonium phosphate (18 N - 46% P), urea (46% N) and triple superphosphate (46% P_2O_5) depending on the ways of treatment. The crops were irrigated after sowing in 2018 due to early seasonal drought. Weed, disease and pests were controlled first manually and then using chemical spray. Data on plant height, plant biomass, pod weight, seed yield per plant, number of pods and number of seeds per plant, biological yield, seed yield, 100 seed weight and harvest index were recorded at harvest.

Data of two years were analyzed separately and pooled, were subjected to analysis of variance, and means were separated using the Duncan's Multiple Range Test (0.05).

Results and Discussions

In the study the effect of fertilizer treatments on chickpea varieties for plant height, plant biomass, pod weight, plant grain yield, number of pods and seeds per plant, biological yield, grain yield, 100 seed weight and harvest index were evaluated.

The effect of fertilizer treatments on for all traits of chickpea varieties was significant, except for 100 seed weight and harvest index. Plant height ranged from 53.3 cm to 57.9 cm. Control, phosphorus (57.9 cm) and diammonium phosphate (57.6 cm) treatments were higher than urea (58.6 cm) and bacteria (53.3 cm) treatments. Cultivar x treatment interaction indicated that the effect of treatments on Aziziye 94, Taek-Sagel and Diyar 95 cultivars were significant, Gokce was non-significant. Urea (57.5 cm) and phosphorus (58.9 cm) treatments in Taek-Sagel, phosphorus (66.0 cm) and diammonium phosphate (65.0 cm) treatments in Diyar 95 were higher than the other treatments. However, Aziziye 94 had the highest value in control group (62.4 cm). Differences among years for plant height were significant. Plant height in the first experiment year (64.6 cm) was higher than that in the second year (47.5

cm) (Table 2).

The most efficient treatment for plant biomass was phosphorus (21.4 g) and diammonium phosphate (20.3 g) treatments. The highest plant pod weight was obtained from diammonium phosphate (10.5 g). The effect of treatments for plant biomass and plant pod weight on varieties was different, and plant biomass and plant pod weight were significant in Gokce and Diyar 95 varieties, and varieties showed high responses to diammonium phosphate fertilizer treatment.

Seed yield ranged from 9.4 g to 11.3 g plant^{-1}, and yield from all treatments were higher than control (9.4 g). The effect of treatments on plant seed yield among varieties was significant in Gokce, and phosphorus fertilizer treatment was an important treatment in Gokce, but other cultivars were not responsive to fertilizer treatments for the plant seed yield (Table 2).

Table 2. The trait means and significance level of variation sources of chickpea genotypes under changing fertilizer treatments

		Plant height (cm)					Plant biomass (g)				
Treatments	**N and P kg ha^{-1}**	**Taek-Sagel**	**Gokçe**	**Aziziye 94**	**Diyar 95**	**Mean**	**Taek-Sagel**	**Gokçe**	**Aziziye 94**	**Diyar 95**	**Mean**
Control	0 - 0	56.1	52.5	62.4	58.8	57.5	14.9	18.1	20.3	18.2	17.9
Urea	40 - 0	57.5	49.3	51.5	58.6	54.2	16.6	20.3	21.7	19.3	19.5
Phosphorus	0 - 80	58.9	52.5	54.2	66.0	57.9	19.3	22.5	19.0	20.3	20.3
DAP	40 - 80	55.4	49.5	60.3	65.0	57.6	17.3	23.8	20.0	24.6	21.4
Bacteria	0 - 0	51.6	47.7	52.7	61.1	53.3	18.6	18.2	20.4	19.9	19.3
Mean		55.9	50.3	56.2	61.9		17.3	20.6	20.3	20.4	
		**	**	ns	**		ns	**	ns	**	
1st and 2nd year means			64.6	47.5				22.4	16.8		
Source of variation											
Cultivar		**					**				
Treatments		**					**				
Interaction		**					**				
		Plant pod weight (g)					**Plant seed yield (g)**				
		Taek-Sagel	**Gokçe**	**Aziziye 94**	**Diyar 95**	**Mean**	**Taek-Sagel**	**Gokçe**	**Aziziye 94**	**Diyar 95**	**Mean**
Control	0 - 0	7.6	7.8	10.3	7.5	8.3	8.3	7.6	10.5	11.5	9.4
Urea	40 - 0	8.6	10.6	9.6	10.2	9.8	9.2	10.5	9.3	12.6	10.4
Phosphorus	0 - 80	10.0	10.1	7.9	9.7	9.4	9.7	12.1	7.5	12.6	10.5
DAP	40 - 80	9.4	11.9	10.8	9.8	10.5	10.1	10.5	10.8	13.9	11.3
Bacteria	0 - 0	8.9	8.2	10.4	7.8	8.8	9.5	8.9	9.8	11.5	10.3
Mean		8.9	9.7	9.8	9.0		9.3	9.9	9.6	12.7	
		ns	*	ns	**		ns	**	ns	ns	
1st and 2nd year means			8.87	9.83				13.5	7.2		
Source of variation											
Cultivars		**					**				
Treatments		**					**				
Interaction		**					**				

**, * and ns: significant at 1 and 5% level, and not significant, respectively

Number of pods per plant ranged from 17.1 to 20.8, and all treatments had the same effect, except for control. The effect of the treatments on plant pod number among varieties was significant. Pod number of Gokce ranged from 14.7 to 23.4, Diyar 95 ranged from 15.4 to 20.0, and both cultivars highly responsive to nitrogen fertilizer (DAP and urea), but control group had low value for this trait. However, Aziziye 94 had the highest value (20.1) in bacteria inoculation and control group. Number of seeds per plant ranged from 17.0 to 19.3 among treatments, and fertilizer treatments had higher compared to control group. Cultivars x treatment interaction indicated that the effect of treatments was significant in only Gokce, and all fertilizer treatments had higher pod number compared to control in Gokce variety. Differences among years were not significant for both traits (Table 3).

Table 3. The effect of fertilizer treatments on number of pods and seeds plant^{-1} in Chickpea

		Number of pods plant^{-1}					Number of seeds plant^{-1}				
Treatments	**N and P kg ha^{-1}**	**Taek-Sagel**	**Gokçe**	**Aziziye 94**	**Diyar 95**	**Mean**	**Taek-Sagel**	**Gokçe**	**Aziziye 94**	**Diyar 95**	**Mean**
Control	0 - 0	18.1	14.7	20.1	15.4	17.1	19.0	16.5	21.5	11.1	17.0
Urea	40 - 0	18.6	23.4	18.7	20.0	20.2	19.4	22.4	19.9	14.9	19.2
Phosphorus	0 - 80	22.4	19.5	15.8	17.5	18.8	21.3	20.0	15.0	14.7	17.8
DAP	40 - 80	19.4	22.9	21.6	19.4	20.8	19.3	20.1	22.0	15.9	19.3
Bacteria	0 - 0	20.4	18.8	20.1	16.7	19.0	21.1	19.5	20.6	13.1	18.6
Mean		19.8	19.8	19.3	17.8		20.0	19.7	19.8	13.9	
		ns	*	**	**		ns	*	ns	ns	
1st and 2nd year means			20.7	17.6				18.3	18.5		
Source of variation											
Cultivars		**					**				
Treatments		**					*				
Interaction		**					**				

**, * and ns: significant at 1 and 5% level, and not significant, respectively.

Biological yield ranged from 3955 kg to 5281 kg per hectare among the treatments. Diammonium phosphate (5281 kg ha^{-1}) and urea (4859 kg ha^{-1}) fertilizer treatments and control group (4699 kg ha^{-1}) had more yields than that of bacteria inoculation (3955 kg ha^{-1}). Variety x treatment interaction was significant, and Gokce, Aziziye 94 and Diyar 95 cultivars had different responses to fertilizer treatments. Diammonium phosphate fertilizer treatment had positive effect on Diyar 95 (7557 kg ha^{-1}) (Table 4). It is generally accepted that the soil nutrients deficiency and alkaline soil reaction (pH) adversely affect the growth of crops. Organic manures or use of inorganic nitrogen fertilizers at sowing is important to ameliorate organic matter and the uptake potential of nutrient elements (Timsina, 2018).

Grain seed yield ranged from 1274 kg to 1479 kg per hectare among treatments. Control group (1479 kg ha^{-1}), urea (1478 kg ha^{-1}) and diammonium phosphate (1449 kg ha^{-1}) treatments had produced more seed yield than those of bacteria inoculation (1274 kg ha^{-1}) and phosphorus (1332 kg ha^{-1}) treatments. Variety x treatment interaction was significant, Gokce and Aziziye 94 cultivars showed different responses to treatments. The highest grain yield for Aziziye 94 was obtained from control while phosphorus and bacteria treatments yielded the lowest (Table 4). Kanwar (1981) reported that phosphorus-deficient soils gave high responses to phosphorus, as the soils with low bacterial activity gave high responses to nitrogen. It has been reported that low rates of starter N (i.e., 30 kg N ha^{-1}) and P (20 kg P2O5 ha^{-1}) may optimize desi type chickpea grain yield (Walley et al., 2005). Gubbels (1992) reported that high rate of phosphorus did not increase the yield. Phosphorus is a nutrient that is effective in nodulation but variable in yield (Chen., 2006), although legumes generally respond well to phosphorus fertilizers (Shukla, 1964), Saxena (1980) stated that this response is variable in chickpea. The phosphorus requirement of chickpea crop is varying by the growth conditions (Johansen and Sahrawat 1991; Riley, 1994; Islam et al., 2011). Bicer (2014) reported that chickpea cultivars showed low response to P and phosphorus fertilization could not be effective in late sown chickpea. Early sown and irrigation supply can be advisable for effective phosphorus uptake in dry regions.

In fact, seed yield had shown little variation among the treatments, although they were statistically significant. The results might have been due to the climatic conditions of two years of trial. The rate of seedling emergence was higher in 2018 compared with 2019 due to the high temperature and the low precipitation in February 2018. Low temperature and rainfall also delayed the time of first emergence and flowering in 2019, some seeds could not even germinate, and had no effect on the duration of vegetative growth and reproductive growth stages. Finally, high precipitation may have caused the draining of fertilizers in the soil. However, biological yield and seed yield were declined by decreasing the seedling emergence rate and vegetative growth rate such as plant height and plant biomass. Although decreased in the second year, this decrease in seed yield was not as sharp as in biological yield. Short and high-branched plants had sufficient number of pods and seeds (Table 1,4). Fertilizer use is the second important factor after water availability in rainfall dependent agriculture (Umrani, 1995), and fertilization increases deeper penetration of roots and therefore fertilizer treatment cause relatively high water extraction (Hedge, 1986). Fertilizer is vital to increase productivity in drylands farming systems (Kanwar, 1981). In this study, our opinion is that we could have better revealed the effect of fertilizer in dry year of the experiment if we had employed a little higher fertilizer dose.

Table 4. The effect of fertilizer treatments on biological yield and seed yield, 100 seed weight and harvest index in chickpea

		Biological yield (kg ha^{-1})					Seed yield (kg ha^{-1})				
Treatments	N and P kg ha^{-1}	Taek-Sagel	Gokçe	Aziziye 94	Diyar 95	Mean	Taek-Sagel	Gokçe	Aziziye 94	Diyar 95	Mean
Control	0 - 0	4857	4361	4620	4956	4699	1628	1517	1506	1264	1479
Urea	40 - 0	5132	5026	3536	5743	4859	1616	1378	1393	1235	1478
Phosphorus	0 - 80	5292	4107	4137	4209	4436	1688	1432	872.4	1335	1332
DAP	40 - 80	5265	4545	3758	7557	5281	1553	1804	1088	1351	1449
Bacteria	0 - 0	4463	3998	3319	4039	3955	1570	1290	878.5	1355	1274
Mean		5002	4407	3874	5301		1669	1484	1148	1308	
		ns	**	**	**		ns	**	**	ns	
1st and 2nd year means			6211	3081				1572	1231		
Source of variation											
Cultivars		**					**				
Treatments		**					**				
Interaction		**					**				

		100 seed weight (g)					Harvest index (%)				
Treatments		Taek-Sagel	Gokçe	Aziziye 94	Diyar 95	Mean	Taek-Sagel	Gokçe	Aziziye 94	Diyar 95	Mean
Control	0 - 0	37.96	39.78	40.96	41.29	40.00	34.50	37.83	36.17	42.50	37.75
Urea	40 - 0	38.10	39.29	41.72	41.57	40.17	37.00	36.17	29.83	35.67	34.66
Phosphorus	0 - 80	38.47	39.72	41.59	42.20	40.49	33.33	32.17	28.33	27.83	30.41
DAP	40 - 80	36.51	39.42	42.26	40.76	39.74	30.67	41.67	32.83	23.50	32.16
Bacteria	0 - 0	39.64	39.28	40.84	40.33	40.02	36.83	35.33	35.50	30.50	34.54
Mean		38.14	39.50	41.47	41.23		34.46	36.63	32.53	32.00	
		ns	ns	ns	ns		ns	ns	ns		
1st and 2nd year means			40.17	39.99				26.13	41.68		
Source of variation											
Cultivars		ns					ns				
Treatments		ns					ns				
Interaction		ns					ns				

** and ns: significant at 1 and 5% level, and not significant, respectively.

100-seed weight and harvest index were not affected by treatments. It ranged from 38.14 g in Taek-Sagel to 41.47 g in Aziziye 94 (Table 4). The reason for the low effect of applications on 100-seed weight is due to the high inheritance of this character (Toker and Canci, 2003). It has been reported that 100-seed weight is positively affected by dry and rainy conditions, but it has decreased in arid years/conditions. Therefore, the seed weight can be improved with an optimum fertilizer dose. The differences among years were significant for harvest index. In the second year of the experiment, seed yield increased instead of vegetative parts (Table 4).

Differences among the two trial years for plant height, plant biomass, plant seed yield, biological yield, seed yield and harvest index may be due to climatic conditions. In 2018 growth period; February, March and April were quite dry and hot, and the experiment was regularly irrigated by sprinkler irrigation. Irrigation supply water, sunny days and warm weather during the vegetation period and high rainfall in May had a positive effect on plant development. In 2019 growth period; since February, March and April were quite rainy and the cloudy weather, the low temperature delayed the plant growth at the beginning of the growing season. Seed germination and emergence delayed due to low soil temperature, emergence rate and seedling vigor was low and weak. Most of seeds that could not emerge due to low soil temperature, could not survive under the soil. As a result, the number of plants per plots decreased. May 2019 was extremely dry during the generative period, and caused negative effects on plants. In 2019, the vegetative period started late and progressed slowly. Finally, the generative period due to drought delayed the development of the plant.

Fertilizer applications could not be useful for crops in wet trial year, 2019, since it applied in sowing time. Because the rainy year increased the nutrient loss by drainage, therefore fertilizer dose, especially for inorganic nitrogen, must be accurately calculated for rainy areas or seasons. In addition, proper combinations of mineral fertilizer and bacteria inoculation would be better for seed productivity, but only bacteria and only mineral fertilizer applications were not very effective in this trial. Zhang et al. (2016) reported that the combined application of organic manures and mineral fertilizers plays an important role in optimizing soil nutrient pool, increasing crop yields or water use efficiency.

Conclusion

The effect of fertilizer treatments on chickpea varieties for all traits were significant, except for 100-seed weight and harvest index. As a result of this experiment, although nitrogen and phosphorus applications gave high values, the effects of fertilizer application in such wet and rainy years were different for yield and its traits. Long-term experiments are required for monitoring the changes in crop yields and soil fertility since short-term studies cannot reveal these changes. In our region, the effect of fertilizer types and doses on legumes crop should separately be investigated in dry and rainy conditions.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Preliminarly Survey Results and Phylogenetic Analysis for *Tomato Yellow Leaf Curl Virus* and *Potato Leaf Roll Virus* on Tomato Grown in Adana

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Abstract

Surveys were conducted at tomato production sites in Adana province in 2019. 53 tomato plants have been collected showing the virus symptom due to determining its prevalence. The collected samples were tested for 13 different viral agents harmful to tomatoes using ELISA and RT-PCR methods. 24.52% TYLCV and 9.43% PLRV were detected from the tested samples. When DNA sequencing comparisons are made from RT-PCR products, 98,74% of PLRV isolates in tomato samples collected from Adana province are homogical similarity with Belgium potato isolate (KX364206.1). Also it clustered at 99.37% similar to same branch with New Zealand (GU002341.1) (BLAST at NCBI).

Keywords: Tomato, TYLCV, PLRV, ELISA, PCR

Introduction

Tomato (*Solanum lycopersicum*) is consumed both as fresh fruit in its own right, as ingredients in many salad recipes or in the form of various foods processed in crushing, whole peeled tomato canning, sliced product, various juices and soups. Tomatoes are consumed in salads, as fresh fruit in their own right, as ingredients in many recipes, or in the form of various processed products. putty, whole peeled tomatoes, chopped produce and various juices and soups. Tomato is a valuable nutrients in many parts of the world and is an economically important agricultural plant.

Tomato is a major plant product and has gained popularity over the last century. It is also grown in almost every country in the world (according to Linnaeus' binomial systematic classification, there are three basic tomato species in them [*L. esculentum, L. peruvianum, L. pimpinellifolium*] and potato (*L. tuberosum*.) is listed under the genus lycopersicon, which also includes. The motherland of tomato is central and South America and its use as a cultured plant started off the coast of Peru (Günay, 1992). Tomato, which was later brought to Anatolia, became a vegetable that was widely grown and consumed (Yazgan and Fidan, 1996). Tomatoes in the Solanaceae family (Solanum lycopersicum) have ranked one of the most important and widely produced crops with a global production increasing by about 45 million tons between 2007 and 2017 (FAO 2017). As of 2017, China is ranked first with 59.6 million tons of world tomato production, India is second with 20.7 million tons of production, Turkey is third with 12.75 million tons (7%) and the United States is fourth with 12.6 million tons of production. 12.75 million tons of tomatoes are produced in Turkey, which includes 8.4 million tons of tableware and 3.7 million tons of tomato paste. In 2018, Antalya is ranked first with 2.410 thousand tons of tomato production, Bursa is ranked second with 1.575 thousand tons and Manisa is ranked third with 975 thousand tons. The highest yield in Turkey is harvested in the Mediterranean Region due to its climate advantage and its greenhouse location. Turkey's tomato production increased by 7% in 2018 compared to 2012 and reached 12.2 million tons. According to TurkStat plant production statistics, tomato production in 2019 increased by 5.7% compared to the former year and amounted to 12.8 million tons.

Despite intensive control efforts to prevent pathogen infec-

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tions in tomatoes, viral diseases are quite difficult to control. Management strategies are becoming increasingly difficult with the constant emergence of new strain or species. Viruses have great potential to adapt to the pressure of natural selection due to their large populations, lack of repair mechanisms facilitating genetic variation in their genomes and their ability to reproduce in a short time (Hanssen et al. 2010). High mutation and recombination ability in viral genomes increases the production of new variants that spread rapidly in the population (Moya et al. 2004). The food content, fruit quality and yield of tomato plants affected by Viral diseases decrease and the shelf life is shortened (Hanson et al. 2016).

In general, virus diseases causes dwarfing, necrosis, deformity, different types of leaf spots (mosaic, ring, speckle, like opening a vein), with a more pronounced effect of the plants by chlorosis and leaf deformation and causes symptoms such as sudden death. These viruses, can be transported vegetatively by production material, pollen and vector insects (Aphids, Whiteflies, Thrips, Mites, Nematodes), mechanically used tools and equipment. There is no effective chemical management control against virus diseases. The absence of a method of combating these diseases in practice increases the importance of virus diseases. Tomato plants grown under cover or under field conditions are particularly exposed to high levels of infections due to the mechanical and seed-borne virus genuses. Tobacco Mosaic Virus (TMV) (Mayer et al. 2009), Pepper Mild Mottle Virus (PMMoV), Tobacco Mild Green Mosaic Virus (MGMT), Tomato Mosaic Virus (ToMV), Tomato Brown Rugose Fruit Virus (ToBRFV) (Fidan, 2020), Tomato Ring Spot Virus (ToRSV), Cucumber Mosaic Virus (CMV), Tomato Spotted Wilt Virus (TSWV), Potato virus Y (PVY), Potato Leaf Roll Virus (PLRV) and Tomato Yellow Leaf Curl Virus (TYLCV), can infect on family Solanaceae.

Tomato Yellow Leaf Curl Virus (TYLCV) is an individual from the genus Begomovirus, in the family Geminiviridae, causing crop losses of over 100% in tomato varieties (Czosnek and Laterrot, 1997). The spread of TYLCV is primarily caused by infected plant material movement or by whiteflies that have TYLCV (Torre et al., 2018). The virus was first spoted in Jordan and Israel in 1939 (Avidov, 1944). It has a wide range of hosts, with several plant families including Solanaceae. TYLCV causes leaf speckling, yellow leaf edges, flower casting and small leaf. In the early stages, infected plants may not bear fruit due to seasonal conditions and variety sensitivity. Its genome formed of circular, single-stranded DNA (ssDNA) about 2.7 kb long (Gronenborn, 2007).

Likewise TYLCV, PLRV is one of the most important viruses that infects members of the Solanaceae family. Among them, the potato culture is the most important host of PLRV. PLRV located within the genus polerovirus in Luteoviridae. Many kind of symptoms depending on the viral strains, host tolerance, duration of infection and also environmental factors. Its symptoms are redness or yellowing in stunted and infected potato plants, and rolling leaves. As well It reduces the number and size of potato tubers with annual world productivity losses of over 20 million tonnes. PLRV formed of single-stranded RNA (+) ssRNA in the (+) sense of the 5.9 kb genome. Potato leaf roll virus can be transmitted experimentally by grafting, aphids in a circulative and non-propagative manner. In aphids, *Myzus persicae* is the most potent vector. Disease management approaches include thermotherapy, tissue culture, resistance breeding, effective vector control and seed potato certification programs. The symptoms of the virus seen in the fields where the crop is grown also show similarities with the symptoms of unblanced fertilization and lack of irrigation in the same cultivated plant (Banttari, 1965).

In field surveys, symptoms caused by viruses and virus-like disease factors were observed in tomato fields. In this study, it is aimed to definitively identify this factor that causes leaf curl symptoms in tomato plants in our region with serological and molecular methods. It was also performed to determine the role of the tomato plant on host virus relationships and to determine the possible risks that PLRV carries in vegetable production.

Materials and Methods

Material

The plant materials were collected from the fields (Çukurova-14; Yumurtalık-12 and Karataş-27) in Adana province in the fall of 2019 (Figure 1.). 53 samples showing symptoms of dwarfing, redness or yellowing and rolling leaves were tested with serological and molecular techniques at the Virology Laboratory of the Dept. of Plant Protection of the Faculty of Agriculture of Akdeniz University.

Method

In the study, Double Antibody Sandwich-Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) and Reverse Transcription Polymerase Chain Reaction (RT-PCR) technique were used Due to the fact that when the tomato samples are examined, the symptoms of viral infection come to the fore. For this reason, serological and molecular assays have been designed to provide more precise results instead of general methods such as dsRNA analysis or electron microscopy.

DAS-ELISA

The study was conducted according to Clark and Adams, (1977). Tomato samples were extracted in phosphate buffer solution (PBS) 1/5 (0.2 g NaN_3, 0.2 g KH_2PO_4, 8.0 g NaCl, 0.2 g KCl, 2.9 g $Na_2HPO_4.12H_2O$, 20 g polyvinylpyrrolidone-25 per L, pH 7.4). ELISA plates were primarily coated with diluted virus-specific antiserum in coatingbuffer (2.93 g $NaHCO_3$, 1.59 g Na_2CO_3, 0.2 g NaN_3 per L, pH 9.6) and incubated for two hours at 37°C. Following incubation, Washing (PBST) buffer (0.2 g KH_2PO_4, 2.9 g $Na_2HPO_4.12H_2O$, 8.0 g NaCl, 0.2 g KCl, 0.2 g NaN_3, 0.5 mL Tween - 20 per L) was added to washed plates from crushed samples and overnight at 4°C. Conjugated (IgG) antibody with alkaline phosphatase specific to the diluted virus in a conjugate buffer (2% polyvniylpyrrolidone-25+ PBST + 0.2% BSA (bovine serum albumin, pH 7.4) was added to the re-washed plate wells after overnight. This process was followed by 2 hours of incubation at 37°C. After repeated washing after incubation, substrate buffer containing P-nitrophenylphosphate in (97 ml diethanolamine, 0.2 g NaN3 L-1, pH 9.8) was added to plate wells and kept for 2 hours in dark conditions at room temperature.

The expected absorption values of the test result were measured at 405 nm in ELISA reader. After twice washing

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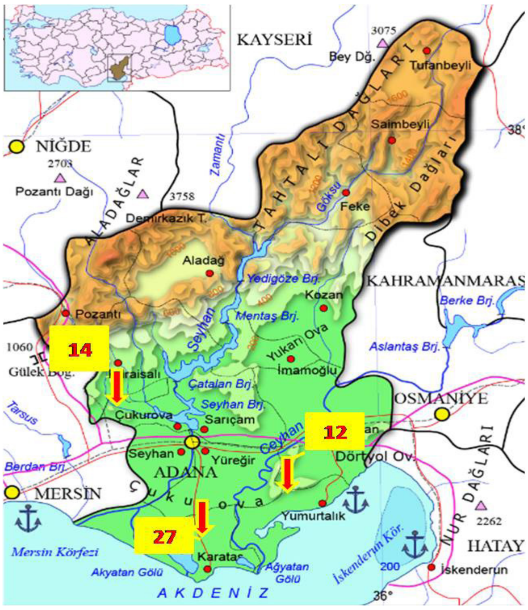

Figure 1 Sample locations and sample numbers of plant materials in Adana (Çukurova-14; Yumurtalık-12 and Karataş-27) http://cografyaharita.com/turkiye_fiziki_harirtalari.html

of plates, the absorbance value of buffer and negative control were considered to be infected with CMV. To diagnose the cause of the disease, leaf samples were assayed with with specific polyclonal antiserum which obtained from BIOREBA AG and Loewe Agdia companies by DAS-ELISA technique against the Cucumber Mosaic Virus (CMV), Pepper Mild Mottle Virus (PMMoV), Tomato mosaic virus (ToMV), Tobacco mosaic virus (TMV), Tomato Ring Spot Virus (ToRSV), Tobacco Mild Green Mosaic Virus (MGMT), Tomato Spotted Wilt Virüs (TSWV), Potato virus Y (PVY), Potato virus X (PVX), and Potato Leaf Roll Virus (PLRV) also known as the common viruses for solanaceae plants.

RT-PCR Method

RT-PCR method was also used in total RNAs extracted according to Astruc et al.,(1996) with total nucleic acid extraction buffer (50mM EDTA pH. 7.0, 500 NaCI, 100 mM Tris-HCI pH.8.0, 10 mM 2. mercapto-ethanol (1/1000)) saturated at 1:2 (w/v). The positive found samples for PLRV in DAS-ELISA were confirmed by the use of total RNAs extracted from 100 mg leaves in RT-PCR. The same samples were tested with PCR against TYLCV due to suspicion of symptom type.

The spesific primer sets TYv2664 (5`-ATTGACCAAGATTTTTACACTTATCCC-3') and TYc138 (5'-AAGTGGGTCCCACATATTGCAAGAC-3`) were used to clone a 316 bp sub-genomic region to identify TYLCV infection in this study (Anfoka et al. 2005) (Table 1). For the same reason of PLRV, cDNA amplicons have been cloned with primer sets Forward 5'-CGCGCTACAGAGTTCAGCC-3`) and Reverse 5` - GCAATGGGGGTCCAACTCAT-3`) reported by Singh et al. (1995).

The PCR and RT-PCR studies were conducted separately. The PCR studies were conducted using the Thermo Sci. Verso 1-Step RT-PCR *ReddyMix Kit*. In total volume of 50 µL; 2X 1-Step PCR ReddyMix 25 µL, 1X RT Enhancer 2.5 µL, Verso enzyme Mix 1 µL, , Reverse primer (10 µM) 1 µL 200 nM, Forward primer (10 µM) 1 µL 200 nM, sample RNA 1-5 µL 1 ng, nuclease free water content to be completed according to total volume of 50 µL has been adjusted as suggested by the company.

One-Step RT-PCR stage for PLRV (50°C 15 min. cDNA synthesis single cycle, 95°C 2 min., verso inactivation single cycle; 95°C, 30 sec. denaturation; 52°C, 30 sec. annealing; and 72°C, 45 sec. extension 35 cycles) and final extension was performed in a single cycle of 10 minutes at 72 ° C (Singh et al. 1995). For TYLCV inactivation single cycle at 94°C for 5 min; 30 cycles at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min.; for the termination of reaction, one cycle at 72°C for 10 min. performed in the form (Anfoka et al. 2005). cDNA bands belonging to the causal agents were observed by staining solution (ethidium bromide) after electrophoresis in agarose gel (1.5%).

Results and Discussions

TYLCV and PLRV symptoms (curling, systemic chlorosis, leaf deformation, lamina narrowing) were determined in the leaves of the tomato plants sampled during the study (Figure 2). Symptoms such as yellowing, stunting and leaf curling on TYLCV infected tomato plants along with important production losses were shown in tomato cultivation (Kil et al., 2016; Papayiannis et al., 2010). Other than tomato, some cultivated plants including Eustoma, Common bean Pepper and Cucurbits have been underlined as TYLCV hosts (Anfoka et al., 2009; Kil et al., 2016). Polston et al.(1994) similarly reported that symptoms (reduced leaf size, yellowing and distortion and cupped inward) on surveyed tomato plants were shown. Severe stunting appears with time. TYLCV is a limiting factor for cultivation (Lapidot and Friedmann, 2002). The infection time can increased yield losses (aproximataly 100%) (Lapidot and Polston, 2006). Dalmon anf Marchoux, 2000 declared that TYLCV has 11 natural and 30 experimental host species. They also underlined susceptible hosts of TYLCV as tomato, zinnia, bean, paprika, tobacco, lisianthus species. In addition to cultivated species, some wild or native species (*Solanum* *nigrum, Datura stramonium, and Malva sp*.) which are common in France, are stand as natural infection reservoir (with or without symptoms).

In Turkey, Yilmaz (1978) studied disease and TYLCV symptoms . Karut et al.2012 have detailed study on vector characterization on different host plant in Adana. Fidan et al.2019 collected some symptomatic samples from tomato plants (with yellowing, stunting, upward leaf curling, distortion and symptoms). the same researchers brought clarity to the connection between tylcv vectors and the transport of virus races. they also emphasized that vector-transportable diseases such as TYLCV can make the transition to non-host species to this day.

Figure 2. In tomato plants found to be infected with TYLCV and PLRV in the survey (A.- Tylcv-induced chlorosis, yellow leaf curl and dwarfing; B., C., D.- PLRV-induced leaf curl and distortion) symptoms

On the potato leaves, yellowing and rolling symptoms of PLRV are common, had been reported by different researchers (less commons are papery and leathery) (Harrison, 1984; Radcliffe and Ragsdale, 2002). In Pakistan for PLRV, the yield losses were dedicated as around 90% (Bhutta and Bhatti, 2002). Batool et al 2011 reported some PLRV symptoms as dwarfing plants with yellowing and rolled leaf inward. PLRV detected on different alternative cultivated host from solanacea which is Tree tomato (*Solanum betaceum*) were showed yellow mosaic and curling inward of the leaves on stunted growth. After the first infection, symptoms of PLRV are recogniseble in leaves with upward rolling, slightly pale and may show purpling arrived to stunting Anoymous, 2018.

In Turkey, Güner ve Yorgancı (2006), detected PLRV from sympytomatic and asymptomatic samples of potato by ELISA and biological indexing in Niğde and Nevşehir provinces. They had been recorded both single infection of PLRV and mixed infections with other potato viruses (PVY, PVX, PVS, PVA). Some critical symptoms as mottling, vein clearing and banding. As well distortion, local lesion were underlined in same study. In an other study, Yardımcı et al. (2015) detected the PLRV on potato plants with common potato viruses as mixed infection by ELISA.

DAS ELISA;

As a result of serology studies, chlorosis, lamina narrowing, leaf deformation and curling in the leaves, symptoms was found to be related with PLRV.

There was no positive response to any other viral agent serologically with other antiserums used during the study. Positive samples were also tested against TYLCV and PLRV by PCR method. Thus, both confirmation of PLRV results and mixed infection cases with TYLCV were examined. However, no mixed infection was detected. In the study, PLRV disease incidence was determined as 9.4% and TYLCV 24.52% in the collected samples (Table 1). In addition to the detection of TYLCV, the detection of PLRV in predominantly potato species rather than tomato, has point out vector transportation. The molecular origin of this factor, which may have important outcomes in terms of virus ecology and epidemiology, was a priority in the study. Similarly, Gugerli (1979) stated that PLRV and PVA with the ELISA method could easily be used in routine testing in potatoes and in test plants. Jafarpour et al. (1988) stated that in their study with the ELISA test they detected 3-4% PVS, PVX and 9% PLRV in certified seeds and commercial potato tubers produced in Australia.

Ali et al.(2002), in a study conducted in different ecoregions of Pakistan, they found that PLRV and PVY viruses, which cause significant problems in potatoes grown, were detected 0-71.0% in autumn respectively. Whitworth et al. (1993) attempted to detect PLRV using observational examination, direct tissue bloting and ELISA tests. In Turkey, Dolar et al., (1976), found PVX, PVY, PLRV, Aucuba Baciliform Badnavirus (AuBV) and witch broom virus and their transmission rates in potato r regions of Adana, Içel, Gaziantep, Hatay, Antalya and Kahramanmaraş provinces. It has been reported by Çıtır (1982) that seed potato tubers in and around Erzurum are infected with PVX, PVY, PLRV, PVS and low percentage PVA. Özbayram ve Yorganci (1989), conducted a survey of PVX, PVS, PVA, PVY and PLRV in 23 centers in Turkey, indicated that 32.08% of PLRV infections were present. Yilmaz et al. (1990), determined that the prevalence of the disease ranged between 0.8% and 17.68% in 1997 and 1987 and that it could be used in tubers in tests as a result of the study conducted by ELISA test for leaf curl virus in early harvest potatoes grown in Çukurova region. Sökmen et al. (2005) in their study, they determined the Potato X Virus (PVX) and Potato Y Virus (PVY) with DAS-ELISA in seed potatoes in the fields. However, none of the potato tuber samples tested were found to be infected with Potato Leaf Curl Virus (PLRV). Al-Ali et al. (2016) reported that 45 out of 50 plants were infected with TYLCV after testing with DAS-ELISA method for symptoms such as upward curling, chlorosis, mosaic, dwarfing in plants and deformation in fruits and leaves, one of the most important vegetables grown in Kuwait. The existence of TYLCV in our country was determined by Yilmaz (1978). Molecular characterization was first performed by Köklü et al, (2006). Yilmaz (2016), in his study in Hatay province, found 26% of infection with TYLCV in samples collected in organic tomato cultivation under cover.

Table 1. Assay results for collected tomato leaf samples

VIRUS SPECIES	ASSAY	*RESULT
TRSV; Tobacco Ringspot Virus	DASELISA	-
ToRSV; Tomato Ringspot Virus	DASELISA	-
PMMoV; Pepper Mild Mottle Virus	DASELISA	-
TMGMV; Tobacco Mild Green Mosaic Virus	DASELISA	-
CMV; Cucumber Mosaic Virus	DASELISA	-
TMV; Tobacco Mosaic Virus	DASELISA	-
ToRSV; Tomato Ring Spot Virus	DASELISA	-
ToMV; Tomato Mosaic Virus	DASELISA	-
PLRV; Potato Leaf Roll Virus	DASELISA/RT-PCR	++(5 sample)
TSWV; Tomato Spotted Wilt Virus	DASELISA	-
PVX; Potato Virus X	DASELISA	-
TYLCV; Tomato Yellow Leaf Curl Virus	PCR	+(13 sample)
PVY; Potato Virus Y	DASELISA	-

*(-) Negative: not infected, (+) Positive: infected

Molecular Studies

All 5 samples that received PLRV positive results in the ELISA test were evaluated in the study with RT-PCR and the same positive results were obtained as the ELISA test results. The 336 bp cDNA amplicons of PLRV were verifed with 1.5 agarose jel electrophoresis (Figure 3). All 53 tomato samples collected in the study were PCR tested against TYLCV and 13 of them showed positive results*.

* Since the detailed data of TYLCV positive samples will be a basis for a separate study, the molecular data in particular is not detailed in the scope of the publication.

The presence of PLRV in tomato culture has been determined by any laboratory technique. However, molecular determinations associated with PLRV were carried out on potato isolates (Bostan et al., 2004; Ghazal et al., 2009). Sivaprasad et al., (2016) found positive results on *Solanum betaceum* in their testing with both ELISA and RTPCR. The study is also one of the rare and special evidence that PLRV can infect different Solanaceae hosts other than potatoes.

Fidan et al. (2011) found that TYLCV-IL, TYLCSV (TYLCV-Sardinia) and TYLCV-Mild strains were present in our country using molecular diagnostic methods. Atalay (2018) identified two strains with TYLCV-specific primary pairs (TYLCV-IL, TYLCV-Mld) in a study conducted in 2017 to investigate variants of TYLCV in Adana and Mersin provinces. The same researcher revealed that many viral variants of TYLCV threatening tomato cultivation in the Mediterranean region.

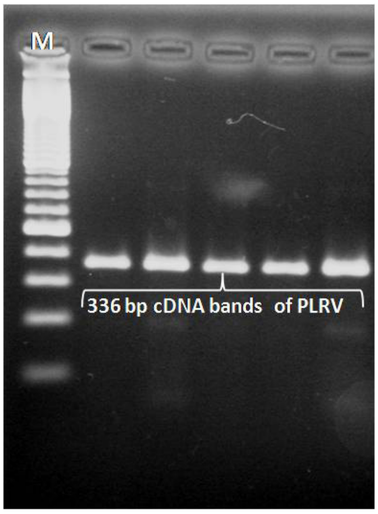

Figure 3. 336 bp cDNA bands of PLRV infected tomato samples by the primer pairs according to Singh et al. (1995); M:;DNA ladder (%1,5 agarose jel)

The evolutionary history was inferred using the Neighbor-Joining procedure. The optimal tree where in the related taxa grouped together in the bootstrap test (1000 repeats) are appeared close to the branches were determined. The evolutionary separations were processed utilizing the p-separation procedure The research has been incorporated 18 nucleotide groupings. All gaps and missings were eliminated with. There were a total of 212 circumstances in the last dataset. Evolutionary assessments were directed in MEGA7.

When the sequences compared, PLRV-Ad isolate, are clustered in Group 1, subgroup 1a with other isolates from NCBI, shown maximum 98,74% similarity with Belgium potato isolate (KX364206.1). Also it clustered in same branch with New Zealand (GU002341.1) 99.37% similarity (Şekil 4.) .

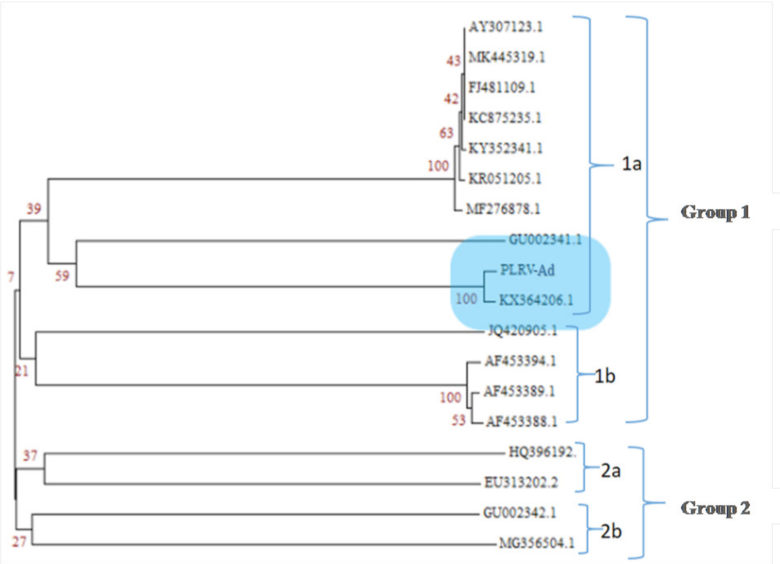

Figure 4. After haplotype examination, phylogenetic trees were built with MEGA 7 program. Related 18 confines were chosen by utilizing

Conclusion

The most important protection methods are to avoid disease resistance of virus diseases, to eliminate sources of inoculum and to fight vectors effectively due to the lack of chemical management. All necessary methods of controlling viruses should be used and economic losses should be reduced at both local and regional levels.

Virus free certified basic material should be used. Weeds that host for viruses and aphids at the same time need to be eliminated in seedling field, greenhouse or land. Plants (tomato, pepper, lettuce, tobacco, etc.) that are mainly the main host of PLRV, including the potato culture) should not be grown side by side. This plays a key role for the epidemiology of all plant virus diseases. Effective vector control must be done by considering transitions through vectors to wild host species around the land.

It is concluded that PLRV was determined for the first time with DAS-ELISA and one step RT-PCR in tomato samples collected from Adana province and was evaluated decisively because it is not only a cultured plant with intermediate host potential but also an important cultured plant in economic terms. Although the Tomato Yellow Leaf Curl Virus is one of the most prominent of these, the Potato Leaf Roll Virus (PLRV), which is one of the rare factors in tomato culture, has the potential to be economically and ecologically important.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

Funding

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Research Article

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Determination of rainfall effects on kaolin clay coverage rates used in prevention plant from sunburn

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Abstract

Sunburn, which is an important problem in agricultural products grown in arid and semi-arid regions, causes economic losses of up to 40-60% in some years. Sunburn occurs as a result of overheating of the surfaces of fruits exposed to high air temperature and solar radiation from the sun. Increase in the temperature stress for plants is observed during months of high temperature, this is one of the main reasons for many negative conditions (changes in fruit colour, cell death in plants, etc.) in fruits and leaves. While the shelf life of damaged agricultural products is shortened, deterioration is observed even in the storage stage. Today, different applications are made to reduce the sunburn in many kinds of fruits and sunburn damage can be significantly reduced as a result of these applications. For this purpose, it was aimed to reduce the temperature on the leaf surface by the application of kaolin clay (Trade name; Güneş Stop) applied to the plant surface at the beginning of the season. In the study; eight different concentrations of kaolin clay with tree application volume were applied and after the application, leaf samples of the citrus trees were taken from the predetermined regions, and the coverage rate was measured in an image processing program. From 8 different regions, 15 pieces of leaf were randomly taken from each region. The same process was carried out again by collecting leaf samples from the same regions after the rain; thus, the performance analysis of kaolin clay was determined after the rainfall. As a result of kaolin clay application; according to the results of image analysis of average leaf surface coverage rates obtained by regions and after the rainfall, the results obtained from the same regions showed that kaolin clay was washed at a rate of 39.64% with precipitation.

Keywords: Image processing, Temperature stress, Solar radiation, Plant Protection

Introduction

With global climate change, high temperatures and frosts may happen in parts where no high temperatures and extreme colds were experienced before. Considering the temperature projections of the century we live in, it is believed that extremely high temperatures and colds will be experienced a lot more. Agricultural products are the leading species that will be most affected by this situation and the food chain will be affected. As the regional high temperatures increase, agricultural products' tolerance to the environmental factors will decrease, thus, the yield and product quality of the agricultural products will inevitably decrease.

It is essential to preserve the plant for the sake of achieving a high yield product in agriculture. It is also vital to heal environmental factors and ensure comfort in terms of the product although pest control is thought of in the first place for plant protection. Product surfaces may burn and color changes may be seen in some fruit types such as apples, pears, grapes, pomegranates, olives, walnuts, citrus fruits and vegetables such as peppers, tomatoes, watermelon that are exposed to sunlight and high temperature directly. Consequently, the product is no longer appealing and rapidly deteriorates and its market value

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decreases. This situation, which is called sunburn, is oxidative damage caused by intense light such as high temperature and ultraviolet (UV) (Finkel and Holbrook, 2000). At extreme temperatures, stains happen due to the damage of the fruit cells that are in the open parts of the crown (Yazıcı and Kaynak, 2006). Considering the high temperature and exposure time, most plant species cannot survive at temperatures above 45^{o}C. The ambient temperature of 27-28 ^{o}C is suitable for an adequate vegetative development. In their study, Wünsche et al. (2002) revealed that sunburn is a defence mechanism performed by the fruits and leaves of the plant due to their efforts to protect themselves. Two examples can be given for this situation: Sunburn in Fuji apples appear as a color change from yellow to brown, and it appears as surface whitening in Granny Smith apples (Yazıcı and Kaynak, 2006).

Since the consequences of sunburn are acknowledged, for the purpose of eliminating this situation, various covering studies are executed in our country, as well as many sunburn prevention studies. One of these is to use kaolin clay on the leaf and fruit surface, which bears the quality of reflecting the sun's heat and rays back. Often the kaolin clay is mixed with water and applied with the high pressure motorized backpack sprayer, by doing so, the fruit and leaf surfaces are covered with a thin layer of clay. Hence, the relationship of the fruit and leaf surfaces with the environment is diminished and the water content of the related parts is protected. Due to the kaolin clay, the leaf part can provide better photosynthesis and respiration since its heat transfer with the environment decreases. Besides, the leaf part draws less water from the root zone so the water will not be lost due to the evaporation. And there are secondary benefits of kaolin clay. As the fruit and leaf surfaces will be covered, it provides protection against some insect and fungus species. It also provides protection against frost since kaolin clay can be applied in cold weather.

In this study, kaolin clay (trade name: Güneş Stop) was applied to the leaf surface with a motorized backpack sprayer in a garden with okitsu tangerine trees; and particularly after the application, the effects of precipitation on kaolin coverage ratio were determined.

Material and Method

The study was conducted in a garden with 5-year-old okitsu tangerine trees in Adana, Turkey. The row width of fruit trees in the garden was 5 m, the tree crown height was approximately 2 m and the tree crown width was 2 m. The trade name of kaolin clay applied to cover the leaf surface was 'Güneş Stop'. This commercial product applied in the study can be started to be used when the fruit diameter reaches 2.5 cm. In terms of frequency of application of kaolin employed in the research, consecutive applications in dry conditions can be performed 14 and 21 days after the first application and it is suggested that the final application should be made approximately 1 month before the harvest. In the research, kaolin was applied in 8 different concentrations (GS1, GS2, GS3, GS4, GS5, GS6, GS7, GS8) and also the producer of the kaolin clay advices to use the concentration between 1.2% to 2.8%. These concentrations were 1.3%; 1.5%; 1.6%; 1.8%; 2%; 2.2%; 2.4%; 2.7 % in the range of GS1 and GS8, respectively. The concentrations prepared were sprayed at a pressure of 15 bar in 2 L / tree volume.

In the research, pH, leaf contact angle, surface tension values of the solution in the tank were measured to identify the effect of different kaolin concentrations on the physical features of the spray liquid in the tank. The contact angle and surface tensions of the drops on the leaf surface were calculated with the Drope Shape Analysis System DSA 10 Krüss brand device to identify the effect of the performed applications and spray solutions of various concentrations on the coverage rate.

Spraying applications were performed with the Palmera OS-768 High Pressure Motorized Backpack Sprayer.The power of this sprayer was 1.9 HP and its tank storage was 25 L (Figure 1).While applying with motorized backpack sprayer , 15 bar operating pressure was preferred. The bypass line of the sprayer was kept open constantly so the relevant concentrations did not accumulate in the storage tank of the sprayer. After each operation, the tank of the sprayer was cleaned and washed with pressurized water. For preparing the concentrations, tap water was used.

Figure 1. Application of the kaolin clay to the trees with the motorized backpack sprayer

Kaolin solutions prepared in different concentrations were sprayed on random parcels with a motorized backpack sprayer according to the trial pattern as 3 replicates. Then 15 leaf samples were randomly taken from each tree, from the outer, inner and different locations of the tree. The coverage rates on leaf samples taken from different points on the tree were measured one by one and the average coverage rate per tree was calculated.

The leaves collected from 8 different parts where kaolin solution was sprayed were put in paper envelopes and preserved in the refrigerator. After taking these leaves out of their packaging in the laboratory, they were photographed with an Olympus (E620, Japan) camera which was placed on a black exposure box (Figure 2). During the photographing session, the camera was calibrated in the following format: ISO: 800; shutter speed: 1/60; aperture: 5.6; resolution: 4032 x 3024 and JPEG. The distance between the camera lens and leaf samples was 25 cm. 12 VDC, 48 led light sources (Cata, TL-4481) were used in the illumination of the exposure box and they were placed around the camera lens for obtaining a homogeneous illumination. A DC power source (Pacific, 2305D +) was used to supply the in-box lighting system. The coverage ratios of the kaolin clay, which created white spots on the leaves, were measured by the image processing technique.

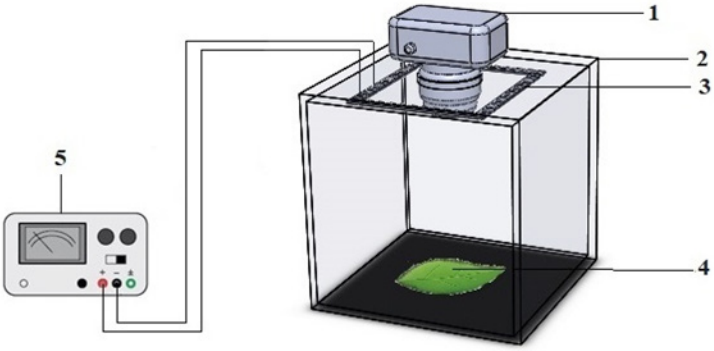

Figure 2. Schematic view of the system (1. Camera, 2. Box of black exposuring, 3. Light Source of LED, 4. Leaf Sample, 5. DC Power Supply)

The image processing technique is frequently used in agriculture today for purposes such as measurement of leaf area, color analysis and classification in fruits; determination of pesticide drop size, drop density and drug coverage rate in spraying applications; determination of leaf area, conducting color analysis in fruits, determination of weeds, determination of grinding degree, monitoring plant growth and root development. In this way, some very difficult measurements are performed easily, faster and precisely (Karabacak, 2007; Mustafa et al., 2008; Zhao et al., 2009; Örge, 2012; Kuncan et al., 2013; Sabancı and Aydın, 2014). The actual shape of the leaf was visually produced by carving the rectangular shaped photographs according to the shape of the leaf by means of the screen snipping tool.

The leaves were photographed in a dark environment and under a constant light source to reveal the coverage area of Güneş Stop containing kaolin clay on the leaves with image processing in the most accurate form. After the threshold values of these pictures displayed with the ImageJ 1.51p program (Özlüoymak and Bolat 2020) were separately identified; RGB was defined as the color space, and particle analysis was conducted to find the coverage rate of the Güneş Stop particles on the leaf (Figure 3).

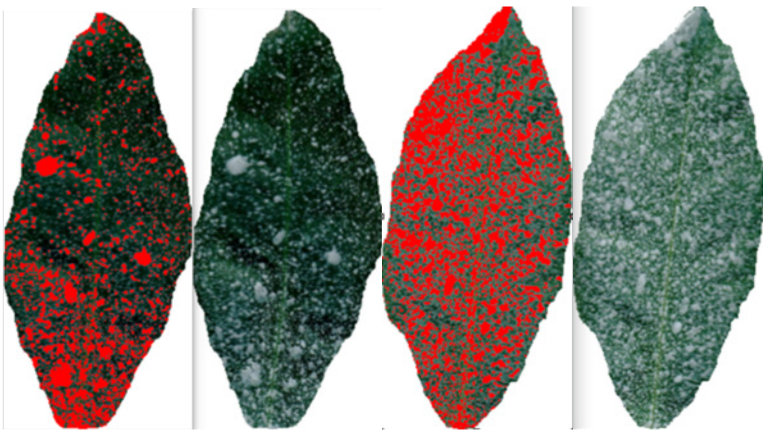

Figure 3. Photographed leaf samples and analysed in ImageJ 1.51

The image processing process was applied to pre-rain leaf samples (15 pieces) and leaf samples which were taken after three days of the rain (15 pieces). These leaf samples were taken from 8 pre-defined locations. Coverage rates of the leaf samples -before and after the rain-taken from each location were compared. Statistical tests were conducted with the SPSS package program.

Results and Discussion

Coverage rates of leaf images were determined by using the image processing program and statistically analysed. Firstly, it was investigated whether 8 different kaolin concentrations applied to the leaf surface demonstrate any differences among themselves; Levene Homogenity Test was carried out before one-way ANOVA test. The Duncan test was conducted to determine the differences (Table 1). According to the Levene test, surface coverage data was distributed homogeneously (P> 0.05). So, there was a difference between the coverage rates achieved in different concentrations. There were also significant differences according to the one-way ANOVA test.

Table 1. One Way Anova results of coverage rates obtained from the image processing program

Levene Statistic	df1	df2	Sig.
2,013	7	112	,060*

One Way Anova Test	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0,331	7	0,047	11,111	0,000*
Within Groups	0,476	112	0,004		
Total	0,807	119			

*P<0,05 significant

In the SPSS program, Duncan test was conducted with the results of the one-way ANOVA test and according to the results, the coverage rate achieved with GS8 (highest kaolin clay concentration) was the best coverage concentration (Table 2). Additionally, the lowest coverage rates were achieved with GS1, GS2, and GS3. Hence, high concentrations produced high coverage rates.

Table 2. Comparisons of the kaolin clay concentrations that cumulated on Leaf Samples

	Concentration	Number of Leaf	GS6	GS7	GS8
Duncan a	GS3	15	0,400		
	GS2	15	0,412		
	GS1	15	0,438		
	GS4	15		0,487	
	GS6	15		0,492	
	GS5	15		0,512	
	GS7	15		0,519	0,519
	GS8	15			0,561
	Sig.		0,140	0,234	0,078

The surface tension and contact angle values of the kaolin clay concentrations with different kaolin clay content employed in the laboratory study are presented in Table 3. In the three-replicate experiments, when the data of the surface tension was examined, it was definitely observed that the concentration did not affect the surface tension however, no significant increase or decrease was observed. When the contact angles of kaolin clay containing droplets on the leaf were assessed, it was noticed that the contact angle did not alter much with higher concentration rates.

In Table 4, the average kaolin coverage rates (%) per leaf noted in the applications before and after the rain are presented. At the GS8 concentration, providing the highest coverage rate before the rain, 39.64% of the kaolin clay washed under rain. Accordingly, it was discovered that more washings occurred at concentrations providing high coverage.

Coverage rates of leaves scanned before and after the rainfall were compared. This comparison aimed to understand washing under the rainfall whether statistically significant or not. In the comparison, pre-rain coverage rate data for each concentration and post-rain coverage rates were compared. Firstly, normality tests were carried out since the binary comparison was applied to the data. Since the number of data was below 50 in the normality test results, the Shapiro-Wilk test result was considered as the basis (Kalaycı, 2016), and it was discovered that the data did not statistically distribute normally according to this result. Therefore, the Mann-Whitney U test, which is a nonparametric binary comparison test, was selected to understand whether the data that did not conform to the normal distribution would reveal a significant difference among themselves. According to the data achieved (Table 5), it was observed that they were seriously washed after the rain and a small amount of kaolin clay coverage remained on the leaf surface. Thus, regardless of the used proportions; kaolin clay, which was sprayed on the leaf surface, flowed statistically with the rainfall.

Table 3. pH, surface tension and contact angle values of each kaolin clay concentrations

	Kaolin Clay Concentration (%)	Surface Tension (N/m)	Contact Angle (º)	pH
GS1	1,3	73,5	72,57	9,55
GS2	1,5	74,53	72,13	9,88
GS3	1,6	74,79	71,13	9,23
GS4	1,8	74,9	70,53	9,7
GS5	2	73,83	71,73	9,73
GS6	2,2	74,16	71,17	9,46
GS7	2,4	74,54	72,6	9,35
GS8	2,7	73,58	72,03	9,87

Table 4. Coverage rates of leaf samples before and after the rainfall (%)

Kaolin Clay Concentration	Coverage rate before rainfall (%)	Coverage rate after rainfall (%)	Coverage Rate Reduction (%)
GS1	35,84	28,24	7,6
GS2	39,27	27,02	12,25
GS3	40,35	25,56	14,79
GS4	44,12	23,88	20,24
GS5	45,7	21,26	23,44
GS6	46,93	18,54	27,39
GS7	48,37	15,09	33,28
GS8	52,11	12,47	39,64

Table 5. Coverage rate comparisons of leaf samples after and before the rainfall

	GS1	GS2	GS3	GS4	GS5	GS6	GS7	GS8
Shapiro Wilk-Asymp Sig.	0,022*	0,013*	0,002*	0,022*	0,00*	0,02*	0,02*	0,013*
Mann Whitney U Asymp Sig.	0,00**	0,00**	0,00**	0,00**	0,00**	0,00**	0,00**	0,00**

* 0,05>P not homogenous

** 0,05>P significant

Conclusion

It is vital that kaolin clay does not harm health in terms of both humans and other living creatures by skin contact or breathing. It was demonstrated that Güneş Stop concentrations prepared in different concentrations did not cause very dissimilar results on pH, surface contact angle, surface tension; and concentrations prepared with high amounts of Güneş Stop, just increased operating cost a little. So, it is proper to employ in high concentration unless it rains and it was observed in applications that a good coverage was achieved on the leaf surface by doing so. When the leaves photographed after the rain, it was seen that there was a certain amount of Güneş Stop remaining on the surface of the leaves despite the heavy rain continuing for three days. Besides, it was found that the stains on these leaves were not statistically significant. With Güneş Stop, the direct contact of the leaves and fruit with the sun was blocked until the first rain subsequent to the application, and moisture content of these sections was preserved. It is believed that Güneş Stop, which ensures improved protection against pests with absorbent mouths, will be beneficial in every rain (until harvest).

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Homocysteine metabolism in rats with metabolic syndrome and the impacts of nigella sativa oil on some biochemical parameters

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Abstract

The high level of fructose taken in the diet is one of the reasons for the increased prevalence of metabolic syndrome, which is increasing day by day globally in association with the effects of genetic and environmental factors. In the study, 21 male Sprague-Dawley rats of 220±20 gr body weight were used. The rats were assigned to 3 groups as the control group, metabolic syndrome group, and the group where *Nigella sativa* oil was administered. The serum homocysteine levels were increased in the metabolic syndrome group compared to the control group but without statistical significance ($p>0.05$). Homocysteine levels decreased significantly after *Nigella sativa* oil compared to metabolic syndrome group. LDH ($p <0.001$) and uric acid ($p <0.05$) levels which were higher in metabolic syndrome group were decreased in *Nigella sativa* oil group. Hyperhomocysteinemia is a risk factor for endothelial dysfunction. In our study, the treatment of the metabolic syndrome and regulation of the increased levels of homocysteine with *Nigella sativa* oil in metabolic syndrome were discussed. Some biochemical parameters and improvements in homocysteine levels with *Nigella sativa* oil has been identified. In this study, we have concluded that the occurrence of elevated levels of plasma homocysteine are closely associated with the development of inflammation, cellular adhesion, hepatic dysfunction, and cell proliferation and that the reduction in the serum levels of homocysteine by the administration of *Nigella sativa* oil will lead to favorable out comes.

Keywords: Homocysteine, Metabolic syndrome, *Nigella sativa* oil, Vanillylmandelic acid

Introduction

Metabolic syndrome (MetS), is a complex condition leading to several disorders, including sympathetic activation, oxidative stress, systemic inflammation, hypercoagulability, endothelial dysfunction, and hyperleptinemia (Scott 2004; Reaven 1988; Fulop et al. 2006). It is a serious health problem which starts with insulin resistance and becomes a combination of several disorders such as obesity or increased waist circumference, hyperglycemia, atherogenic dyslipidemia, hypertension, and proinflammatory and prothrombotic disorders (Iannucci et al. 2007; Brent 2004). Gerald M Reaven described the resistance as the stimulation of glucose intake by insulin. He called the entire combination of the following findings as “Syndrome X”, including hyperinsulinemia, glucose intolerance, decreased HDL-cholesterol levels, elevated VLDL-cholesterol levels, hypertension, and the increased risk for ischemic cardiac diseases (Reaven 1988). The definition of this table has become broader day by day with a variety of different definitions introduced by several scientists. “Syndrome X plus” defines the additional four findings called “the fatal four”, which are upper body obesity; glucose intolerance, hypertriglyceridemia, and hypertension. The inclusion of erythrocytosis and elevated uric acid levels into the clinical table is called “fatal six” (Sencer 2001). In this way, the definition of the syndrome has been improved day by day, being named with a variety of different terms along with the quality and

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comorbidity of various factors.

It is well known that the risks for developing Type 2 diabetes mellitus (DM) and the cardiovascular disease are remarkably high in MetS patients (Özgen 2006). The prevalence of this major public health issue, MetS, is on the rise gradually all around the world due to the contributions of increasing rates of obesity and sedentary life, leading to the increasing rates of several diseases, especially Type 2 DM and cardiovascular diseases (Carr and Brunzell 2004).

In addition, another cause of the increased prevalence of MetS is the intake of high-level dietary fructose, which is another individual risk indicator (Ford et al. 2002; Pyorala et al. 2000; Bruce and Byrne 2009). Fructose is a natural sugar found in honey and several fruits. Today, it has a wide spread use as a sweetener in the form of corn syrup in the food and industrial sector (Miller and Adeli 2008). The corn syrup rich in fructose contains 55-90% fructose, and being an ingredient as a sweetener in ready-made foods, it is the major source of dietary intake of fructose. Due to its low cost and easier miscibility, the corn syrup high in fructose is very commonly used in all sweetened ready-to-drink beverages (fruit-flavored sodas, ice tea, fruit juices etc.), in a variety of confectionery types, jam, marmalade, chocolate, cake, crackers, and other food products with a jelly content. Because of this increase in its use, fructose consumption per capita (excluding the natural consumption of fructose found in fruits and vegetables) was less than 0.5 g per day in the 1970s, however, today this figure has become 85-100 g per day with an astonishing increase (Hanson et al. 2002; Gaby 2005; Korkmaz 2008; Reddy et al. 2009).

Today, the use of medicinal plants against diseases and associated research has gained importance in European countries. In this study, we used the oil of *Nigella sativa* plant which has an important place in traditional medicine. *Nigella sativa* plant of the Ranunculaceae family has a remarkable history as it has been referred to in a number of references on history and religion (Salem 2005). The main origin of *Nigella sativa* is Egypt and it has been well known since ancient times. It has been popular in the Eastern and European countries as a medicinal plant rich in hundreds of active ingredients. *Nigella sativa* is known to be commonly used in for many years in folk medicine as a traditional medicine for the treatment of hypertension, rheumatismal disorders, gastrointestinal diseases, burns, cutaneous diseases, liver and kidney diseases, diabetes, constipation, asthma, bronchitis, diarrhea, dyspepsia, headache, dizziness, jaundice, and fever in Middle and far East (Hajhashemi et al. 2004; Ramadan 2007). Today, it is suggested by the medical community that the oil extracted from the black cumin "*Nigella sativa*" grown naturally in some parts of Egypt shows favorable effects in the treatment of cancer and several other diseases. In this study, we aimed to determine the change of homocysteine, vanillylmandelic acid and some serum parameters after fructose-induced metabolic syndrome before and after *Nigella sativa* oil administration.

Materials and Methods

This study was carried out in accordance with standard experimental animal studies after the approval of the Ethics Committee (Firat University Ethics Committee of Animal Experiments; 06.09.2017 date, protocol number 2017/83 and decision number179).

Approved by the Ethics Committee, the study was conducted in compliance with the ethical principles applied to the standard experimental animal research. In this study, 21 eight-week-old Sprague-Dawley male rats were used, each weighing 220±20 grams on average. Standard pellet feed and drinking water were supplied to the animals. The rats were assigned to three groups, each group consisting of 7 rats. During the study, the standard rat diet, fructose, and *Nigella sativa* Oil (NSO) were administered orally. A standard pellet diet and % 10 fructose added into the tap water was administered to the groups, but the control group, to induce MetS (Sánchez-Lozada et al. 2007).

Group 1 (n=7): Control group

Group 2 (n=7): MetS group

Group 3 (n=7): NSO group

Mode of administration of the oil of *Nigella sativa*

After inducing MetS in group 3, by adding fructose of % 10 into the drinking water for a period of 10 weeks, the rats in this group were administered 0.1 ml NSO daily by oral gavage for four weeks (Perveen and Hainder 2013). The rats in group 1 and group 2 were decapitated 10 weeks after the start of the experiment and the rats in group 3 were decapitated 14 weeks after the start of the experiment.

Laboratory Analyses

The blood samples were collected into plain biochemistry tubes containing gel. These samples were centrifuged at 4000 rpm for 5-10 minute sob taining the sera. Pinkish red or red serum, which is a sign of hemolysis, has not been observed in the study samples. The levels of urea, creatinine, uric acid, inorganic phosphorus, alkaline phosphatase (ALP), sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), lactate dehydrogenase (LDH), creatine kinase (CK), and CK-MB (creatine kinase-muscle/brain) were determined by the colourimetric method using a autoanalyser (Advia 2400 Chemistry Analyzer, Siemens Healthcare, Germany) device and appropriate kits. In order to collect urine samples to be analyzed for vanillylmandelic acid (VMA), funnels of appropriate size were placed under the floors of metabolic cages. The volumes of each of the 24-hour urine samples were documented individually. From each animal individually, a urine sample of approximately 2 ml (mixture) was collected into the sample tubes and then centrifuged at 3000 rpm for 10 minutes. The elicited supernatants were transferred to another group of clean and dry tubes and stored at -20°C until the time of analysis with high-performance liquid chromatography (HPLC system, Shimadzu Corporation, Kyoto, Japan).

The amount of VMA in urine was determined using a commercial kit and the HLPC method at a column temperature of 25°C, flow velocity of 1.0 ml/min, and an injection volume of 10 µL.

Serum homocysteine levels were determined with HPLC device Agilent, 1200 series, with fluorescence detectors by means of a reverse-phase C18 ODS column and a mobile phase column for tri-n-butyl phosphyne, dimethylformamide, 7-floro-2,1,3-bensoxadiasole-4-sulfonamide and acetonitrile

(Sridhar et al. 2016).

Histopathological analysis

Liver tissue samples were fixed in % 10 formalin and embedded in paraffin. 5-µm-thick paraffin sections were cut from the paraffin-embedded tissue blocks and stained with haematoxylin and eosin and picrosirius red F3BA (% 0.5 saturated picric acid solution). The paraffin sections were deparaffinized by immersing in xylene and rehydrated through a series of graded alcohols (100%, 95% and 75%), for 15 min each. The slides were stained with haematoxylin and eosin as well as picrosirius red F3BA and mounted with coverslip using distyrene plasticizer and xylene (Maulik et al. 2012). The slides were examined under light microscope by a pathologist blinded to the study groups. Images were taken at magnification × 20.

Statistical Evaluation

The data collected in the study have been presented in means±standard deviation. KruskalWallis test was used to evaluate the data in the groups and Mann Whitney-U test was used for binary comparisons between groups. The level of statistical significance was accepted at a level of $p<0.05$. SPPS statistical package program (IBM SPSS Version 22.0) was used for the analysis of data.

Results and Discussion

Determination of biochemical parameters

The levels of serum homocysteine, urinary VMA, and the results of the biochemical tests were compared statistically (Table 1 and 2). It was observed that the elevated levels of homocysteine after the occurrence of MetS were decreased statistically significantly by the administration of NSO ($p<0.05$) (Table 1 and figure 2).

The serum levels of LDH, CK-MB, uric acid, and Ca statistically significantly increased in the MetS group compared to the control group and those levels were decreased by the administration of NSO similarly ($p<0.05$) (Table 2).

Effect of NSO on histopathological studies

Haematoxylin and eosin-stained liver tissue Figure 1: 1a control group with normal histology, 1b MetS group showing micro- and macrovesicular fatty change in hepatocytes and 1c NSO group with normal histology. In the MetS group caused micro- and macrovesicular fatty changes of the hepatocytes. No infiltration of inflammatory cells, necrosis or fibrosis was observed in the MetS group. NSO group led to protection of micro- and macrovesicular fatty changes of hepatocytes caused by MetS (Figure 1).

Table 1. The Levels of Homocysteine and VMA by group

Parameters	Groups			p
	Group 1	Group 2	Group 3	
Homocysteine (Mmol/L)	9.64 ± 0.25^{ab}	11.06 ± 0.55^{a}	9.51 ± 0.42^{b}	0.033 or *
VMA (µg/L)	21.53±0.99	20.31±0.87	20.56±1.50	0.737 or NS

The data are presented as means and standard deviation. a, b, c: There is a statistically significant difference between the measured levels of the parameters when they are marked with different letters on the same line. Different letters in the rows represent statistically significant difference *p<0.05; **p<0.01; ***p<0.001; NS: Not significant.

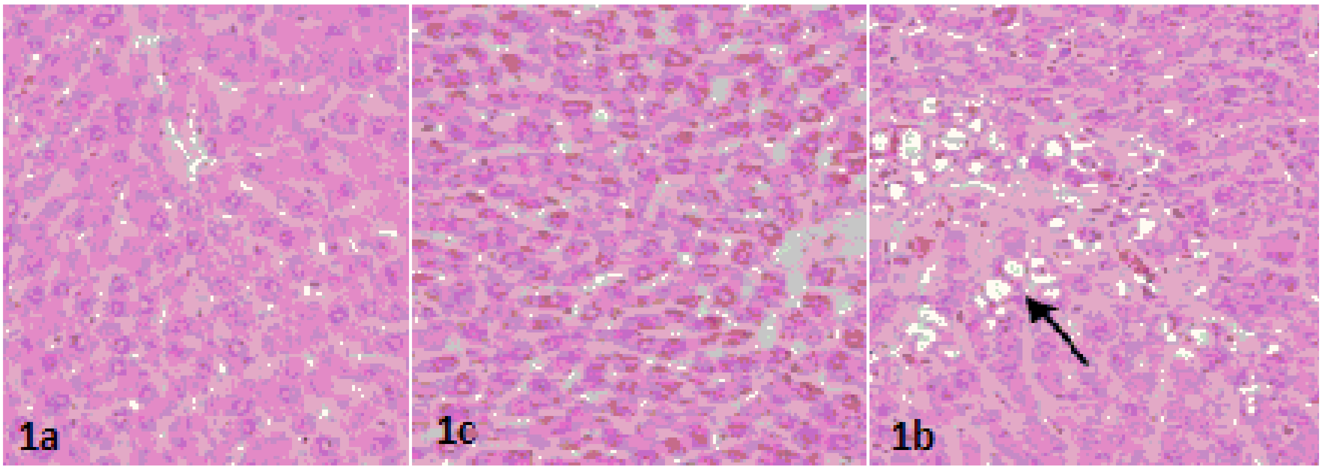
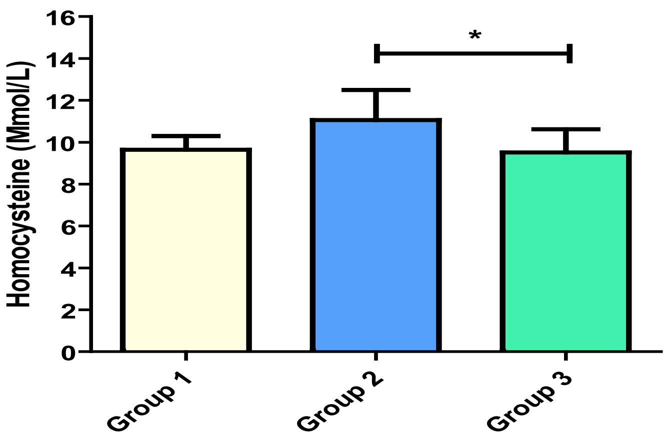


Figure 1 (1a, 1b, 1c). Haematoxylin and eosin-stained liver tissue

Table 2. Biochemical Parameters

Parameters	Groups			p
	Group 1	Group 2	Group 3	
Urea (mg/dL)	45.29±2.12[a]	36.86±1.30[b]	43.57±2.66[ab]	0.026 or *
Creatinine (mg/dL)	0.33±0.03	0.35±0.01	0.39±0.02	0.242 or NS
Uric acid (mg/dL)	1.33±0.05[b]	1.97±0.27[a]	1.31±0.07[b]	0.016 or *
Inorganic P (mg/dL)	7.19±0.16	7.43±0.53	7.37±0.17	0.869 or NS
ALP (mg/dL)	155.86±6.49	175.86±15.06	141.71±5.96	0.081 or NS
Na (mEq/L)	145.86±0.83[a]	145.00±0.87[a]	141.57 ± 0.53[b]	0.002 or **
K (mEq/L)	7.30±0.12	7.77±0.22	7.27±0.19	0.113 or NS
Cl (mEq/L)	107.57±0.37[b]	104.43±0.78[c]	110.14±0.34[a]	0.000 or ***
Ca (mg/dL)	9.44±0.04[b]	9.67±0.04[a]	9.43±0.08[b]	0.010 or *
LDH (u/L)	1787.57±27.83[c]	2786.71±192.27[a]	2292.43±119.06[b]	0.000 or ***
CK-MB (u/L)	1713.71±13.36[b]	2304.71±147.32[a]	1782.00±146.74[b]	0.005 or **
Ck (u/L)	13780.00±225.55[b]	26375.86±319.99[a]	12203.43±263.96[c]	0.000 or ***

The data are presented as means and standard deviation. a, b, c: There is a statistically significant difference between the measured levels of the parameters when they are marked with different letters on the same line. Different letters in the rows represent statistically significant difference *p<0.05; **p<0.01; ***p<0.001; NS: Not significant.



Different letters in the rows represent statistically significant differences (* P < 0.05).

Figure 2. Plasma homocysteine levels by group.

It is known that drinking water rich in fructose results in the occurrence of oxidative stress, which is the major factor involved in the progression of the cardiovascular disease (Busserolles et al. 2002). Studies demonstrate that a 6-week administration of fructose leads to oxidative stress due to the emergent imbalance between the ROS production and antioxidant capacity (Panda et al. 2015). In another study, it was reported that chronic consumption of fructose might lead to increased ROS production and oxidative stress (Sreeja et al. 2014).

It is known that fructose intake might induce hypertriglyceridemia and lipogenesis (Basciano et al. 2005). Fructose is absorbed in the intestines by the glucose transporter GLUT 5 and then it is delivered to the blood vessels by GLUT 2. In contrast to glucose, the absorption of fructose from the intestinal lumen does not require ATP hydrolysis and it is independent of sodium absorption. Fructose uptake by the liver is followed by its conversion into the following molecules in a row, which are fructose 1-phosphate, and then dihydroxyacetone phosphate and glyceraldehyde via aldolase respectively. The metabolites produced by these processes consequently lead to the synthesis of triglycerides. Exposure of liver to excessive amounts of fructose induces lipogenesis and accumulation of triglycerides leading to decreased insulin sensitivity.

The levels of VMA and homocysteine provides a unique overview of the progression of the disease, which may lead to the development of new treatments. VMA and homovanillic acid (HVA) are the biomarkers of sympathetic system activity and can easily be determined in the urine. The metabolic end-product of epinephrine and norepinephrine is VMA. The levels of VMA provide information on the general activity of the sympathetic nervous system. VMA is usually found in small quantities in the urine and its levels are elevated shortly after the exposure of the body to stressors (Csaba 2014). When the levels of VMA were compared (Table 1), no significant differences were observed between the groups (p>0.05).

It is known that homocysteine is the degradation product of the amino acid methionine. Hyper homocysteinemia is an independent risk factor for the development of cardiovascular diseases. High plasma homocysteine levels can lead to insulin resistance or can be involved in atherogenesis actively (Hayden and Tyagi 2004). Fructose-fed rats are reported to develop significant hyper homocysteinemia compared to normal rats (Panda et al. 2017). Homocysteine should be converted to methionine by remethylation and to cysteine by trans sulfuration. Water-soluble vitamin B preparations are known to affect these pathways significantly and decrease the levels of homocysteine. In our study, it was observed that the elevated homocysteine levels in the fructose-fed rats were reduced significantly by NSO (Figure 2). We are of the opinion that the elevated but not statistically significant levels of homocysteine in MetS, compared to the levels in the control group, might have occurred due to the stress which was experienced by all rats during the experiment (Table 1).

When there is a cardiac injury, the levels of LDH and CK-MB, which are abundantly found in the heart, increase and these enzymes are released into the bloodstream. Therefore, the high levels of these enzymes, released into the bloodstream indicate a myocardial necrosis. In this model, the emergent hyperglycemia after the fructose overload causes the over production of ROS, leading to developing damages in the myocardial membrane (López and Hernández, 2013). Therefore, it is reported that the activities of LDH and CK-MB increase in the serum of the fructose-fed rats (Panda et al. 2017). Our study findings support the reports in the literature (Table 2). Because, the reduction in the elevated levels of LDH and CK-MB enzymes in fructose-induced MetS after NOS administration indicates that this plant has a significant cardio protective activity.

A study has reported that the serum levels of uric acid, urea, creatinine, and lactate dehydrogenase increased significantly in the rats treated with fipronil (FPN) compared to the control group. The occurrence of the oxidative damage after FPN-induced changes in all study parameters was alleviated with thymoquinone, probably by enhancing the tissue antioxidant defense system (Ohamed et al. 2018). In our study, it was observed that the elevated levels of uric acid and LDH were reduced after NSO administration (Table 2). Severity sympathetic in metabolic syndrome it is a fact that the system is activated and increases the level of plasma catecholamines (Cornier et al. 2008). Muscle contraction in a study performed by applying catecholamine level of exercise type, the severity of and duration was reported to be important (Cornier et al. 2008). In a study with animals, treadmill after the exercise, epinephrine level increased 2-fold was determined (Jobidon et al. 1985). Literature There was no statistically significant difference between VMA results.

Conclusions

In this study, we have concluded that the occurrence of elevated levels of plasma homocysteine are closely associated with the development of inflammation, cellular adhesion, hepatic dysfunction, and cell proliferation and that the reduction in the serum levels of homocysteine by the administration of NSO will lead to favorable out comes. Since there was not a significant difference in the VMA levels, we have been prevented from having an opinion whether the sympathetic system activity and MetS were associated. Supporting the reports in the literature, the elevated levels of LDH, CK-MB, and uric acid occurring after the development of MetS were favourably reduced by the administration of NSO. Our study findings may provide important information about the pathophysiology and the course of the disease after the development of MetS.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Experimental procedures were approved by Animal Experiments Local Ethics Committee of Fırat University (Decision No: 179, Protocol No: 2017/83, Date: 06.09.2017).

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Data availability

Not applicable.

Consent for publication

Not applicable.

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