SEPTEMBER
2021

INTERNATIONAL JOURNAL OF AGRICULTURE, ENVIRONMENT AND FOOD SCIENCES

YEAR : 2021
VOLUME : 5
ISSUE : 3

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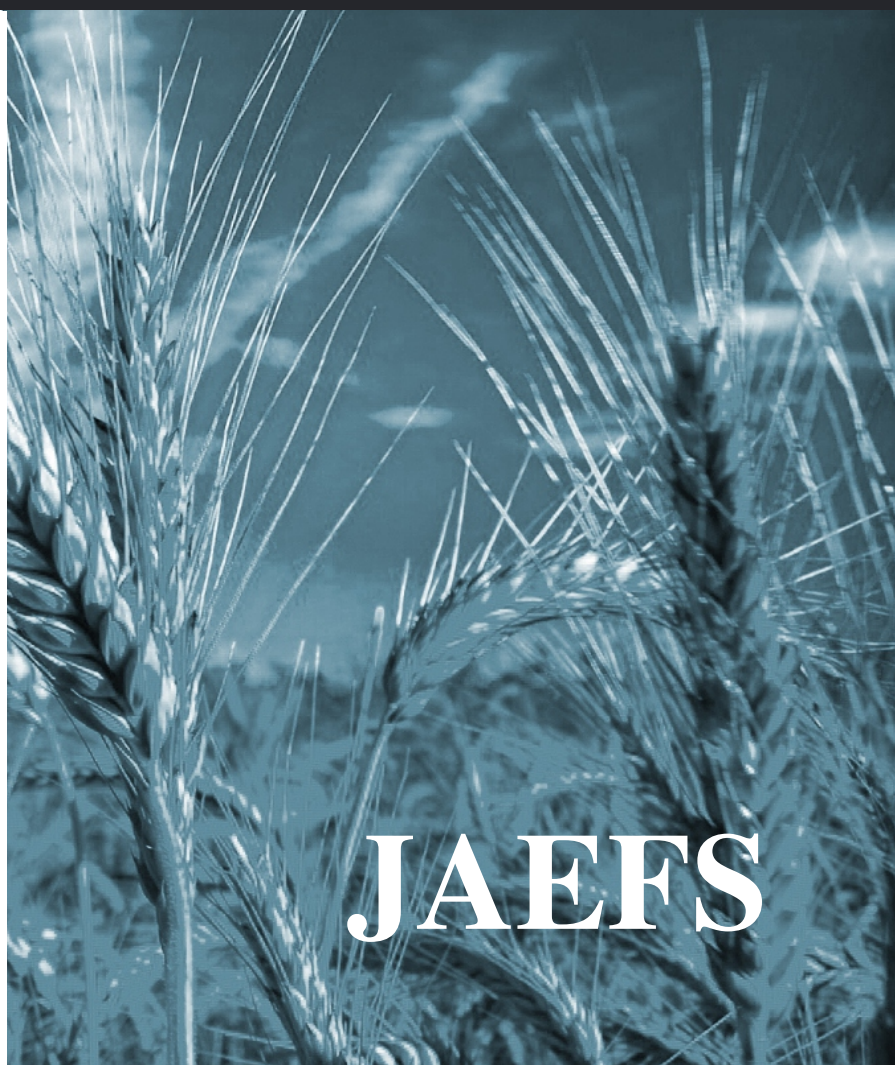

JAEFS

e-ISSN : 2618-5946
doi : 10.31015/jaefs

JAEFS

International Journal of Agriculture, Environment and Food Sciences

Int J Agric Environ Food Sci

e-ISSN : 2618-5946
DOI: 10.31015/jaefs

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September

Volume : 5 Issue : 3 Year : 2021

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Reviewer Board
September 2021

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DOI: 10.31015/jaefs

Production Information

Journal Name	International Journal of Agriculture, Environment and Food Sciences
Abbreviation	Int J Agric Environ Food Sci
Subjects	Agriculture, Environment and Food Sciences
e-ISSN	2618-5946
Publisher	Gultekin Ozdemir
Owner	Gultekin Ozdemir
Language	English
Frequency	Quarterly (March, June, September, December)
Type of Publication	International, Scientific, Open Access Double-blinded peer review Widely distributed periodical
Manuscript Submission and Tracking System	JAEFS uses the submission system of TUBITAK-ULAKBIM JournalPark Open Journal Systems - http://dergipark.gov.tr/jaefs
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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 5 (3) September 2021

DOI: 10.31015/jaefs

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Journal publishes Quarterly (March, June, September, December).
The publication language of the journal is English and continues publication since December 2017.
A Digital Object Identifier (DOI) number has been assigned for each article accepted to be published in JAEFS, starting from December 2017.
Journal of JAEFS welcomes article submissions and does not charge any article submission or processing charges.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 5 (3) September 2021

DOI: 10.31015/jaefs

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Research Articles

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.1 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):251-259 (2021)

Screening for *in vitro* antioxidant activity and antifungal effect of *Artemisia campestris* L.

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Abstract

In this study, the methanolic extract (ME) and the essential oil (EO) of the medicinal plant *Artemisia campestris* L. were investigated for their antioxidant activity and their antifungal efficacy on the postharvest storage decays; *Botrytis cinerea* Pers. and *Penicillium expansum* Link. The total polyphenolic and flavonoid contents were determined. The ME had higher total polyphenolic and flavonoid contents (400.64 µg GAE/mg and 43.13 ± 0.14 µg QE/mg, respectively) than EO (27.47 ± 0.44 µg GAE/mg and 14.04 ± 0.82 µg QE/mg, respectively). The ME presented higher radical scavenging power than the BHT and its IC_{50} values were 11.71, 40.96 and 23.32 µg/mL for the DPPH, β-carotene bleaching and reducing power respectively. In the antifungal activity, the EO had the stronger effect on both molds, particularly at concentrations > 15 µL, ≥ 800 µL/L and ≥ 15 µL by fumigation, incorporation and disc-diffusion methods respectively, resulting in higher than 80% inhibition of *B. cinerea* mycelial growth, and from 50 to > 80% inhibition on *P. expansum* mycelial growth. Methanolic extract showed nearby 50% inhibition on both fungi. The EO MIC was less than 2.5 µL/mL which was shown as MFC for both molds. The bio-autography test has shown separated compounds of the ME having an inhibitor effect on spore germination. These results offer an advantage of suggesting *A. campestris* could be used as a material for extraction of certain antifungal chemicals for preventing spoilage in food items.

Keywords: Antifungal activity, antioxidant activity, *Artemisia campestris, Botrytis cinerea, Penicillium expansum*

Introduction

Yield losses and food decay caused by insects, fungi, bacteria and viruses are of a great economic importance in crop and food production (Kordali et al., 2008; Zabka et al., 2009). *Penicillium expansum* Link (blue mold is the most common postharvest rot of apple fruit in storage, transit and market (Mari et al., 2002; Larous et al., 2007). It produces mutagenic toxins (Leggott et al., 2000). Among other, patulin is of high hazard to human health (Andersen et al., 2004; Quaglia et al., 2011). *Botrytis cinerea* Pers. (gray mold) causes disease in over 200 plant species around the world (Nakajima and Akutsu, 2014). The application of synthetic fungicides remains the main strategy to control these moulds, but other methods are required, due to public concerns about human health and environmental risks, besides the continuous appearance of fungicide resistant strains (Spadaro et al., 2004; Bi and Yu, 2016).

The plants become the suitable and a promising source of natural compounds as effective alternatives to synthetic chemical pesticides (Zabka et al., 2011). Plant extracts have

Cite this article as:
Hendel, N., Sarri, D., Sarri, M., Selloum, M., Boussakra, F., Driche, O. (2021). Screening for in vitro antioxidant activity and antifungal effect of Artemisia campestris L.. Int. J. Agric. Environ. Food Sci., 5(3), 251-259
Doi: https://doi.org/10.31015/jaefs.2021.3.1
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Received: 12 February 2021 **Accepted:** 29 May 2021 **Published Online:** 26 July 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 251-259
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

 DOI: 10.31015/jaefs.2021.3.1

been used worldwide for control of plant disease and extracts from many plant species were found to be involved in many pathogenic fungi control. (Apisariyakul et al., 1995; Zabka et al., 2009; Prakash et al., 2012; Askarne et al., 2013; Ozbek et al., 2020; 2021).

Artemisia campestris L. is widespread in Algeria and commonly known as "D'gouft" (Quezel and Santa, 1963). The traditional use of this plant includes the infusion, decoction or powder forms to treat digestive, respiratory, metabolic, allergic, cutaneous disorders (Dib et al., 2017), and for the antimicrobial, antiinflammatory, antivenom, antirheumatic and antioxidant properties (Akrout et al., 2001; Nikolova et al., 2010; Ghlissi et al., 2016; Nigam et al., 2019).

In Algeria, apple production reached 400.000 tons in 2011, and pears were estimated to be 211.000 tons in 2012. The strawberry cultivation has increased markedly (Agroligne, 2014). Although gray and blue molds affecting these products are reported, there is no statistics available.

The objective of this work was to determine the antioxidant activity of wild-growing *Artemisia campestris* L. in Algeria and to evaluate the susceptibility of blue and gray molds of Algerian apples and strawberries to its essential oil and methanolic extract, using different methods.

Materials and methods

Plant material

Artemisia campestris L. spontaneously growing in Kef Maâfer, at the station 35°50'58.7"N 5°23'58.4"E, was harvested in September 2015 during its flowering period. The plant identification was done by the botanist Dr SARRI Djamel and voucher specimen (N°: AC15M19) was deposited in the herbarium of the nature and life sciences department. After being dried in the shade in a dry and ventilated room for 15 days, the plant aerial parts were recovered and stored in paper bags and retained protected from light and heat until use.

Extraction of essential oil (EO)

Plant material (100 g) was subjected to the extraction of the essential oil by hydrodistillation, for 3h, in a Clevenger type apparatus. Recovered EO (yield: 0.45 %, v/w) was dehydrated over anhydrous sodium sulfate and stored in dark glass vial at - 4 °C until tested.

Preparation of the plant methanolic extract (ME)

Powdered plant material (50 g) was extracted with 500mL of methanol using Soxhlet extractor for 8 h at 40 °C. The methanol extract was filtered through Whatman filter paper and then concentrated at 40 °C in a rotary evaporator until dryness. The final obtained extract (4.8 g) was stored at 4 °C until use (Erkan et al., 2008).

Total phenolic content

The total phenolic content of the ME or EO was determined by the Folin-Ciocalteu reagent (FCR), according to the method described by Maity et al. (2013) with slight modification, using gallic acid as standard. 40 µL of methanolic solution of the ME or EO were mixed with 200 µL of the FCR and 1160 µL of distilled water. After 3min incubation, 600 µL of 7.5% Na_2CO_3 solution was added to the mixture which was subsequently kept in the dark for 2 h at room temperature. The absorbance was measured at 760 nm. The results were expressed as µg of gallic acid equivalents per mg (µg GAE/mg) of ME or EO.

Total Flavonoid content

The total flavonoid content was determined by the aluminum trichloride method reported by Meda et al. (2005) with minor modification. Briefly, 600 µL of $AlCl_3$ (2% in methanol) were mixed with the same volume of the methanolic solution of ME or EO. After 15 min, the absorbance was measured at 415 nm against a blank composed of the same volumes without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin and was expressed as µg quercetin equivalents per mg (µg QE/mg) of ME or EO.

Antioxydant activity

DPPH radical scavenging activity assay

The DPPH free radical scavenging activity was carried out as described by Hazzit et al. (2009). 50 µL of different concentrations of ME (10, 20, 50, 10, 200, 300, 400, 500 µg/mL) or EO (1, 2, 4, 8, 12, 16, 20µL/mL), prepared in methanol, were added to 2 mL of 60 µM methanolic solution of DPPH. After 30 min incubation in the dark at ambient temperature, the absorbance was measured at 517 nm against a control (same amount of methanol and DPPH solution without EO or ME). Butylated hydroxytoluene (BHT) was used as positive control. The tests were carried out in triplicate. The inhibition of DPPH free radical was calculated as follows:

$$\% \text{ Inhibition} = (A_c - A_t / A_c) \times 100$$

where, A_c is the absorbance of the control sample, and A_t is the absorbance of the tested sample. Percentages of inhibition were plotted against concentrations of EO or ME to calculate the concentration providing 50% inhibition (IC_{50}).

β-Carotene bleaching assay

This assay was applied as described by Shukla et al. (2012). A stock solution of ß-carotene and linoleic acid was prepared (0.5 mg of ß-carotene in 1ml chloroform, 25 µL linoleic acid and 200 mg Tween 40). The chloroform was evaporated under vacuum and 100 mL of aerated distilled water was then added to the residue. The resulting mixture was vigorously stirred. Aliquots (2.5 mL) of the ß-carotene-linoleic acid emulsion (freshly prepared before each experiment) were transferred to test tubes, each containing 350 µL of various concentrations of the plant ME or EO diluted in methanol. The absorbance was measured at 470 nm immediately (zero time). The test tubes were incubated in a hot water bath at 50 °C together with blanks, BHT as a positive control and the other contained the same volume of methanol instead of extract as a negative control. After 120 min incubation the absorbance was measured again. All tests were carried out in triplicate and inhibition percentages were averaged. Antioxidant activity (inhibition percentage, I%) values were calculated using the following formula:

$$I\% = (A_t - C_t / C_0 - C_t) \times 100$$

where A_t and C_t are the absorbance of the sample and control at 120min, respectively, and C_0 is absorbance of the control at t = 0 min. The results are expressed as IC_{50} values, the concentration required to cause 50% inhibition of β-carotene bleaching.

Reducing power assay

The reducing power was determined by evaluating the

transformation of Fe^{3+}– Fe^{2+} according to Esmaeili and Sonboli (2010). The ME or EO (0.75 mL) at various concentrations (in methanol) was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (w/v, 1%). The mixture was incubated at 50 °C for 20 min followed by the addition of 0.75 mL of trichloroacetic acid (10%) and then centrifuged at 3000 rpm for 10 min. A volume of 1.5 mL of the upper layer of the solution was collected and mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. The EC_{50} (RP) value, which represents the concentration at which absorbance is 0.5, was calculated for ME, EO and BHT.

Evaluation of the antifungal activity

The antifungal effect of *A. campestris* ME and EO was carried out on potato dextrose agar (PDA) or potato dextrose broth (PDB) media depending on the applied method.

Fungal strains

Botrytis cinerea and *P. expansum* molds were isolated from decayed strawberry and apple fruits respectively. They were identified according to the identification technique of Pitt & Hocking (2009) based on the cultural characteristics on Czapek Yeast Extract Agar (CYA), Malt Extract Agar (MEA) and 25% Glycerol Nitrate Agar (G25N), and keys for determination (colony diameter, color and texture; microscopic characteristics: hyphae and conidiophore appearance, size and shape of vesicles, metulae, phialides, and conidia…) described elsewhere (Botton et al., 1990). They were cultured on PDA slants and kept at 4 °C until use.

Preparation of inocula

The conidial suspension was prepared as described by Hendel et al. (2016); conidia were harvested from 10-day-old culture using sterile 0.01% Tween 80 saline solution. After well mixing the conidial suspension, the final concentration was adjusted to 10^4 conidia/ml using a Haemocytometer. The fungal discs were taken from the margins of 7 and 4 days fungal cultures grown on PDA at 25 °C of *P. expansum* and *B. cinerea* respectively.

Measurement of inhibition average

The inhibition of spore germination was evaluated by measuring the inhibition zone diameter (mm) and the percentage of mycelial growth inhibition (I%) was measured according to the formula (1).

$$I\% = (DC-DT) / DC \times 100 \text{ --------(1)}$$

Were

DC: Diameter of the control fungal colony (mm)

DT: Diameter of the treated fungal colony (mm)

Antifungal activity assays

Essential oil fumigation assay

The Fumigation bioassay was carried out as described by (Li et al. 2014); PDA medium (15 mL/Petri dish 90 mm) was inoculated at its center by a 6 mm fungal disc. The petri dishes were reversed and a 9 mm diameter sterile filter paper disc was placed in the center of the lid and soaked with the EO. Different volumes (10, 15, 20 µL) were applied. The dishes were immediately sealed with parafilm and then incubated at 25 °C for 7 days. The paper discs in the control dishes were treated with distilled water instead of the EO. Each test was performed in triplicate. Colony diameters were recorded daily until the 7th day. Growth inhibition was calculated according to formula (1).

Contact assay

This test was applied according to Shukla et al. (2009). The ME or EO, dissolved in dimethyl sulfoxide (DMSO) or Tween 80 (0.05%), was incorporated into a melted PDA to obtain the desired final concentrations (100, 200, 400 µg/ml for the ME and 400, 800, 1200 µL/L for the EO). After solidification in a Petri dish, the medium was aseptically seeded at its center by a fungal disc (6 mm). The control plates were supplemented with DMSO instead of ME or Tween 80 (0.05%) instead of EO. Each test was performed in triplicate. Colony diameters were recorded daily up to the 7th day. Growth inhibition was calculated according to formula (1).

Agar-well diffusion assay

For the effect of the plant on the mycelial growth of fungi, a fungal disc (6 mm) was deposited in the center of PDA Petri plate. Three wells (8 mm) were made using a cork borer, at three points equidistant from the center and the edge of the Petri plate (90mm), then each well was filled by 20 µL of the ME (100, 200, 400, 600 and 800 mg/mL, in DMSO). Each plate represents a triplicate and each test was carried out twice. The ME was replaced by DMSO in the control. Colony diameters were recorded daily up to the 7th day (Talibi et al., 2012).

Disc diffusion assay

The PDA was seeded by spreading 100 µL of a spore suspension (10^4 spores/mL). Sterile filter paper discs (6 mm) were placed at three points equidistant from the center and the edge of the Petri plate (90 mm), then each was impregnated with 20 µL of the ME (200, 400, 600 and 800 mg/ml, in DMSO). Each plate represents a triplicate and each test was carried out twice.

To test the effect of the EO, the PDA was seeded as above. On a single disc, placed in the center of the Petri plate, the EO was applied at different volumes (10, 15, 20, and 25 µL). In the controls, the ME was replaced by DMSO and discs without EO were applied. The test was carried out three times. The inhibition diameters around the discs were measured after incubation of the fungi at 25 °C for 48 h (Shirazi et al., 2008).

Bioautography

To detect biologically active compounds with antifungal activity in ME or EO, bioautography was carried out on silica gel-based thin-layer chromatography plates (TLC) (silica gel 60 F254, 0.2 mm thick) according to Kumar et al. (2012). Twenty microliters of the ME (800 mg/mL, in MeOH) or pure EO were spotted onto the activated TLC plate and then developed in the hexane: ethyl acetate (60:40) system. After the total evaporation of the solvent from the chromatogram, a spore suspension (10^4 spores/mL) of the tested fungi in the PDB was plated on the dried chromatogram and incubation was carried out in a sterile moist chamber for 24 to 72 h at 25 °C. The clear inhibition zones on the TLC plate are indicative of the antifungal activity of the separated compounds on the TLC plate.

 DOI: 10.31015/jaefs.2021.3.1

Micro-well dilution assay and determination of the minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC)

This test was conducted using the EO. This latter was dissolved in DMSO then diluted to a concentration of 10 µL/mL in the PDB, and then serial two-fold dilutions were made to obtain concentrations ranging from 10 to 0.312 µL/mL in 5 mL sterile test tubes containing PDB. MIC values of the EO against fungal isolates were determined on the basis of the micro-well dilution method as described by Sokmen et al. (2004). Ninety-five microliter of PDB and 5 µL of the inoculum (10^4 spores/mL) were dispensed into each well of a 96-well plate. An aliquot (100 µL) of initially prepared EO stock solution at the concentration of 10 µL/mL was added to the first wells. From the prepared serial dilutions, 100 µL was transferred into the five consecutive wells. The control contained 195 µL of PDB without EO and 5 µL of the inoculum. The final volume of each well was 200 µL. The plate was covered with parafilm and incubated at 25 °C for 24 to 48 h. The MIC was defined as the lowest concentration of EO at which the tested fungus exhibited no visible growth. To determine the MFC, samples from the broth of each well, showing no growth were plated on PDA and incubated at 25 °C for 48 h. MFC was defined as the lowest concentration of EO at which the incubated fungus was completely killed. Each test was performed in duplicate.

Statistical analysis

All data are expressed as mean ± SD (n = 3). The analysis of variance (ANOVA), Tukey's and Sidak's multiple comparison were considered significant at $p < 0.05$. (Statistical analyses were done using GraphPad prism 6.05 for Microsoft Windows).

Results

The ME revealed high levels of polyphenols and flavonoids (400.64 ± 12.97 µg GAE/mg and 43.13 ± 0.14 µg QE/mg) and low contents were registered in the EO (27.47 ± 0.44 µg GAE/mg and 14.04 ± 0.82 µg QE/mg).

This research investigated the antioxidant efficacy of *A. campestris*' essential oil and methanolic extract by *in vitro* testing. As Table 1 presents, the ME showed more capacity to scavenge the DPPH free radical followed by the standard BHT and the EO. In contrast, regarding the effect of the linoleic acid oxidation inhibition and reducing power test, the BHT appeared showing higher activity than the ME and the EO.

The evaluation of *A. campestris* antifungal activity was conducted by measuring the mould mycelial growth under the effect of progressive concentrations of the EO or the ME, applied by different methods. The inhibition percentage was calculated on the 7th day by the measurement of the inhibition zones, and the EO MIC values were determined. Table 2 summarizes the inhibition percentages of the tested moulds by the EO or the ME, applied at higher concentrations and by different methods.

The EO of *A. campestris* applied as fumigant had the very effective effect on the growth of *B. cinerea*, reaching 87.06% with total inhibition till the 6th day (with 20µL), and had the less effective effect on *P. expansum*, reaching 49.75% . Incorporation of the EO into the culture medium results in 3 to 5-days total inhibition of *B. cinerea*, according to the concentration tested, to up to 80.39% on the 7th at the higher concentration (1200 µL/L). The mycelial growth of *P. expansum* was delayed for up to 4 to 5-days with 81.44% reduction at the end of the incubation period. It should be noted that *B. cinerea* was sensitive to EO applied by both methods and exhibited mycelial constriction with very weak sporulation and secretion of exudates and pigments on and into the culture medium. *Penicillium expansum* exhibited yellow exudates and pigments and was more sensitive when EO was incorporated than fumigated.

The ME of *A. campestris* applied by incorporation into the culture medium had strongly reduced *B. cinerea* mycelial growth, reaching 56.47% inhibition, to a stable inhibitory level from the 6th day for all applied concentrations. *Penicillium expansum* mycelial growth was reduced up to 51.96% at the 7th day. In agar-well diffusion assay *B. cinerea* showed a slow mycelial growth rate with a reduction value up to 56% on the 4th day. The mould reached its maximum growth from the 5th day to the end of the incubation period without registered inhibition. All concentrations showed low inhibitory effect towards *P. expansum*; around 30% with no significant difference between the applied concentrations. It should be noted that the well-diffusion technique has shown less effectiveness when compared to the incorporation technique.

In the disc diffusion test, the *A. campestris* EO was less effective against spore germination of both moulds when applied at 10 µL/disc (inhibition zones were 11.66 mm and 9.42 mm for *P. expansum* and *B. cinerea* respectively), to highly effective at 15 µL/disc on *P. expansum* (59 mm) and cause complete inhibition of *B. cinerea*. The other concentrations caused a total inhibition of both moulds. The extract caused a slight inhibition of the spore germination of both moulds. The 200 and 400 mg/mL concentrations had no inhibitory effect. The 600 mg/mL concentration had a low effect on both mould spores (the respective inhibition zones were 11.17 mm and 13.42 mm for *P. expansum* and *B. cinerea*) and 800 mg/mL was significant on *P. expansum* (16.42 mm) but had the same effect as 600 mg/mL on *B. cinerea* (13.53 mm).

In the micro-well dilution test only the EO was tested for its sporostatic or sporicidal potency by applying regressive concentrations (10 - 0.312 µL/mL) in micro-wells. The results indicated that the MIC is strictly lower than 2.5 µL/mL and greater than 1.25 µL/mL for both moulds since no visible growth was observed at 2.5 µL/mL and the complementary test (plating from this concentration showed no growth on PDA free of EO), confirmed that this concentration (2.5 µL/mL) is the MFC of both moulds.

The bio-autography test allowed well separation of ME components. A sporal germination inhibition of both fungi was observed. We registered clear inhibition zones at the R_f values 0.85, 0.79, 0.61, 0.32 and 0.17. No evident separation was observed with the EO.

Discussion

Our plant ME shows high polyphenol and flavonoid contents compared to those obtained by Djeridane et al. (2007) from *A. campestris* aqueous ethanol (80%) extract (103.40 mg

Table 1. Antioxidant activities of the methanolic extract and the essential oil of *A. campestris*, and the synthetic antioxidant BHT measured in DPPH, b-carotene–linoleic acid and RP assays. [a,*]

	DPPH (IC_{50}, µg/mL)	β-carotene bleaching (IC_{50}, µg/mL)	Reducing Power (EC_{50}, µg/mL)
Methanolic extract	11.71 ± 0.22 [b]	40.96 ± 0.93 [b]	23.32 ± 0.18 [b]
Essential oil	2713 ± 293[c]	6613 ± 60	2613 ± 186 [c]
BHT	26.07 ± 0.75 [d]	4.54 ± 0.13 [d]	5.56 ± 0.66 [d]

[a] Values are means ± SD (n = 3).
[*] Values with the same superscript letter in each row are not statistically different at 5% level, according to the Tukey's multiple comparisons test.

Table 2. Percentage of the growth inhibition of *B. cinerea* and *P. expansum* at the 7th day incubation by the EO and the ME of *A. campestris* applied by different methods at different concentrations each.

Test method	Concentration[#]	Percentage of the growth inhibition [a,*]	
		B. cinerea	*P. expansum*
EO Fumigation (µL)	10	26.67 ± 0.68 [b]	28.42 ± 1.71 [b]
	15	65.10 ± 1.36	42.30 ± 1.13
	20	87.06 ± 0.00	49.75 ± 4.99
EO incorporation (µL/L)	400	52.55 ± 0.68	75.49 ± 3.05
	800	72.55 ± 0.68	81.44 ± 3.13
	1200	80.39 ± 1.80 [c]	81.44 ± 3.13 [c]
ME incorporation (µg/mL)	100	55.69 ± 2.45	35.51 ± 1.64
	200	56.86 ± 1.80	40.13 ± 0.99
	400	56.47 ± 1.18 [d]	51.96 ± 2.59 [d]
ME agar-well diffusion (mg/mL)	200	0	16.08 ± 0.51
	400	0	14.23 ± 2.70
	600	0	14.23 ± 2.70

[a] Values are means (n = 3) ± SD.
[#] In fumigation method, the EO was used without being diluted.
[*] Values with the same superscript letter in each row are not statistically different at the 5% level, according to the Sidak's multiple comparisons test.

GAE/g) and those determined by Al Jahid et al. (2016) from hydro-alcoholic maceration (72 h) of the same plant and mentioned as optimal amounts obtained, among some tested processes and solvents (≈124 µg GAE/mg and ≈ 25 µg QE/mg). Our plant also presented higher polyphenolic content than the halophyte Tunisian one (158.75 ± 12.5 µg GAE/mg) (Megdiche-Ksouri et al., 2015). Akrout et al. (2011) found higher phenolic content (463.2 µg EAG/mg) in 50% ethanol extract. These differences may be attributed to climatic conditions, altitude, soil characteristics and extraction methods. For isolation and identification of the extract compounds, further studies should be conducted.

High antioxidant activity was also registered as estimated by 3 methods. The high polyphenol content of the ME may be responsible for this phenomenon. Flavonoids and phenolics form a group of plant chemicals well known as contributors to the antioxidant activity (Baykan Erel et al., 2012; Pereira et al., 2018). These phytochemicals prevent the lipid oxidation by acting as hydrogen atom donors to free radicals (Al Jahid et al. 2016). Although the effect on linoleic acid oxidation was weaker, ME showed strong free radical scavenging power. This is mainly due to its richness in phenolic compounds. These form a low content of the essential oil. The ME activity towards the radical DPPH was shown to be higher when compared to that obtained by Nikolova et al. (2010) (DPPH IC_{50} = 225 µg/mL), nevertheless the activity of the ethyl acetate fraction issued from this latter ME was comparable to that obtained from our ME (DPPH IC_{50} = 12.50 µg/mL). These extracts were found rich in flavonoids with antioxidant properties. Our plant also showed high reducing power compared to the halophyte one studied by Megdiche-Ksouri et al. (2015). Akrout et al. (2011) pointed out a positive relationship between the polyphenol and flavonoid levels and the antioxidant and the antitumor activities of *A. campestris*.

Plant extracts and essential oils have been listed as a significant means of preventing spoilage of food (Dib and El Alaoui-Faris, 2019). Our results evidently proved the potent antifungal activity of *A. campestris* EO and ME against two most important decay moulds; *P. expansum* blue mould and *B. cinerea* gray mould. The EO was better than the ME since its application by the above different methods resulted in stronger inhibition of the mycelial growth besides the total inhibition of spore germination by disc diffusion method and its fungicidal effect at 2.5 µL/mL by well-dilution test. This latest result is clearly in concordance with that mentioned by Houicher et al. (2016) about *P. expansum* and other pathogenic and toxicogenic fungi. Secondary metabolites of plants have been tested for their antimicrobial activity and a significant number of plant extracts and essential oils have been showen to possess antimicrobial activity (Rahman et al., 2011). *Artemisia campestris* EO, having high levels of germacrene D and β-pinene, has shown clear antibacterial activity (Al Jahid et al., 2016). This is partially in concordance with our findings since our *A. campestris* EO, analysed previously (Sassoui et al., 2020), contains 15.2% of β-pinene and 9.0% of germacrene D, but also other important levels of α-pinene, myrcene (Z)-β-ocimene, and γ-curcumene. Nevertheless, the essential oil biological activity might be attributable to their major components or synergistic/antagonistic interaction between oil components; the main component of *Callistemon lanceolatus* EO (1,8-cineole), used alone has no completely inhibition on *Aspergillus flavus*, but the EO causes total fungal inhibition (Shukla et al., 2012). *Artemisia campestris* aqueous extract has shown to be effective against clinical isolates of yeasts, dermatophytes and other filamentous fungi (Webster et al., 2008). Zabka et al. (2011) investigated the antifungal activity of 46 medicinal plants and found that 14 plant species, among them *A. campestris*, have shown to possess the fungicide character. Methanolic extract of *A. campestris* has a median inhibitory concentration (MIC_{50}) higher than 2 mg/mL on 6 significant pathogenic and toxicogenic fungal species namely, *P. expansum*, *P. brevicompactum*, *Fusarium oxysporum*, *F. verticillioides*, *A. fumigatus* and *A. flavus*. In our study, the application of the ME by the well-diffusion method resulted in low effect on mycelial growth compared to the application by incorporation technique; this may be attributed to the low diffusion capacity of the ME from the well into the culture medium (It remains concentrated at the agar well). Flavonoids are found mainly in plants and may play an important role in controlling fungi. Isolated flavonoids from citrus species were found to have an inhibitory effect on fungal food contaminants *A. parasiticus*, *A. flavus*, *F. semitectum* and *P. expansum* (Salas et al., 2011). *Artemisia campestris* is also known to have a high inhibitory effect on bacteria and yeasts (Naili et al., 2010; Karabegović et al., 2011; Ghorab et al., 2013; Al-Snafi, 2015), and the EO is commonly most effective antimicrobial than the other extracts.

Conclusion

The results of this work have shown that the essential oil and the methanolic extract of *A. campestris* possess significant antioxidant and antifungal properties. Knowing that *A. campestris* is a local medicinal plant with a great abundance in Algeria, it will form a source of natural antimicrobials and antioxidants since public continued demands for reduced pesticide usage and the emergence of fungicide resistant pathogens. Qualitatively, our plant has shown to contain compounds with promising antifungal activity against grey and blue molds. Further study is needed to obtain pure compounds and to reveal more quantitative data. Furthermore, testing the biological activities under *in vivo* conditions is also necessary to confirm the above-cited properties and prevent against these important moulds.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.2 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):260-270 (2021)

Determining the yield responses of maize plant under different irrigation scenarios with AquaCrop model

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Abstract

The AquaCrop simulation model is a significant implementation used to determine the response of crop yield to water and accordingly build up new strategies to improve agricultural irrigation management. Since determining the appropriate irrigation program in the field researches will require many years and labor; it becomes convenient with the AquaCrop to determine the adaptation of crops to the cultivating conditions and to examine the impact of possible variables such as drought on crop production. In this study, different irrigation scenarios were created, and yield predictions were made with the AquaCrop 6.1 model for maize plant which irrigated by drip irrigation method in Adana conditions, Turkey. These scenarios were created by determining four different depletion levels of readily available water (RAW) amount in the soil. These depletion levels were 25%(S1), 50%(S2), 75%(S3) and 100%(S4). The highest grain yield value was found in S1 as 10.075 ton/ha and the lowest grain yield in the S4 as 9.837 ton/ha. The amount of seasonal irrigation water simulated for different irrigation schedules varied between 348.5–390.7 mm, and the evapotranspiration (ET) varied between 411.5-426.5 mm. As a result, S3 scenario has been recommended considering the amount of irrigation water and the yields achieved.

Keywords: AquaCrop, Maize, Modelling, Irrigation scheduling, Yield estimation

Introduction

Maize (*Zea mays* L.) has a significant role among the grains found in the world. Since it can grow in tropical, subtropical and temperate climates, it can be cultivated almost anywhere in the world except Antarctica. The production quantity of maize has shown a significant acceleration in the rate of increase at the beginning of the 20th century, and the production data for the world is 1147 million tons in 2018 and Turkey has 0.49% of this amount with 5.7 million tons (FAO, 2018). One of the most suitable areas for maize agriculture in Turkey is Çukurova plain. According to the statistical data of 2019; Adana (located in Çukurova Plain) is the one of the provinces (second one after Konya) with highest amounts of harvested area (66 564 ha) and production (717 802 tons) of maize in Turkey (TURKSTAT, 2019). Since maize plant grows in the hottest period of the year, water consumption is high. Besides, it is a plant that uses water most effectively among field crops; that is, produces the highest amount of dry matter per unit of water.

It is essential to determine the appropriate irrigation time for the plant. When irrigation is delayed, because the plant is sensitive to water stress, the yield decreases even if the amount of water applied at the next irrigation time increases. Rather than planning the irrigation to a fixed irrigation calendar, the

Cite this article as:
Aşık, M., Yetik, A.K., Candoğan, B.N., Kuşçu, H. (2021). Determining the yield responses of maize plant under different irrigation scenarios with AquaCrop model. Int. J. Agric. Environ. Food Sci., 5(3), 260-270
Doi: https://doi.org/10.31015/jaefs.2021.3.2
Orcid: Mehmetcan Aşık: 0000-0002-1816-747X, Ali Kaan Yetik: 0000-0003-1372-8407, Burak Nazmi Candoğan: 0000-0001-9898-5685 and Hayrettin Kuşçu: 0000-0001-9600-7685
Received: 26 November 2020 **Accepted:** 20 May 2021 **Published Online:** 26 July 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 260-270
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

irrigation time and the water needs differ according to the plant's development periods should be determined (Doorenbos and Pruitt, 1977). Some researchers have been conducted to determine these values for the maize plant. Braunworth and Mack (1989) investigated the effect of water deficit on maize yield and quality. They determined that the yield value was close to each other in irrigation conditions without consuming 50% of the RAW. Oğretir (1993) conducted a study on the water-yield relationship in maize under Central Anatolian conditions in Turkey and determined the effect of water deficit on grain yield. The researcher determined the irrigation water need as 440 mm and the crop evapotranspiration as 659 mm in the treatment with full irrigation, and the highest efficiency is obtained by irrigating four times. Gençoğlan and Yazar (1999), conducted a study in Adana to determine the effects of deficit irrigation applications on water use efficiency and yield values. As a result of the study, they reported that the amount of water they applied varied between 102 mm and 823 m, and the plant water consumption values varied between 343 mm and 1052 mm. Besides, the yield values achieved 10.02 ton/ha in the first year and 10.04 ton/ha in the second year of the study. Shaozhong et al. (2000) found the crop evapotranspiration of maize to be 442.72 mm in their studies conducted in Hong Kong. Vural and Dağdelen (2008) investigated the effects of different irrigation programs on agronomic properties in their studies. The amount of irrigation water they reached at the end of the research varied between 234 mm and 571 mm for different treatments, and the crop evapotranspiration values varied between 130 mm and 609 mm. Yuan et al. (2019) determined the effect of 3 different irrigation levels on the maize plant yield in their study. They reported the highest yield from the treatments with 370 mm irrigation water.

The effects of different irrigation strategies can also be understood using agricultural-hydrological simulation models (Li et al., 2020). With the models' help, irrigation schedule scenarios can be created. The dynamics of crop growth under different meteorological factors and soil water content can be followed. Together with the necessary calibrations and accurate data, these simulation models provide you with access to yield and irrigation programs according to climatic conditions anywhere in the world. The models involving plant water relationships; AquaCrop, SWAP, soil and water balance simulation model, Hydrus are popular simulation models used to simulate the water needs for the growth periods of the plant or the water consumption/supply of farmland (Xu et al., 2019; Ran et al., 2018; Li et al., 2017a; Li et al., 2017b). The AquaCrop model was developed in 2009 by the Food and Agriculture Organization of the United Nations (FAO). The model's main primary purpose is to predict plant growth and yield in restricted, supportive irrigation levels and rainfall-dependent conditions (Steduto et al., 2009). The model has already been run for different crops and obtained acceptable results compared to yields in field conditions. However, it is important to determine the AquaCrop model's suitability by testing it under alternative irrigation programs in different climatic, soil or plant conditions (Yiğit and Candoğan, 2019). This study's objectives were to estimate yield and irrigation water need and to create different irrigation schedules using the AquaCrop model for maize plant and Adana province conditions in Turkey.

Materials and Method

AquaCrop

The model used in the study is AquaCrop version 6.1 programmed in Delphi by FAO in 2009. The AquaCrop estimates the yield that can be obtained as a function of water consumption under dry conditions with convenient, deficit irrigation or full water applications (Steduto et al., 2009; Andarziana et al., 2011). The model requires input climate data, crop characteristics, soil properties and irrigation parameters. Inputs; climate, plant, soil and management files are kept and can be easily accessed and changed by the user (Raes et al., 2018a). The main inputs and outputs of the complete model are shown in Figure 1.

Climate

In Adana, located in the Mediterranean Region's south-east, summers are sweltering and dry; winters are warm and rainy. The long-term climate data (1929-2019) (MGM, 2020; Gaisma, 2020) of some climate elements provided from the Adana Province central meteorology station affiliated to the General Directorate of State Meteorology Affairs and the NASA-Langley-Gaisma weather database given in Table 1. The total annual precipitation is 645.7 mm.

To obtain the climate data required running the model, average lowest and highest air temperatures, reference crop evapotranspiration (ETo), precipitation, and annual average CO_2 concentration in the atmosphere were used as inputs. CO_2 concentration data in the atmosphere were taken from the Hawaiian Mauna Loa Observatory records, which included in the program (Raes et al., 2018a). The AquaCrop 6.1 model does not include the reference crop evapotranspiration calculation. Therefore, to get the reference crop evapotranspiration values, the ETo calculator on the FAO's official website was used in the calculation. To calculate ETo, relative humidity; insolation, the monthly average for many years' highest and lowest temperature values were used as inputs (Raes, 2012). Different methods have been developed to calculate ETo and the performances of different these equations have been analyzed. The FAO Penman-Monteith method has been recommended as the standard method for ETo calculations (Allen et al., 1998). The original Penman-Monteith method used by the ETo calculator is the following equation.

In the equation;

ETo : Reference evapotranspiration (mm day^{-1}),
R_n : Net radiation at the crop surface (MJ m^{-2} day^{-1}),
G : Soil heat flux density (MJ m^{-2} day^{-1}),
T : Mean daily air temperature at 2 m height (°C),
u_2 : Wind speed at 2 m height (m s^{-1}),
e_s : Saturation vapor pressure (kPa),
e_a : Actual vapor pressure (kPa),
$e_s - e_a$: Saturation vapor pressure deficit (kPa),
Δ : Slope vapor pressure curve (kPa $°C^{-1}$),
γ : Psychometric constant (kPa $°C^{-1}$).

 DOI: 10.31015/jaefs.2021.3.2

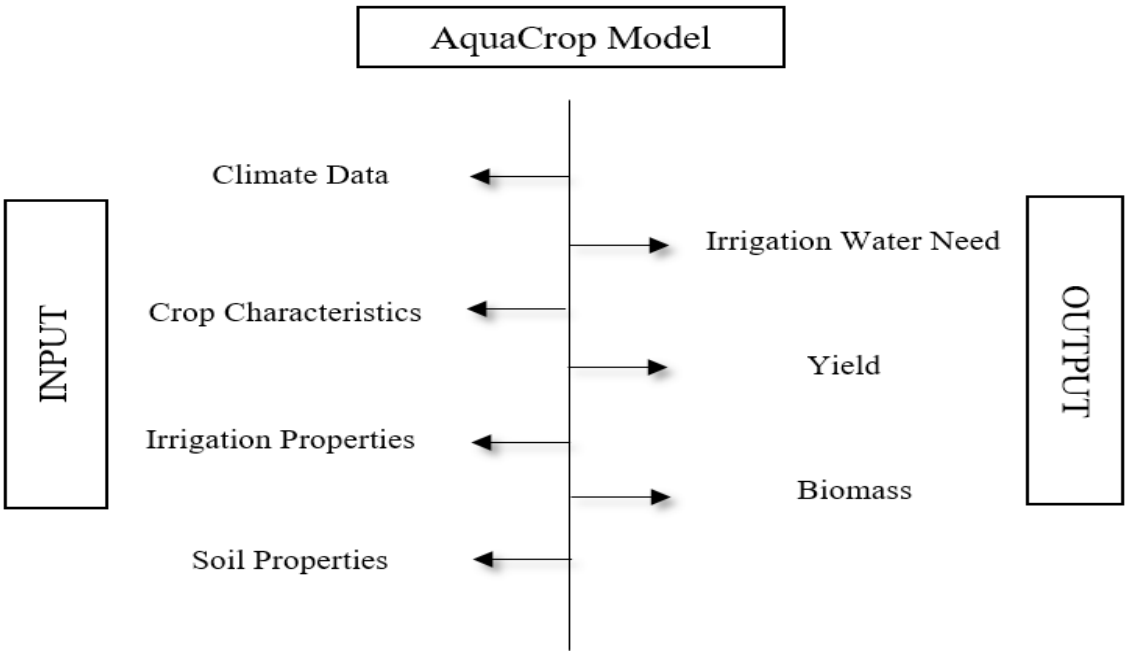

Figure 1. Inputs and outputs of the model

Table 1. Long term (1928-2019) monthly averages climate data

Months	Avg. max. temperature (°C)	Avg. min temperature (°C)	Avg. Insolation (hours)	Rainfall (mm)	Wind speed (m s^{-1})	Relative humidity (%)
January	15.1	5.5	4.4	105.1	3.33	66.0
February	16.1	5.9	5.1	85.1	3.02	64.0
March	19.5	8.5	5.5	60.4	3.00	65.0
April	23.8	12.3	6.5	50.3	2.63	68.0
May	28.2	16.2	8.5	42.8	2.55	67.0
June	31.7	20.4	10.2	19.3	2.52	68.0
July	33.7	23.9	10.2	9.4	2.66	71.0
August	34.6	24.2	9.6	7.0	2.58	71.1
September	33.2	21.0	8.3	15.1	2.58	65.0
October	29.2	16.4	7.1	47.9	2.50	62.0
November	22.0	10.7	5.3	82.6	2.19	66.0
December	16.8	7.0	4.2	120.7	2.63	68.0
Avg./Total	25.3	14.3	7.08	645.7	2.68	66.75

Plant Material

In this study, the maize which is one of the most used plants in agricultural production in Turkey, is defined as plant material. AquaCrop version 6.1 offers some data which contains parameters suitable for the simulation of maize. Some of these parameters are not universal and need to be adjusted to similar climate conditions or the soil contents. To minimize the margin of error of the results, the data was collected from different sources. The default parameters for the maize plant taken from the AquaCrop model are given in Table 2. In contrast, the parameters taken from different researches to adapt to study site conditions are given in Table 3. The values of the AquaCrop default database parameters were also created by taking into account the previous studies for maize. It was assumed that there were not too many variables and did not affect the results (Raes et al., 2009).

Table 2. Conservative parameters used in simulation (Raes et al., 2009)

Parameters	Values
Minimum effective rooting depth (Z_n) (m)	0.30
Shape factor describing root zone expansion	1.30
Crop coefficient when the canopy is complete but prior to senescence ($K_cT_{r,x}$)	1.05
Water productivity normalized for ETo and CO_2 (WP) (g/m^2)	33.70
Water productivity normalized for ETo and CO_2 during yield formation	100
Reference harvest index (HI_0) (%)	50
Possible increase (%) of HI due to water stress before flowering	None
Excess of potential fruits (%)	Small
Coefficient describing the positive impact of restricted vegetative growth during yield formation on HI	Small
Coefficient describing the negative impact of stomatal closure during yield formation on HI	Strong
The allowable maximum increase of specified HI (%)	15
Soil water depletion level for canopy expansion - Upper level	0.14
Soil water depletion level for canopy expansion - Lower level	0.72
Shape factor for Water stress coefficient for canopy expansion	2.90
Soil water depletion level for stomatal control - Upper level (p_{sto})	0.69
Shape factor for Water stress coefficient for stomatal control	6
Soil water depletion level for canopy senescence - Upper level	0.69
Shape factor for Water stress coefficient for canopy senescence	2.70
Soil water depletion level for the failure of pollination - Upper level	0.80
The electrical conductivity of the saturated soil-paste extract: lower threshold ($ECe_{n)}$	1.70
The electrical conductivity of the saturated soil-paste extract: upper threshold ($ECe_{x)}$	10

Table 3. The crop characteristics of maize

Parameters	Values	References
Base temperature (°C)	10	Lee, 2007
Upper temperature (°C)	28	Sanchez et al., 2014
Planting date	02 June	Sahin, 2001
Max. effective rooting depth (Z_x) (cm)	90	Kuscu et al., 2013
Leaf Area Index	6	Koca and Turgut, 2012
Emergence (days)	8	Ritchie et al., 1993
Crop Coefficient ($K_{c)}$	0.6	Piccini et al., 2009
Senescence (days)	80	Borrás et al., 2003
Maturity (days)	120	FAO, 2020
Duration Flowering (days)	20	Durand et al., 2012
From day 1 after sowing to flowering (days)	60	Durand et al., 2012
1000 seed mass (g)	345.7	Kılınc et al., 2018
Row space (cm)	70	Kuscu and Demir, 2012
Inter-row spacing (cm)	20	Kuscu and Demir, 2012
Germination rate (%)	95	Khodarahmpour et al., 2012

Irrigation Management Data

In this study, the drip irrigation method was used to estimate the yield of the maize plant. The 25%, 50%, 75% and 100% depletion levels of ready water available (RAW) were determined to starting irrigation applications in the model as scenario 1 (S1), scenario 2 (S2), scenario 3 (S3) and scenario 4 (S4), respectively. To put it another way, the irrigation scheduling for the S1, S2, S3 and S4 scenarios were set to start irrigation applications when 25%, 50%, 75% and 100% of the RAW value is depleted. Soil water content has been reached to field capacity after all irrigation applications, and the percentage of wetting soil surface was accepted as 30%.

In order to ensure the uniformity between scenarios during the emergence periods and to keep the soil water content at the field capacity, first 4 irrigation applications were applied to all scenarios at the same time and amount. Following the irrigation application on June 24, different irrigation scenarios were started according to the soil water content.

It is assumed that the RAW value lies between field capacity and the stomatal closure point (p_{sto}.TAW) by model. Besides, when the upper level of soil water depletion for stomatal closure is multiplied by field capacity, the found value is an upper level of depletion of root zone ($Dr_{sto,\ upper}$) (Raes et al., 2018b). As seen in Table 2, the upper level (p_{sto}) was accepted as 0.69 for maize plants (Raes et al., 2018c).

Soil Data

The soil texture used in the study has been assumed considering the soil characteristics of Adana. The AquaCrop model includes different soil textures and soil texture has been taken from these available profiles, and its properties are given in Table 4. 30 cm soil layers were taken into account for 0-120 cm soil depth.

Table 4. Soil properties used in the model as input

Type of Soil	Soil Depth (cm)	FC[1] (%)	PWP[1] (%)	SAT[2] (%)	TAW (mm/m)	Ksat[3] (mm/day)	τ (Tau)[4]
Sand	0-30	13	6	36	70	3000	1
Silty Clay	30-60	50	32	54	180	100	0.43
Silty Clay	60-90	50	32	54	180	100	0.43
Silty Clay	90-120	50	32	54	180	100	0.43

[1]In percentage of volume, [2]Water content in soils as percentage by volume, [3]Hydrolic Conductivity [4]Drainage coefficient

Monitoring the soil water content is an important part of the model. In the study the soil water content (Wr) in the root zone and the corresponding water stress were followed daily by model to calculate soil water balance. The root zone was defined as the part where the soil was held and calculations were made on the amount of water retained in this part. The change of soil water content is determined by tracking the amount of water incoming to the root and outgoing. Incoming water can be defined as rain and irrigation, and some section of the rain disappears due to runoff. Another option for water transportation is a capillary rise, which is transported from a shallow groundwater layer to the root zone. The outgoing waters are; soil evaporation, crop transpiration, and deep percolation losses. The soil water balance used by the model is based on this logic and is expressed in the following equation (Raes, 2017).

$$Wr_{t+1} = Wr_t + (P - RO) + I + CR - E - Tr - DP$$

In the equation;

Wr_{t+1}, Wr_t : Water contents in the root zone (at the time "t" and "t+1")
P : Rainfall
RO : Runoff
I : Irrigation
CR : Capillary rise
E : Evaporation
Tr : Transpiration of the crop
DP : Deep percolation losses

After severe rainfalls or over irrigations; If the water content in the soil exceeds the field capacity (Wr_{FC}), deep percolation losses (DP) will occur.

$DP = Wr_{FC} - Wr$

Root zone depletion (Dr); refers to the difference between the WR_{FC} value and the soil water content (Wr) after water depletion in the root zone.

$Dr = Wr_{FC} - Wr$

Results and Discussion

Reference Crop Evapotranspiration

Daily average ETo values for each month were calculated with the ETo calculator software developed by FAO, using Adana's monthly average climate data for long term years as input. As a result of these calculations, it was found that the highest ETo value was in July with 6.2 mm/day, while the lowest ETo value was in December with 1.7 mm/day. The annual ETo changes calculated with the ETo calculator are given in Figure 2. The annual average ETo value was found to be 1425 mm.

Simulation Results for Different Scenarios

The biomass and grain yield values calculated by model is given in Figure 3. As a result of the simulations, biomass yields varied between 20.842-21.435 ton/ha and grain yield values changed between 9.83-10.07 ton/ha. For both yield parameters, the highest values were obtained at S1 scenario while the lowest yields were obtained at S4 scenario.

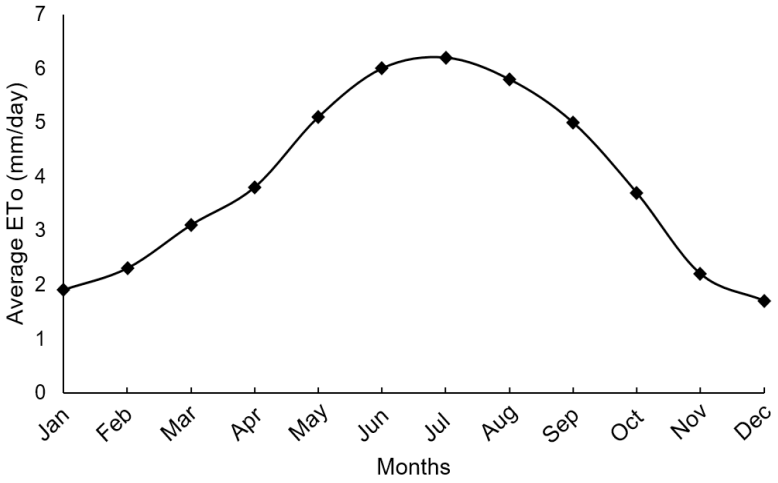

Figure 2. Annual reference crop evapotranspiration (ETo) changes of the study site

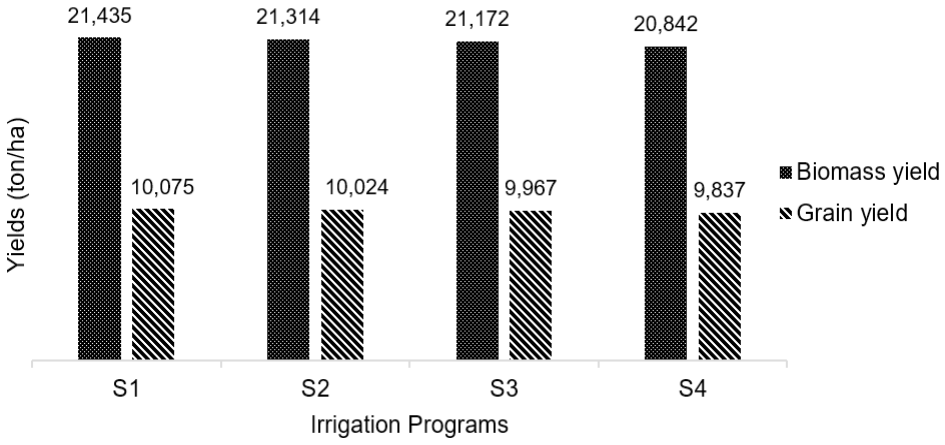

Figure 3. The biomass and grain yields obtain from different scenarios

The net amount of irrigation water for the S1 scenario was estimated to be 390.7 mm. This amount is distributed over 20 irrigation applications and is the highest irrigation water amount reached among allscenario. After the start of irrigation applications considering the soil water content, the amount of water simuleted in each scenario varied between 4.1 mm and 26.7 mm and the irrigation intervals were minimum 4 and maximum 8 days. The irrigation water applications for the S1 scenario are given in Table 5 in detail. The total evapotranspiration (ET) has been estimated as 426.5 mm and water use efficiency (WUE) as 2.38 kg/m^3. Also, evaporation and transpiration values in the model were estimated at 56.6 mm and 370.3 mm, respectively. Total yields, transpiration (Tr), cover percentage development (CC), root zone water depletion (Dr) simulations of the S1 irrigation scenario are given in Figure 4.

According to the model's simulation results, there were 12 irrigation events in S2 scenario and net irrigation water amount to be applied in the total was calculated as 378.4 mm. After the start of irrigation applications considering the depletion values of RAW, irrigation intervals were formed as minimum 7 and maximum 12 days and the lowest irrigation water amount applied was 35.5 mm and the highest was 48.7 mm. The amount of irrigation water to be applied for S2 is given in Table 6 in detail. When the data obtained were evaluated, the total ET was found to be 418.3 mm and the WUE was calculated as 2.41 kg/m^3. Evaporation and transpiration values for scenario S2 were estimated as 50.4 mm and 368.1 mm, respectively. The simulation results of yields, Tr, CC and Dr for scenario S2 are given in Figure 5.

In the scenario S3, 9 irrigations were made and a total of 348.5 mm irrigation water was calculated. The maximum irrigation water amount applied were obtain 69.4 mm on 21 August and the minimum irrigation water amount was 53.3 mm on 18 July. In the applications made after the start of different irrigation simulations for each scenario, the irrigation intervals were between 11-14 days. Detailed information about irrigation is given in Table 7. The total ET was found to be 415.5 and the highest WUE value of the study was calculated as 2.42 kg/m^3 in the S3 scenario. According to the data obtained, evaporation and transpiration values for the S3 scenario were 49.4 mm and 365.6 mm, respectively. Biomass and grain yields, Tr, CC and Dr simulations of S3 irrigation scenarios are given in Figure 6.

 DOI: 10.31015/jaefs.2021.3.2

Table 5. The irrigation applications of S1 scenario

Number of applications	Days after sowing	The Date	Net irrigation water application (mm)
1	2	03 June	9.0
2	6	07 June	4.1
3	11	12 June	5.0
4	23	24 June	9.8
5	34	05 July	14.2
6	40	11 July	15.5
7	45	16 July	21.2
8	49	20 July	21.4
9	53	24 July	23.4
10	57	28 July	24.1
11	61	01 August	24.4
12	65	05 August	24.4
13	69	09 August	24.2
14	73	13 August	23.7
15	77	17 August	23.3
16	81	21 August	22.8
17	86	26 August	26.7
18	91	31 August	24.5
19	97	06 September	25.3
20	105	14 September	23.9
Total			390.7

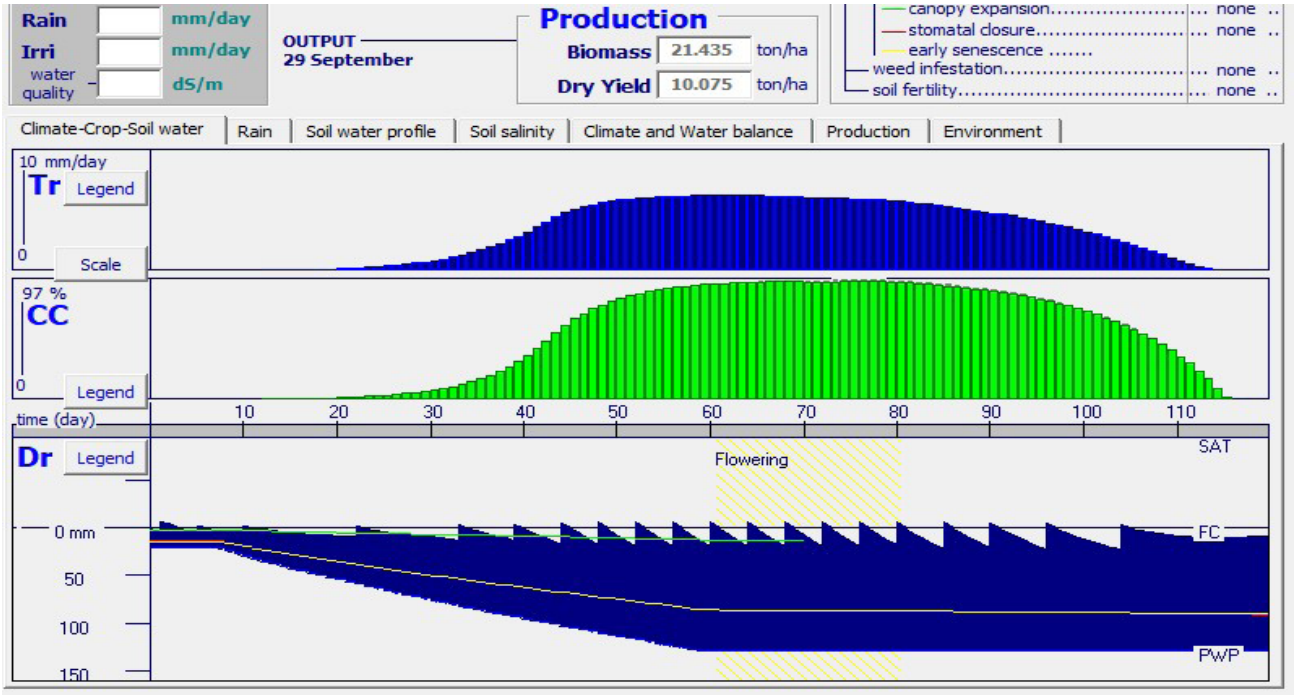

Figure 4. Simulation of crop development for scenario S1

Table 6. The irrigation applications of S2 scenario

Number of applications	Days after sowing	The Date	Net irrigation water Application (mm)
1	2	03 June	9.0
2	6	07 June	4.1
3	11	12 June	5.0
4	23	24 June	9.8
5	43	14 July	35.5
6	51	22 July	41.4
7	58	29 July	41.7
8	66	06 August	48.7
9	74	14 August	47.6
10	82	22 August	45.6
11	91	31 August	45.6
12	103	12 September	44.3
Total			378.4

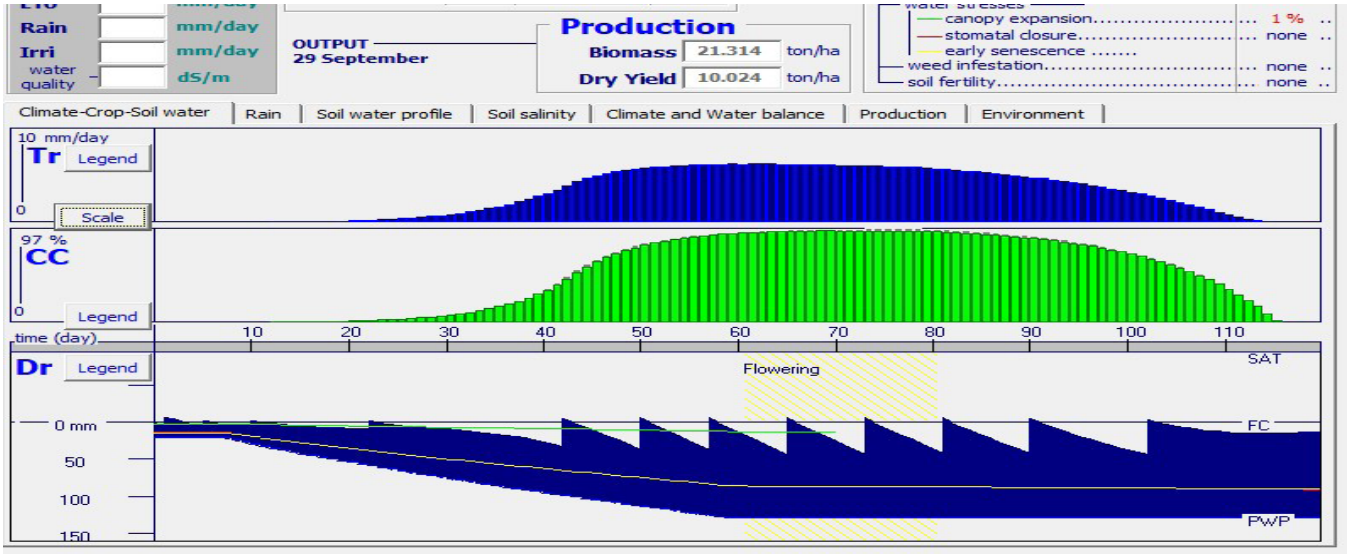

Figure 5. Simulation of crop development for scenario S2

Table 7. The irrigation applications of S3 scenario

Number of applications	Days after sowing	The Date	Net irrigation water Application (mm)
1	2	0 June	9.0
2	6	07 June	4.1
3	11	12 June	5.0
4	23	24 June	9.8
5	47	18 July	53.3
6	58	29 July	63.1
7	69	09 August	66.5
8	81	21 August	69.4
9	95	04 September	68.3
Total			348.5

 DOI: 10.31015/jaefs.2021.3.2

When the results for scenario 4 are examined, it is seen that a total of 8 irrigations were made and 376.4 mm of irrigation water was found. Following the irrigation applied according to depletion levels, the irrigation intervals of these 8 application varied between 3-15 days and the irrigation water amount applied at once maximum 91.4 mm and minimum 79.1 mm. Detailed information on irrigation practices is given in Table 8. The ET for scenario 4 was determined as 411.5 mm and the WUE was calculated as 2.41 kg/m^3. According to the data obtained, evaporation and transpiration values for the S4 scenario were 51.2 mm and 359.9 mm, respectively. Simulations results of S4 irrigation scenarios are given in Figure 7.

Table 8. The irrigation applications of S4 scenario

Number of applications	Days after sowing	The Date	Net irrigation water Application (mm)
1	2	0 June	9.0
2	6	07 June	4.1
3	11	12 June	5.0
4	23	24 June	9.8
5	52	23 July	79.1
6	67	07 August	88.5
7	83	23 August	91.4
8	106	15 September	89.5
Total			376.4

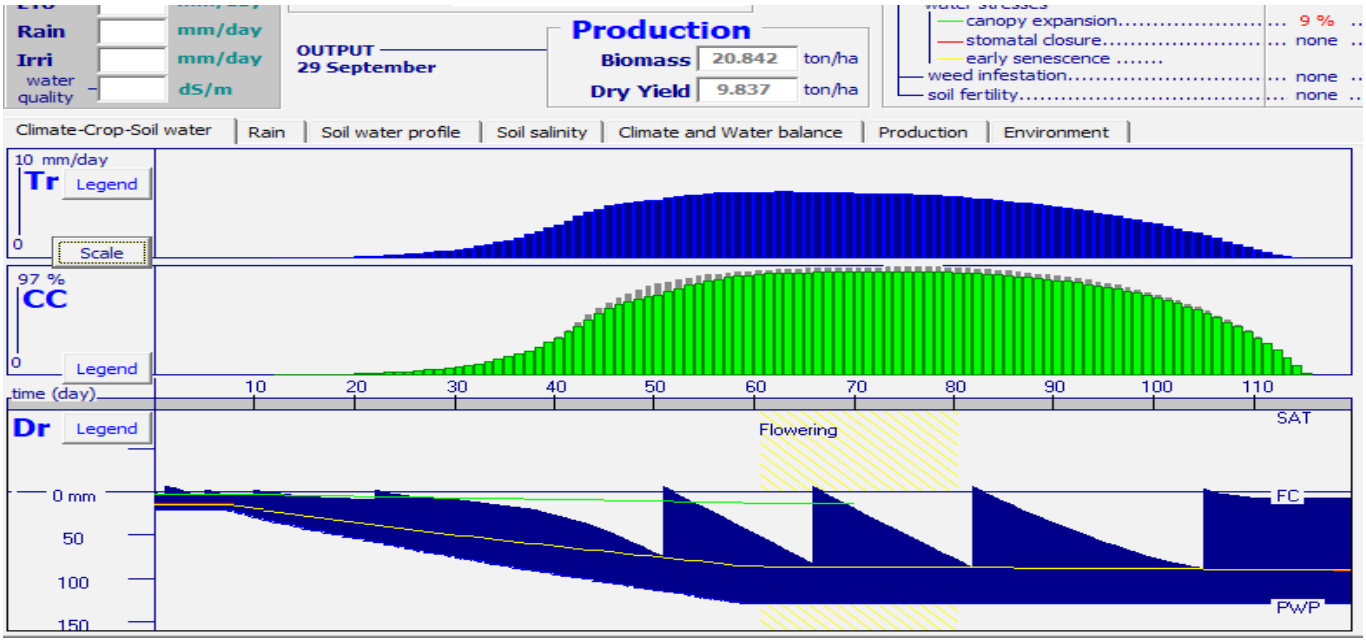

Figure 7. Simulation of crop development for scenario S4

When the data of 2019 are examined, the yield obtained from Adana maize production is seen as 11.4 t/ha TURKSTAT, 2019). Demirok and Tuylu (2019), in their study with the drip irrigation method under the conditions of the Harran Plain, varied between 6.98 t/ha and 11.51 t/ha. Gönülal and Soylu (2020) achieved a yield of 9.93 t/ha in their study. Gençoğlan and Yazar (1999), achieved yield values of 10.02 ton/ha in the first year of their research and 10.04 ton/ha in the second year of their research stated in Adana. Uçak et al. (2010), stated that the average yield of maize plant under Çukurova conditions in the years between 1996-2006 as 11.37 ton/ha. The results show that the obtained yields are parallel with the simulation results obtained from the AquaCrop model.

ET values calculated by the model for S1, S2, S3 and S4 scenarios are respectively 426.5, 418.3, 415.5 and 411.5 mm. Zhang et al. (2018) stated after their study were carried in China, total evapotranspiration values varied between 430.0 and 497.4 mm. Barbieri et al. (2012) stated that as a result of their research seasonal ET ranged from 389 to 486 mm. Gökçel and Yazar (2008), in their study carried in Adana, determined

ET values for maize plant with different irrigation methods and their results were varied between 375 mm and 677 mm. ET values predicted by the AquaCrop model with irrigation programs are similar to the above study.

Conclusion

The results obtained from the AquaCrop 6.1 model simulation show that there are no remarkable differences in yields between the 4 scenarios. When the simulation result graphs of each scenario are examined, it is seen that the root zone depletion (Dr) values of S3 scenario are the most efficient irrigation program. For the case where irrigation intervals are set as specified in the study, S3 scenario, which has the lowest irrigation water amount and the highest water use efficiency can be recommended as a proper irrigation program. There was 108 kg grain yield difference between S1 with the highest yield value and the recommended S3 scenario. In regions where there is no water shortage and in conditions where irrigation applications can be performed more frequently, the irrigation schedule of the S1 treatment can also be used. Even in cases where there is no water shortage, it is expedient to use the irrigation schedule of the S3 scenario in terms of effective use of water resources and reduction of labor with long irrigation intervals. The biomass and grain yield values obtained in the S3 scenario are respectively 21.172 t/ha and 9.967 t/ha. Besides, the total evapotranspiration was estimated to be 415.5 mm and WUE 2.42 kg/m3. It can be said that the irrigation program applied in the S3 scenario applicable under similar climate and soil conditions for the maize plant.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.3 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):271-278 (2021)

Profit Forecasting in Crop Production: The Case of Gazipaşa

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Abstract

Agriculture sector in Turkey is among the largest, employing one out of every five working people. In this study agricultural crop income of Turkey's Gazipaşa district is analyzed. Located on the coast of the Mediterranean, the district's main economy is based on agriculture with 81 different crops currently cultivated in 43 regions. For each crop type, total planted land size, yield, wholesale price and operating costs are determined using the data from Turkish Statistical Institute and the district's Directorate of Agriculture. Crop types are ranked based on their economic returns and top 30 that corresponds to 96.45% of the total agricultural income are determined. Profit forecasts are made for those 30 crop types for each of the next 15 years. Future wholesale prices are forecasted using linear trend projection. The annual agricultural loan interest rate of 7.5% is used to estimate the increase in operating costs. Results show that the annual total profit increases slowly in the next 11 years and then decreases. Moreover, profitability increases only for 18 out of the 30 crop types. Internal rate of return is also found to be 15%. Findings suggest that the current crop diversity is not economically sustainable and a better agricultural production plan is required.

Keywords: Agricultural production, Crop profitability, Forecasting

Introduction

Agricultural production maintains its importance in today's world with domestically increased crop consumption rates and fierce competition for reaching global food markets. Agriculture is among the most important economic sectors also in Turkey. According to the Turkish Statistical Institute (TUIK), the sector employs 20% of the national active work force. The area of the total agricultural land in Turkey is around 23.3 million ha with an average value of 683 Turkish Lira per decare. Turkey is also among the major exporters of agricultural products in Eastern Europe, Near East and North Africa (Ucak, 2006).

As in other countries, demand for agricultural products in Turkey has increased not just in quantity but also in variety in recent years. Country's one of the important regions that can provide crop variety is the Gazipasa district. Located in southern Turkey, the district is within the boundaries of Antalya province and its population is mostly engaged in agricultural production. The altitude of Gazipasa is between 0-2200 meters and this allows a wide variety of climates from tropical to continental. As a result, crops like wheat as well as fruits like mango and dragon fruit can well be grown in the district. It is also one of the rare regions in Turkey where products like banana and avocado are commonly grown. According to TUIK's 2018 Agricultural Production Data, more than 80% of Turkey's outdoor banana production comes from Gazipasa. Moreover, around 141.5 thousand decares of land in the district are cultivated for agricultural activities with an average of 5,335 TL agricultural production value per decare. The district's agricultural output corresponds to 755 million TL per year which constitutes 0.5 % of the total produced agricultural value in Turkey.

Cite this article as:
Ersoy, M.C., Gümüş, M. (2021). Profit Forecasting in Crop Production: The Case of Gazipaşa.
Int. J. Agric. Environ. Food Sci., 5(3), 271-278
Doi: https://doi.org/10.31015/jaefs.2021.3.3
Orcid: Mehmet Caner Ersoy: 0000-0003-0195-6648 and Mehmet Gümüş: 0000-0003-2588-0270
Received: 09 April 2020 **Accepted:** 06 June 2021 **Published Online:** 26 July 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 271-278
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

DOI: 10.31015/jaefs.2021.3.3

According to the district's Directorate of Agriculture, agricultural activities are carried out in 43 different regions, and 85 different types of crops are produced. The average value of production per decare in Gazipasa is higher than most of the other parts of Turkey because of the availability of fertile land, water and required climate. Despite this, the district may have a lower agricultural income than its potential due to the possible mistakes made in product selection. The aim of this study is to analyze the economic sustainability of existing crop production activities in the Gazipasa district of Turkey.

There are a number of examples in the literature that evaluate the economics of agricultural production for certain crop types. Gayak et al. (2020) consider the production and marketing of apple in a certain district of Nepal. Using the data from 100 farmers, they use statistical analysis to identify the factors that affect the production as well as the marketing of apples. They also conclude that apple farming has a 1.84 ratio of benefit over cost and thus profitable. Ugurlu (2019) uses survey data obtained from 82 producers to study to the economic evaluation of pomegranate production in Manisa province in Turkey. He finds that pomegranate production has a cost of 1950.4 TL per decare and a net profit of 834.5 TL per decare.

There are also studies conducted in the form of regional-based analysis of the agricultural sector. Uzunöz and Çiçek (2003) consider the effects of social and agricultural structure of agricultural enterprises on revenue generation in Kazova and Artova regions of Tokat province in Turkey. Their research includes the analysis of data obtained through a survey and the comparison of these two regions. They determine that the factors related to the agricultural structure of the regions have significant effects on agricultural income. Doğan and Gürler (2015) examine the supply sensitivity of products grown in the Yesilirmak basin in Turkey within the scope of agriculture support programs. They make recommendations on price, use of technology, and support policies. On a broader scope, Arun and Ghimire (2018) provide the strengths, weaknesses, opportunities, and threats for the Nepalese agriculture based on a national policy set in 2004.

Increasing operational costs in agricultural production makes the economic analysis of existing agricultural production even more important today than ever. Evaluating the profitability of current agricultural production in a certain region and making future estimates can be considered as useful approach that helps future planning. In this study, profit forecasts are made for the next 15 years based on the crop production in the Gazipasa district. Results will help identify the group of products that offer better economic returns. The next section explains the research methodology used.

Materials and Methods

Forecast studies are not new in the research area of agricultural production. Pandey et al (2008) use neural networks to forecast the production quantity of wheat for years 1996-2001. The inputs in their study are average rainfall, temperature and sunshine. Niyigaba and Peng (2020) forecast Rwanda's agricultural production in US dollars up until 2030. They use the grey model and ARIMA methods of forecasting with economics data obtained from National Institute of Statistics of Rwanda (NISR) and the World Bank dataset. Rana (2020) uses a fuzzy time series method with crop-yield data gathered from a university farm. He compares actual yield (kg/ha) with his forecast values to test the effectiveness of the model.

There are also studies that estimate crop profitability. McBride and Greene (2009) compare the prices and costs of conventional versus organic soybean production in order to evaluate the effect on profitability. Urfi et al. (2011) assess the similarities and differences between organic and conventional farming in Hungary in terms of costs and profits. They use data of agricultural business from two different regions and consider products that were cultivated both by organic and conventional means. In addition to unit yield, cost and price, they also include subsidies in profit calculations. Hrytsiuk and Babych (2017) study the profitability of grain production. Using two-year data of yield, price and costs, they use regression analysis and fuzzy simulation principles for predicting profits. Klima et al. (2020) access the impact of subsidies on the yield and profitability of a number of crop types cultivated in a mountainous region of Poland.

In this study, agricultural production's profit estimation for the next 15 years is made using time series analysis. The study consists of three phases which are picturing the current crop production, calculating current revenues, and making profit forecasts. Firstly, the current agricultural production is revealed by compiling the 2018 data from Farmer Registration System of district's Directorate of Agriculture. As a result of this stage, the types of products produced and the total decare where each type is cultivated are determined. Raw data included 85 different crop types cultivated in 43 different regions of Gazipasa. After extracting the data with zero production output, 81 different crop types and their cultivation areas are identified and listed in Table 1.

In all the tables following, the abbreviations GG, PG, TG, HTG and LTG refer to glass, plastic, tunnel, high tunnel and low tunnel greenhouse, respectively. Table 1 shows the size of the total land used for crop production in Gazipasa. It is seen that the most cultivated product is wheat with an area of 34000 decare (DA), followed by olives and banana.

In the second stage, the economic value of existing agricultural production is calculated by using the following formula:

$$TR_i = DA_i R_i P_i \qquad (1)$$

Where i is the index for crop type, $i = 1,\ldots,81$, and

TR_i : current total revenue for crop type i,

DA_i : total decar cultivated for crop type i,

R_i: harvest amount in kg for crop type i per decare,

P_i: current wholesale price for crop type i per kg.

In order to run Equation (1), data on the quantity produced per decare and the annual average sales price per kilogram for each type of product are collected from the district's Directorate of Agriculture. Based on that data, the current total revenue for each type of product was determined and a list was made according to annual turnover. As a result of the

listing, 30 products that make up 96.5% of the total turnover are determined. The last stage of the study continued with the cost and turnover values of the 30 products that generate the most revenue since the remaining 51 products' total economic value is assumed negligible.

In the last stage, profit calculations are made for the next 15 years based on the forecasts of prices and costs. It is predicted that both sales prices and operating costs will vary over time. For crop prices, linear trend projection is employed based on the actual prices per kilogram of the last 5 years using Equation (2) below.

$$P_{it}=a_i+b_i t \quad (2)$$

Where *t* is the time period in years, and

P_{it} : forecast of the wholesale-price per kg for crop type *i* in period *t,*

a_i : wholesale price at time zero (the y-intercept of the trend line) for crop type *i*,

b_i: slope of the trend line for crop type *i*.

In determining the annual operating costs per decare for each crop type, the current costs are first estimated by taking the expert opinion of 5 agricultural engineers and 7 producers present in the district. An annual average interest rate for agricultural support loans is then used to project the future rate of increase in operating costs. Future cost figures are thus estimated using the equation below.

$$v_{it} = v_{i0} (1+r)^t \quad (3)$$

Where *r* is the annual average interest rate for agricultural loans, and

v_{i0} : current operating cost per decare for crop type i, estimated by taking expert opinions,

v_{it} : estimation of the operating cost for crop type *i* per decare in year *t.*

Finally, the annual profit for each crop type is calculated using the area of the land planted, quantity produced per decare, forecast of the sales price per kilogram, and the estimation of the operating cost per decare. Equation (4) below is used for profit calculations.

$$TP_{it} = DA_i R_i P_{it} - DA_i v_{it} \quad (4)$$

Where TPit is assumed 0 if (4) returns negative, and

TPit : total profit for crop type i in year t.

Table 1. Gazipasa's crop production data in 2018.

#	Product	DA	#	Product	DA	#	Product	DA	#	Product	DA
1	Wheat	34.000	**22**	Eggplant GG	1.200	**43**	Jujube	385	**64**	Nectarine	110
2	Olive	20.000	**23**	Bean PG	1.200	**44**	Eggplant TG	380	**65**	Vetch type2	110
3	Banana	12.850	**24**	Eggplant PG	1.100	**45**	Carob	330	**66**	Pepper Capia PG	100
4	Cucumber GG	6.360	**25**	Orange Valencia	850	**46**	Pear	330	**67**	Watermelon GG	80
5	Strawberry HTG	6.175	**26**	Eggplant	850	**47**	Apple Golden	320	**68**	Cabbage	80
6	Almond	5.720	**27**	Peanut	800	**48**	Artichoke	310	**69**	Potato	70
7	Barley	4.800	**28**	Peach	770	**49**	Vetch type1	310	**70**	Pepper Pointed PG	60
8	Tomato GG	3.900	**29**	Green Pea	755	**50**	Pumpkin PG	290	**71**	Lettuce	60
9	Beans	3.700	**30**	Strawberry LTG	750	**51**	Apricot	280	**72**	Lemon	55
10	Triticale	3.550	**31**	Broad Bean	580	**52**	Tomato TG	270	**73**	Cauliflower	55
11	Banana PG	2.600	**32**	Pepper Pointed	560	**53**	Oat	260	**74**	Spinach	55
12	Cherry	2.400	**33**	Corn (Grain)	550	**54**	Kidney Bean Type 2	250	**75**	Okra	55
13	Bean GG	2.330	**34**	Plum	540	**55**	Chickpea	250	**76**	Mandarin Klamantin	35
14	Cucumber PG	2.220	**35**	Cucumber Tunnel	530	**56**	Peas (Dry)	230	**77**	Mulberry	30
15	Pomegranate	1.950	**36**	Orange Washington	500	**57**	Apple (Other)	210	**78**	Pepper Pointed GG	25
16	Tomato	1.500	**37**	Bean TG	500	**58**	Zucchini	190	**79**	Mandarin Satsuma	22
17	Onion	1.500	**38**	Kidney Bean	470	**59**	Strawberry	160	**80**	Apple Gransimit	20
18	Broad Bean	1.500	**39**	Rye	470	**60**	Kiwi	150	**81**	Quince	3
19	Walnut	1.400	**40**	Watermelon	460	**61**	Broccoli	150			
20	Avocado	1.240	**41**	Corn (Dent)	450	**62**	Stuffed Pepper	140		Total Land Cultivated: 141.550 decare	
21	Tomato PG	1.200	**42**	Grape	420	**63**	Pepper Capia GG	130			

Data obtained from Gazipasa Directorate of Agriculture.

DOI: 10.31015/jaefs.2021.3.3

Results and Discussion

As the second phase of the study, the economic value of 81 different products grown in Gazipasa in 2018 is analyzed. Using Equation (1), total revenue in Turkish Lira for each type of product is calculated. After finding the revenues, all products are listed in descending order according to their returns and divided into groups for classification purposes. Results are presented in Table 2.

As seen in Table 2, the district achieved approximately 583 million TL agricultural revenue in 2018. Among all types of products, 30 that are in the first three groups generate 96.45% of the total revenue. The commercial return of the remaining 55 types of products remains very low at 3.55% of the total revenue. Therefore, it is assumed that these products are planted for hobby or trial purposes and hence are not included in the rest of the study. Table 3 provides the names and revenues of the top 30 products.

The top 10 products in Table 3 constitute 76.35 % of the total revenue of the district with a value of around 445.4 million TL. The total area planted for these products is 59,765 decare. This amount corresponds to 42.22% of the total agricultural production area of Gazipasa. Open field banana, cucumber grown in glass greenhouse, tomato grown in glass greenhouse, and banana grown in plastic greenhouse are the top four revenue generating products with an annual return of at least 50 million TL.

The second 10 products make up 14.99% of the total revenue with an annual return of around 87.4 million TL. Although their yields are far behind compared to the top ten products, this group includes products with high long-term yield potential such as avocado, pomegranate, cherry, walnuts and almond. This group of products constitutes 38.36% of the total cultivated land. Almond, which has a harvest time of around 7 years after plantation, is the on the top of the list. Plastic greenhouse tomato and bean take the second and third place respectively. Avocado, which has been commercially produced in the district for the last 6 years only, ranks seventh.

The last 10 products in Table 3 constitute 5.1 % of the total revenue with a return of around 29.8 million TL and occupies 3.34% of the total cultivated area. Excluding orange-valancia and peaches, the remaining eight in this group are high tonnage products in the district and can be yielded in the same season. This shows that although these products do not bring as much income as the ones in the first and second ten, they have a potential for future.

Table 2. Groups of products according to generated revenue.

Groups	1st Ten	2nd Ten	3rd Ten	4th Ten	5th Ten	Rest 51-81	TOTAL
Total Revenue (Million TL)	445.41	87.44	29.79	10.90	5.34	4.49	583.37
% Share	76.35	14.99	5.11	1.87	0.92	0.76	100

Table 3. Top 30 revenue generating products.

#	Product Name	Revenue (TL)	#	Product Name	Revenue (TL)	#	Product Name	Revenue (TL)
1	Banana	100,230,000	11	Almond	14,157,000	21	Strawberry LTG	3,952,500
2	Cucumber GG	84,588,000	12	Tomato PG	13,680,000	22	Cucumber TG	3,524,500
3	Tomato GG	51,870,000	13	Bean PG	11,520,000	23	Eggplant TG	3,458,000
4	Banana PG	50,544,000	14	Pomegranate	9,630,150	24	Peach	3,368,750
5	Strawberry HTG	36,741,250	15	Cherry	8,880,000	25	Pepper Capia GG	3,328,000
6	Bean GG	27,960,000	16	Wheat	7,514,000	26	Bean TG	3,000,000
7	Cucumber PG	25,308,000	17	Avocado	6,820,000	27	Watermelon	2,622,000
8	Olive	24,750,000	18	Tomato	5,700,000	28	Orange Valencia	2,244,000
9	Eggplant GG	23,400,000	19	Beans	4,921,000	29	Pepper Capia PG	2,240,000
10	Eggplant PG	20,020,000	20	Walnut	4,620,000	30	Tomato TG	2,052,000
	TOTAL	445,411,250		TOTAL	87,442,150		TOTAL	29,789,750

Considering all the products in Table 3, greenhouse production seems to be very important for generating district's revenues. Excluding banana and strawberry, greenhouse production includes vegetables only. Among those, cucumber provides the highest yield, followed by tomato and eggplant. Table 3 also shows that return on field vegetables produced in Gazipasa are not very high. There are three products that stand out in terms of revenue generated from field vegetables and these are tomato, beans and watermelon in sequence.

For any product that has an economic value, profit can be calculated as revenue minus cost. In this part of the study, profits for the 30 types of products listed in Table 3 are calculated using unit prices, yields, and costs. Based on the data gathered, highest yields are eggplant-glass greenhouse with 15 tons/decare, followed by cucumber-glass greenhouse, tomato-glass greenhouse, and eggplant-plastic greenhouse each with 14 tons/decare. The lowest yields per decare are wheat with 0.26 tons, walnuts with 0.30 tons, almond with 0.45 tons, and olives

with 0.83 tons. In general, yield per decare for greenhouse products is high whereas it is low for fruits produced from trees that do not require an irrigation system.

The third phase of the study is forecasting the profitability in future years. First, as in Equation (2), linear trend projection is employed for each of the 30 crop types to predict future prices using the wholesale-price data of the last 5 years. Results for selected product types are depicted in Figure 1 where the 6th time period refers to year 2020. MS Excel's function of adding a trend line and equation was used in the graphs.

In terms of costs, the current operating cost for each crop type is determined by taking the expert opinion. The average annual agricultural loan interest rate of 7.5 % is then used to increase the costs annually as in Equation (3). Results for selected crop types are shown in Figure 2.

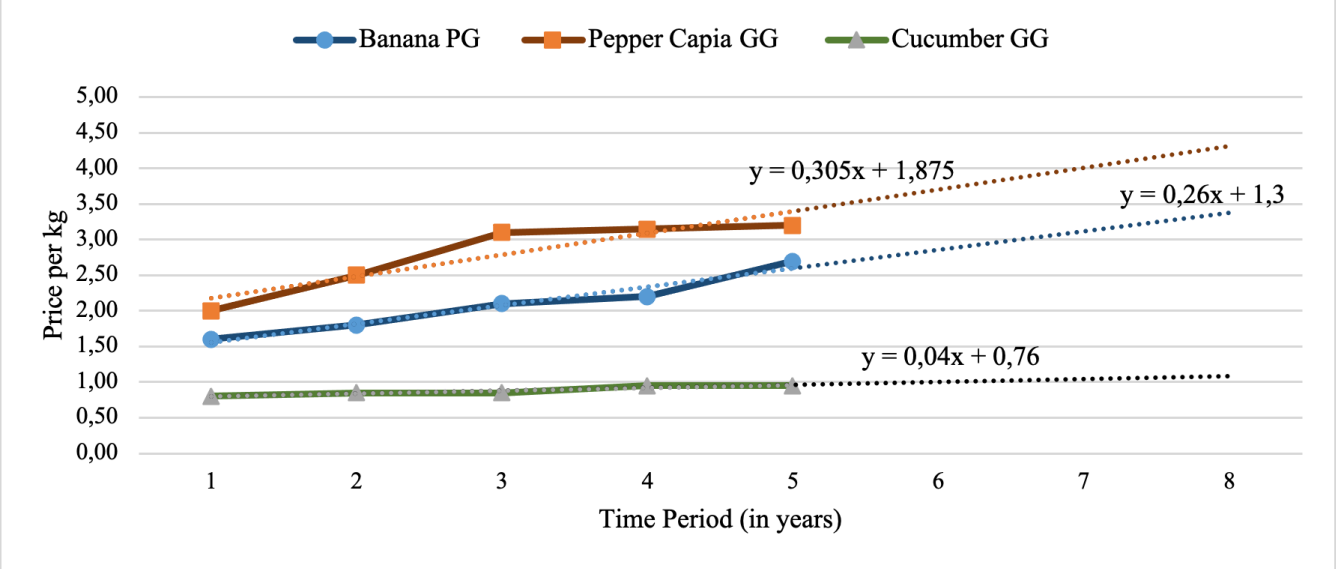

Figure 1. Linear trend projection for selected crop types

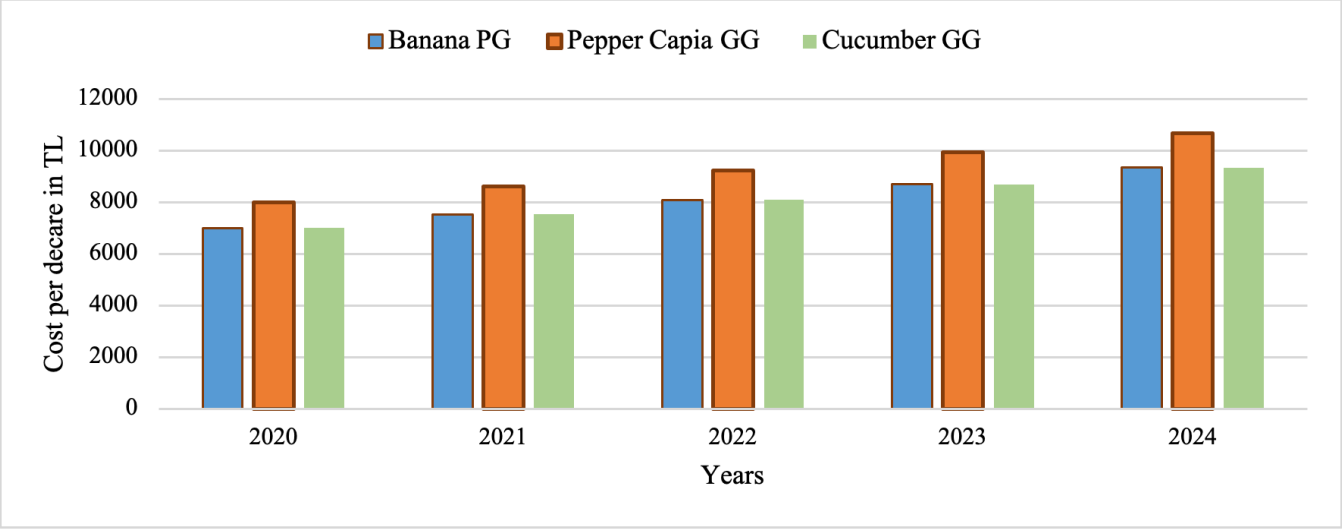

Figure 2. Estimated operating costs over years for selected crop types

Yield per decare for a product type is assumed constant over years. The total profit for each crop type in each of the next 15 years is then calculated using Equation (4). Results are presented in Table 4 where Year 1 refers to 2020. The first column of Table 4 represents the 30 most revenue generating products named in Table 3 where the column DA indicates the total decare of the cultivated land. Remaining columns show the profits per year in million TL for years from 1 to 15.

The last row of Table 4 shows the total profit for the next 15 years if the current agricultural production does not change. It is seen that the annual total profit, which is around 298 million TL in the first year, gradually increases to around 385 million TL in the 11th year and then starts to decrease. Aggregated values for the timespans of 1, 5, 10 and 15 years are given in Table 5. The table shows, in case the current planting scheme continues, the total profit generated, annual average profit, the total area of land cultivated and its percentage compared to total arable land for each of these timespans.

Table 4. Total estimated profit per year for the next 15 years for each crop type (Million TL).

#	DA	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Year 9	Year 10	Year 11	Year 12	Year 13	Year 14	Year 15
1	12800	37.95	41.41	44.50	47.21	49.51	51.35	52.72	53.56	53.86	53.55	52.61	50.97	48.59	45.41	41.38
2	6360	44.52	44.74	44.71	44.42	43.83	42.93	41.70	40.11	38.13	35.74	32.90	29.58	25.74	21.35	16.36
3	3900	28.39	29.07	29.60	29.97	30.15	30.15	29.94	29.51	28.84	27.92	26.73	25.24	23.43	21.28	18.77
4	2600	33.85	37.22	40.48	43.64	46.67	49.58	52.36	54.98	57.45	59.75	61.86	63.78	65.49	66.97	68.21
5	6175	7.81	7.44	6.90	6.17	5.23	4.09	2.71	1.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	2330	17.59	19.51	21.34	23.08	24.70	26.21	27.60	28.86	29.98	30.94	31.74	32.37	32.80	33.03	33.05
7	2220	11.10	11.00	10.81	10.53	10.15	9.66	9.05	8.32	7.45	6.44	5.27	3.93	2.41	0.70	0.00
8	20000	17.70	18.45	19.13	19.74	20.27	20.72	21.08	21.34	21.50	21.54	21.47	21.26	20.92	20.43	19.77
9	1200	16.98	18.15	19.27	20.34	21.36	22.32	23.22	24.04	24.80	25.48	26.07	26.57	26.97	27.27	27.46
10	1100	14.01	14.98	15.90	16.77	17.59	18.36	19.07	19.72	20.30	20.81	21.24	21.59	21.85	22.02	22.08
11	5720	11.04	11.60	12.14	12.66	13.17	13.65	14.12	14.56	14.98	15.37	15.73	16.06	16.35	16.62	16.84
12	1200	6.29	6.38	6.42	6.41	6.35	6.23	6.04	5.79	5.47	5.06	4.58	4.00	3.32	2.54	1.65
13	1200	5.57	6.23	6.85	7.42	7.93	8.39	8.78	9.10	9.35	9.53	9.62	9.61	9.51	9.31	8.99
14	1950	8.11	8.26	8.39	8.52	8.63	8.73	8.81	8.87	8.92	8.96	8.97	8.96	8.93	8.87	8.79
15	2400	4.94	5.59	6.21	6.81	7.38	7.94	8.46	8.96	9.42	9.85	10.24	10.60	10.91	11.18	11.40
16	34000	2.81	2.56	2.28	1.97	1.63	1.25	0.84	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	1240	4.26	5.16	6.05	6.93	7.79	8.63	9.46	10.26	11.04	11.80	12.54	13.25	13.93	14.57	15.19
18	1500	2.55	2.75	2.93	3.08	3.21	3.32	3.39	3.44	3.45	3.43	3.37	3.27	3.13	2.94	2.70
19	3700	0.37	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	1400	4.02	4.33	4.64	4.94	5.23	5.51	5.78	6.04	6.28	6.51	6.73	6.94	7.12	7.29	7.44
21	750	1.16	1.15	1.11	1.06	0.99	0.90	0.79	0.65	0.49	0.29	0.07	0.00	0.00	0.00	0.00
22	530	1.59	1.58	1.56	1.52	1.47	1.41	1.33	1.23	1.12	0.98	0.82	0.65	0.44	0.21	0.00
23	380	1.09	1.16	1.21	1.24	1.26	1.26	1.24	1.20	1.13	1.04	0.93	0.78	0.61	0.40	0.15
24	770	3.44	3.93	4.41	4.88	5.36	5.83	6.31	6.77	7.24	7.70	8.15	8.61	9.05	9.50	9.94
25	130	3.15	3.53	3.91	4.28	4.65	5.01	5.36	5.70	6.03	6.36	6.67	6.97	7.26	7.54	7.80
26	500	0.14	0.21	0.27	0.30	0.31	0.30	0.26	0.19	0.10	0.00	0.00	0.00	0.00	0.00	0.00
27	460	3.61	4.23	4.84	5.45	6.05	6.65	7.25	7.85	8.44	9.03	9.61	10.19	10.76	11.33	11.89
28	850	1.49	1.58	1.67	1.75	1.82	1.89	1.96	2.01	2.06	2.10	2.13	2.16	2.17	2.17	2.16
29	100	2.12	2.38	2.63	2.88	3.13	3.37	3.61	3.84	4.06	4.28	4.49	4.69	4.89	5.07	5.25
30	270	0.31	0.28	0.24	0.18	0.11	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
∑	117735	297.97	314.92	330.41	344.16	355.97	365.68	373.21	378.37	381.89	384.46	384.53	382.01	376.60	368.01	357.27

Table 5. Aggregated profit figures and land usage in future years.

	Total Profit (TL)	Average Annual Profit (TL)	Total Cultivated Land (Decar)	Arable Land Usage (%)
Year 1	297,974,060	297,974,060	117,735	%83.18
Year 5	1,643,447,113	328,689,423	114,035	%80.56
Year 10	3,527,064,507	352,706,451	73,090	%51.64
Year 15	5,395,483,530	359,698,902	69,590	%49.16

As can be observed in Table 5, the total area of land currently cultivated and hence the percentage of arable land used are decreasing over years. At the end of the first year, economic return is obtained from 117,735 decares of the potential 141,550 decares, and approximately 17 % of the arable land remains idle. In case the current agricultural crop diversity continues, the rate of idle land increases in the following years as the production of some crop types will cease. Although the total annual profit decreases after the 11th year, the average annual profit keeps increasing due to the rising prices of crops that continue to be produced in the future years. Production sustainability of the crop types based on the profits they generate is summarized in Table 6.

Table 6. Production sustainability of existing crop types based on profitability.

	Production Stops	Profitability Decreases	Profitability Increases	
Product Name & Number	Strawberry HTG (# 5)	Cucumber GG (# 2)	Banana (# 1)	Cherry (# 15)
	Cucumber PG (# 7)	Tomato GG (# 3)	Banana PG (# 4)	Avocado (# 17)
	Wheat (# 16)	Tomato PG (# 12)	Bean GG (# 6)	Tomato (# 18)
	Bean (# 19)	Eggplant TG (# 23)	Olive (# 8)	Walnut (# 20)
	Strawberry LTG (# 21)		Eggplant GG (# 9)	Peach (# 24)
	Cucumber TG (# 22)		Eggplant PG (# 10)	Pepper Capia GG (# 25)
	Bean TG (# 26)		Almond (# 11)	Watermelon (# 27)
	Tomato TG (# 30)		Bean PG (# 13)	Orange Valencia (# 28)
			Pomegranate (# 14)	Pepper Capia PG (# 29)
Total Count	8	4		18

Table 6 shows that only 18 out of 30 types of products remain profitable over years. On the other hand, the cultivation of 8 types of products will need to be ended after some years. For example, the profitability of bean and bean-tunnel greenhouse will be zero after 2 and 9 years respectively, and hence their production will stop accordingly. Moreover, there are four types of products whose profit decrease continuously even if not stopped indicating that their production will also cease sometime after 15 years.

A profit greater than zero doesn't necessarily mean sufficient rate of return is achieved. In order to calculate the return on investment, land setup costs for plantation is estimated by taking expert opinion. It is found that the total current setup cost of the agricultural production for the 30 types of products considered is approximately 2 billion TL. Since the total profit in the first year is approximately 298 million TL, return on investment in the first year is 14.9 %. As the planning period in the study is 15 years, internal rate of return (IRR) is also calculated. IRR is the rate that makes the net present value of all cash flows equal to zero. Setting 2 billion TL as the initial investment and the total profits in Table 4 as net cash flows, IRR is found to be 15 %. Current interest rate in the Turkish financial markets is above 16%, and the policy interest rate set by the Central Bank of Turkish Republic is above 17%. Hence, the IRR found is considered to be low compared to the annual return of alternative investment instruments in financial markets. In order to increase it, a better agricultural planning is required in Gazipasa. That plan should take into account the future profitability while selecting crop diversity in the district.

Continuing with the plantation of the 18 profit-making products in Table 6 in all arable land does not necessarily mean that optimal plan is achieved. Each product type may require different soil characteristics, land sizes, climate, and irrigation. Future cultivation planning should also consider these exterior factors.

Conclusion

In this study, the economic analysis of the agricultural crop production of Turkey's Gazipasa district for the next 15 years was made. Out of 81 different products currently grown in 43 regions of the district, 30 products with the highest revenues were determined. Current total return of these products corresponds to 96.45 % of the total revenue and covers 83.18% of the existing agricultural areas. Total income and expenses were determined for those 30 types of products and the profitability was forecasted on a yearly basis. It was found that the total profit increases slowly in the next 11 years and then decreases. It was observed that the profits achieved would increase for only 18 out of 30 product types. Results show that such analysis may help shape future plans in agriculture in order to increase profitability and maintain economically sustainable production. Conducting similar studies on different regions of the country and coordinating their results may also be useful to identify production policies on a national basis. Future studies can also include identifying the optimal product types for a certain region with taking into consideration the factors affecting agricultural production.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.4 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):279-286 (2021)

Pollen characteristics of some grape cultivars (*Vitis vinifera* L.)

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Abstract

Pollen morphology is an important parameter. In this study, the pollen morphology of selected in 10 grape cultivars were examined by SEM (scanning electron microscopy). The pollen surface features were observed, such as length, width, and P/E ratio of pollen. The pollen differed in some microstructural characteristic. Pollen width exhibited significant according to the varieties (10.12-22.44 µm). Smilarly, the statistical difference occurred among the ten *Vitis* genotypes in terms of mean pollen length (16.26-29.91 µm). P/E ratio determined to the varieties (1.08-2.55 µm) and, grape varieties have the longest pollen, in general. The without furrows are surrounded in exine were determined in cultivars of "Alicante Boushet", "Cardinal", "Syrah". Areolat pollen was determined in among other cultivars. Depending on the cultivars there was a statistical difference from the point of pore diameter. Pollen viability was determined between 11.75 % and 84.25 % in TTC tests. The present research is a contribution to amore detailed analysis of grapevine cultivars.

Keywords: Palynological, Pollen, Pollen viability, Pollen germination, Scanning electron microscope (SEM), TTC test, Classification

Introduction

Pollen has hereditary properties that determine the genotype of plants. Pollen morphology confirms phylogenetic relationships among genera, species and varieties. Thus, it is used in systematic studies due to similarity and diversity of pollen. The morphology of pollen can be examined in detail via the scanning electron microscope (Tanaka et al., 2004).

On the basis of surface ornamentation and pollen grain dimensions, different classifications have been made on various plant species (Erdtman, 1952; Faegri and Iversen, 1989; Hyde and Adams, 1958; Wodehouse, 1935). Studies in grapevines have mainly focused on cultivars. In the description of the pollen exine microrelief, separate elements, polar and equatorial axis, mesocolpium, apocolpium, and length and width of the colps are used (Roytchev, 1995). For example, Uzun and llter (1987) and Kharitonashvili et al. (1989) studied pollen grains in different types of flowers of *Vitis vinifera* L., using SEM. Ahmedullah (1983) characterized different grape cultivars based on pollen morphology. Martens et al. (1989) studied pollen size variability within genotypes of *Vitis*, Ben Slimane (1990) characterized 30 grapevine varieties based on pollen size, Roytchev et al. (1994) obtained information on the ultrastructure of exine surface in 27 Bulgarian and foreign seedless grape cultivars seedless grape cultivars and Gallardo et al. (2009) studied fourteen Spanish *Vitis vinifera* L. subsp. *sylvestris* populations. Palynology has presented considerable opportunities for newly released hybrid varieties identification in grapevines (Marasalı et al. 2005). Jovanovic-Cvetkovic et al. (2016) analysed the pollen morphology of indigenous cvs. Žilavka and Blatina to determine their morphological specificities.

The objective of the present study was to classify the ten

Cite this article as:
Isci, B. (2021). Pollen characteristics of some grape cultivars (*Vitis vinifera* L.). Int. J. Agric. Environ. Food Sci., 5(3), 279-286
Doi: https://doi.org/10.31015/jaefs.2021.3.4
Orcid: Burçak İşçi: https://orcid.org/0000-0002-6542-0271
Received: 11 February 2021 **Accepted:** 19 May 2021 **Published Online:** 26 July 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 279-286
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

grape cultivars according to the shape and microrelief of pollen grains and to establish the possibilities for using the parameters of the different apertures as classification indices using SEM.

Materials and methods

Pollen samples:

This study was carried with pollen from ten different of *Vitis vinifera* L. (Table 1) are located at the Department of Horticulture, Agriculture Faculty, Ege University (38° 27′ 15.8652" ve 27° 13′ 32.5272"). The flower clusters were isolated and these inflorescences were collected in the morning hours at the beginning of the blooming period (when the calyptras fall from the flower a cloud of pollen is released from the anthers of the stamens which move away from the pistil) of Eichorn and Lorenz (1977) classification. Since bloom may progress for several days over a vine and an individual cluster, one must estimate the percentage of cap fall to designate the stage of development. In the varieties examined in the study, full bloom occurred between 25 May- 8 June. Samples from "Cardinal" were collected on 25 May, "Foça Karası" and "Yuvarlak Çekirdeksiz" on June 1, "Alicante Bouschet", "Cinsaut", "Mahrabaşı", "Malbec", "Syrah" on June 5, "Alphonse Lavallee" and "Italia" on June 8, respectively. The pollen was collected by cutting flowers and brushing the anthers and pollen into an Eppendorf tube using a soft brush (Gökbayrak and Engin, 2016). The study materials were stored a refrigerator in controlled conditions until analysis (Storey, 1975).

Dry pollen was sputter-coated (Leica model) with 10 µm of gold-palladium, for this pollen grains were air dried for SEM investigations. Pollen grains were measured directly on the screen of the scanning electron microscope. Each of the tested samples were observed with Thermo Scientific Apreo S model scanning electron microscope were photographed at 10000 x for whole grain (Central Research Test and Analysis Laboratory Application and Research Center (EGE MATAL) for help on SEM operation). The pollen length, width, length/width ratio and pore diameter, distance between pori and colpi length were measured on 10 pollen grains for each genotypes. The pollen shape was stated by considering the length/width ratio (Erdtman, 1952). The types of aperture found in pollen were described by Wang et al. (2014). The polar and equatorial axes, the pollen grain equatorial diameter relationship were determined by Erdman (1952) and La-Serna Ramos (1996) was used for ultra morphological descriptions of the pollen grains.

Pollen viability:

1% of TTC was used for the viability capacity of pollens in this study (Norton, 1966). The pollen samples were carried out through eight replications in four fields chosen randomly on each microscope slide. In TTC test, they were classified into dark red pollen grains, light red pollen or colourless pollens according to their colours. Pollen were considered as active, semi-active, and lifeless, respectively (Kelen and Demirtaş, 2003).

Pollen germination:

To calculate the *in vitro* germination percentage of 10 grape cultivars, a medium containing 10 mg of boric acid per ml, 20 % sucrose, and 0.6 % agar was used. Pollen grains were placed agar in petri dishes containing the germination medium that were maintained at 30°C in the dark for 24 h (a thermos controlled dryer). To determine the germination percentage, 3 fields per sample (each one containing around 100 pollen grains) were counted using a light microscope (Kelen and Demirtaş 2003).

The data were subjected to analysis of variance using SPSS 20 statistical package program. The mean, minimum, maximum, and standard error values of the properties were found out. The relationship among these values was revealed by conducting Pearson's correlation analysis. Cluster analysis (CA) was also realized and indicated by dendrogram (Marasalı et al., 2005).

Table 1. List of the cultivars studied

Cultivars	Type	Cultivars	Type
Vitis vinifera L. "Alphonse Lavallee"	T, S	*Vitis vinifera* L. "Italia"	T, S
Vitis vinifera L. "Alicante Bouschet"	W, S	*Vitis vinifera* L. "Mahrabaşı"	T, S
Vitis vinifera L. "Cardinal"	T, S	*Vitis vinifera* L. "Malbec"	W, S
Vitis vinifera L. "Cinsault"	W, S	*Vitis vinifera* L. "Syrah"	W, S
Vitis vinifera L. "Foça Karası"	W, S	*Vitis vinifera* L. "Yuvarlak Çekirdeksiz"	T, SE

Abbreviations: T: table grape, W: Wine grape, S: Seeded, SE: Seedless grape

Results

Given the characteristics of ten grape cultivars, a general description was established for all, according to the values of the various parameters corresponding to the max. and min. records. The statistical difference appeared in terms of the pollen grains (length, width and length/width ratio of pollen) (Table 2 and Table 3).

Mean pollen width differed statistically significant according to the varieties. Thus, the highest mean values for this feature was in "Mahrabaşı" (22.44 µm, a) and the lowest mean values for this pollen width were found in "Syrah" (10.54 µm, f) and "Alicante Bouschet" (10.12 µm, f) varieties, respectively. Pollen length ranged from 29.91 µm (a) "Alphonse Lavallee" to 16.26 µm (e) "Foça Karası" (Table 2).

On the other hand, when the pollen is examined in terms of symmetry and shape, the length/width ratio ranged from 2.55

µm "Alicante Bouschet" to 1.08 "Foça Karası" (Table 2). The morphological description of pollen was made using Erdman (1952)'s terminology. Among the grape varieties examined four different shapes. The pollen grains were prolate-spheroidal "Foça Karası", subprolate "Mahrabaşı", perprolate (Alicante Bouschet, Syrah, Cardinal, Italia) and prolate (Alphonse Lavallee, Malbec, Cinsault, Yuvarlak Çekirdeksiz) (Table 6).

According to aperture, typically two types were observed. Among the grape varieties examined, it was determined that there was no diaphragm opening in the pollen of a group. Inaperturate pollen grains were observed in some cultivars such as "Foça Karası", "Mahrabaşı", whereas "Alphonse Lavallee", "Cinsaut", "Alicante Bouschet", "Malbec", "Italia", "Syrah" "Cardinal", "Yuvarlak Çekirdeksiz" were tricolporate (Figure 1).

Circular openings were detected on the pollen grains and such grains are called porate. The pollen had circular apertures on the exine surface, these ere not uniformly distributed and the pollen grains were said pantoporate. The pollen grains surface has elongated or furrow-like apertures. These were called colp. Also, the circular apertures on the pollen has circular apertures on the exine surface, they were called pori. The pollen shape and exine patTterns of the studied varieties were given in SEM images. There were statistical differences in terms of these properties. For pore length, "Cinsaut" (397.45, a) located at the first group, while the "Alphonse Lavallee" (112.40, d) was the last group. Thus, pore width differed statistically significant according to the varieties. For this value, the "Cardinal" (245.68, a), variety was the first group, among the varieties examined, "Syrah" and "Alphonse Lavallee" were the smallest diameter of the pore width and, "Syrah" (97.06, e) and "Alphonse Lavallee" (102.32, e) located at the last group. In terms of this feature, it was found in different statistical groups in other varieties (Table 3).

Pollen of "Alicante Bouschet", "Syrah", "Cardinal" cultivars did not furrows. Areolat is surrounded in exine was observed in "Foça Karası", "Mahrabaşı", "Alphonse Lavallee", "Cinsaut", "Malbec", "Italia", "Yuvarlak Çekirdeksiz" (Figure 2).

It was determined that to pollen germination ranged from 29.25 % and 87.25 %. In respect to pollen viability levels varied between 11.75 % and 84.25 % in TTC tests. "Cardinal", Alicante Bouschet" and "Syrah", cultivars were without furrows and TTC test result in these cultivars was found 84.25 %, 75.75 % and 74.25, respectively (Table 5).

The correlation coefficients of the features are shown in Table 4. Accordingly, the highest positive correlation was determined between TTC and pollen length/width ratio ($r= 0.700$; $p<0.05$). From the other side, a negative correlation occurred between the pollen length/width ratio and pollen width value ($r= -0.796$; $p<0.01$) and TTC and pollen width ($r= -0.700$; $p<0.05$). The similarities or differences among the grape cultivars examined with Pearson correlation coefficienties showed a correlation with those examined with TTC test in terms of examined characteristics.

Cluster analysis was used to determine the degree of similarity of grape cultivars, is located in Figure 4 as dendograms. Consequently, the cultivars were classified under two main groups. "Alicante Bouschet", "Yuvarlak Çekirdeksiz", "Foça Karası", "Mahrabaşı", "Malbec", "Cinsaut", "Cardinal" were included in the first group while "Italia", "Syrah", "Alphonse Lavallee", were collected in the second group. First and second groups divided into different sub-groups.

Discussion

A number of palynological investigations into cultivated *Vitis* varieties have shown that pollen shape and P/E ratio change from one sample to another (Reille ,1966; Roytchev, 1997; Cabello et al., 1994). Our results appeared that the ten cultivars of *V. vinifera* L. examined were differences in terms of pollen morphology. There were difference in the size (pollen width, pollen lenght), shape of pollen grains, pore on pollen surface and pollen ornamentation.

There were significantly differences in pollen width grains sizes in the cultivars studied. The lenght of pollen grains ranged from 16.26 µm to 29.91 µm (Table 2).

Table 2. Morphological characteristic of pollen of grape cultivars (µm)

Cultivars	Pollen Size											
	Pollen width (µm)				Pollen length (µm)				Length/width ratio			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Alphonse Lavallee	14,50	16,76	15,66 cd	0,75	28,28	32,32	29,91 a	1,64	1,75	2,08	1,91 cd	0,12
Alicante Boushet	8,18	13,07	10,12 f	1,49	21,43	29,02	25,43 c	2,69	2,09	3,35	2,55 a	0,41
Cardinal	11,43	16,90	13,96 de	1,74	26,98	32,27	29,43 a	1,74	1,78	2,60	2,14 bc	0,27
Cinsault	14,06	17,00	15,90 c	1,18	24,34	32,86	28,42 ab	2,64	1,18	1,50	1,79 d	0,15
Foça Karası	14,17	16,89	15,04 cd	0,94	15,70	17,26	16,26 e	0,62	1,02	1,13	1,08 f	0,04
Italia	9,65	17,03	12,94 e	2,80	20,82	29,52	25,74 c	2,99	1,46	2,95	2,08 cd	0,54
Mahrabaşı	20,82	24,25	22,44 a	1,03	23,55	29,87	26,86 bc	2,12	1,08	1,39	1,20 ef	0,13
Malbec	9,59	18,56	12,77 e	2,56	20,44	26,11	22,59 d	1,95	1,41	2,44	1,82 d	0,36
Syrah	9,11	12,28	10,54 f	0,97	20,25	31,90	25,04 c	3,46	1,70	3,06	2,40 ab	0,39
Yuvarlak Çekirdeksiz	14,83	23,81	19,09 b	3,15	23,19	34,47	26,47 bc	3,37	1,03	1,67	1,42 e	0,26

Abbreviations: Min: minimum values; Max: maximum values; SD: standard deviations

Table 3. The length and width values of pori in grape varieties (µm)

Cultivars	Pore width (µm)				Pore length (µm)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Alphonse Lavallee	84,91	135,83	102,32 e	20,13	99,46	146,52	112,40 d	20,15
Alicante Boushet	167,56	200,18	185,69 bc	16,11	194,20	280,33	217,18 bc	35,64
Cardinal	165,72	281,35	245,68 a	46,05	178,39	398,80	265,36 bc	91,58
Cinsault	124,56	176,55	158,09 cd	22,20	322,00	492,54	397,45 a	71,91
Foça Karası	141,95	260,77	196,85 abc	48,74	152,91	276,75	204,06 bc	51,24
Italia	94,86	136,80	118,63 de	21,29	159,30	305,50	219,18 bc	63,19
Mahrabaşı	149,30	315,66	220,11 ab	70,65	109,80	332,63	208,54 bc	111,45
Malbec	180,51	250,54	209,07 abc	25,73	217,20	349,90	298,82 b	52,02
Syrah	86,00	133,50	97,06 e	20,43	111,20	288,90	178,48 cd	75,82
Yuvarlak Çekirdeksiz	135,81	228,64	161,62 cd	37,84	147,30	295,60	214,60 bc	57,80

Abbreviations: Min: minimum values; Max: maximum values; SD: standard deviations

Table 4. Pearson correlation coefficienties among traits in cultivars

	Pollen width	Pollen length	Pollen length/width ratio	Pore width	Pore length	TTC
Pollen length	0,178					
Pollen length/width ratio	-0,796**	0,410				
Pore width	0,273	-0,175	-0,329			
Pore length	-0,009	0,046	-0,028	0,395		
TTC	-0,700*	0,021	0,700*	0,137	-0,069	
Agar in petri dishes (%)	0,176	-0,071	-0,098	0,290	-0,197	0,411

Abbreviations: * Significant at P<0.05, ** Significant at P<0.01

Table 5. Pollen viability capacity determined in the TTC tests (%) and germination rates obtained from the agar in petri dishes (%)

Cultivars	Viability				Germination			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Alphonse Lavallee	0,00	57,00	24,25 cd	23,79601	0,00	70,00	38,25 bc	29,64653
Alicante Boushet	50,00	100,00	75,75 a	21,10884	33,00	75,00	53,75 abc	17,38534
Cardinal	80,00	86,00	84,25 a	2,87228	60,00	81,00	69,25 abc	8,84590
Cinsault	0,00	23,00	11,75 d	9,42956	0,00	92,00	29,25 c	43,46167
Foça Karası	9,00	50,00	26,50 cd	18,77054	0,00	100,00	50,00 abc	57,73503
Italia	22,00	75,00	56,75 ab	23,99132	50,00	100,00	79,50 ab	21,07922
Mahrabaşı	4,00	33,00	20,25 cd	13,67175	83,00	90,00	87,25 a	2,98608
Malbec	42,00	85,00	67,00 ab	18,38478	42,00	100,00	70,75 abc	23,96351
Syrah	66,00	86,00	74,25 a	8,65544	50,00	77,00	67,00 abc	12,30176
Yuvarlak Çekirdeksiz	25,00	71,00	42,25 bc	20,12254	50,00	100,00	65,00 abc	23,80476

Table 6. Mean value for P/E ratio

	< 0.50 peroblate	0.50-0.75 oblate	0.76 - 0.88 suboblate	0.89 – 0.99 oblate-spheroidal	1.00 spherical	1.01 – 1.14 prolate-spheroidal	1.15 – 1.33 subprolate	1.34 – 2.00 prolate	> 2.00 perprolate
Cultivars									
"Alicante Bouschet"									X (>8:4)
"Syrah"								X (8:4)	
"Cardinal"									X (>8:4)
"Italia"									X (>8:4)
"Alphonse Lavallee"								X (8:4-8:6)	
"Malbec"								X (8:4-8:6)	
"Cinsaut"								X (8:4-8:6)	
"Yuvarlak Çekirdeksiz"								X (8:4-8:6)	
"Mahrabaşı"							X (7:8-6:8)		
"Foça Karası"						X (8:7-8:8)			

P/E ratio

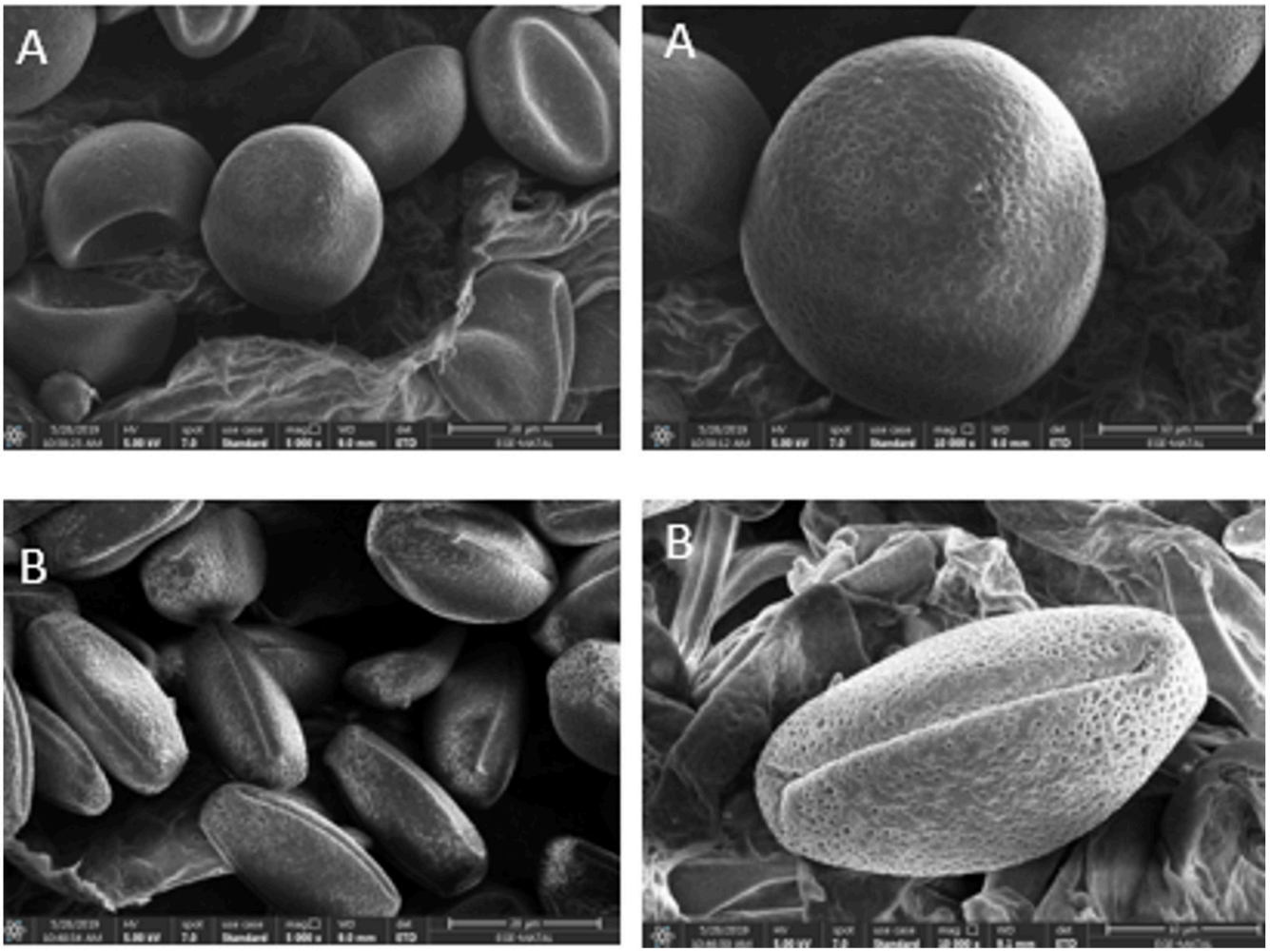

Figure 1. Scanning electron microscope image of pollen
A: Inaperturate pollen in "Foça Karası" and "Mahrabaşı".
B: Tricolporate pollen in "Alphonse Lavallee", "Cinsaut", "Alicante Bouschet", "Italia", "Syrah", "Cardinal", "Yuvarlak Çekirdeksiz" and "Malbec".

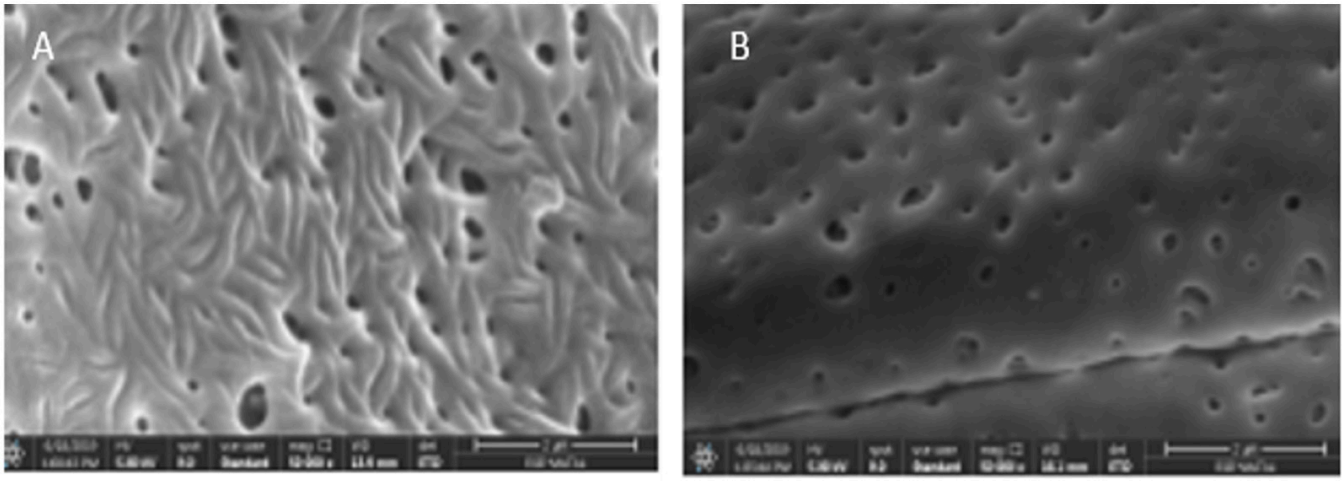

Figure 2. Pollen exine ornamentation image
A: Areolat pollen in "Foça Karası", "Mahrabaşı", "Alphonse Lavalee", "Cinsaut", "Malbec", "Italia" and "Yuvarlak Çekirdeksiz".
B: Without furrows pollen in "Alicante Bouschet", Syrah" and "Cardinal".

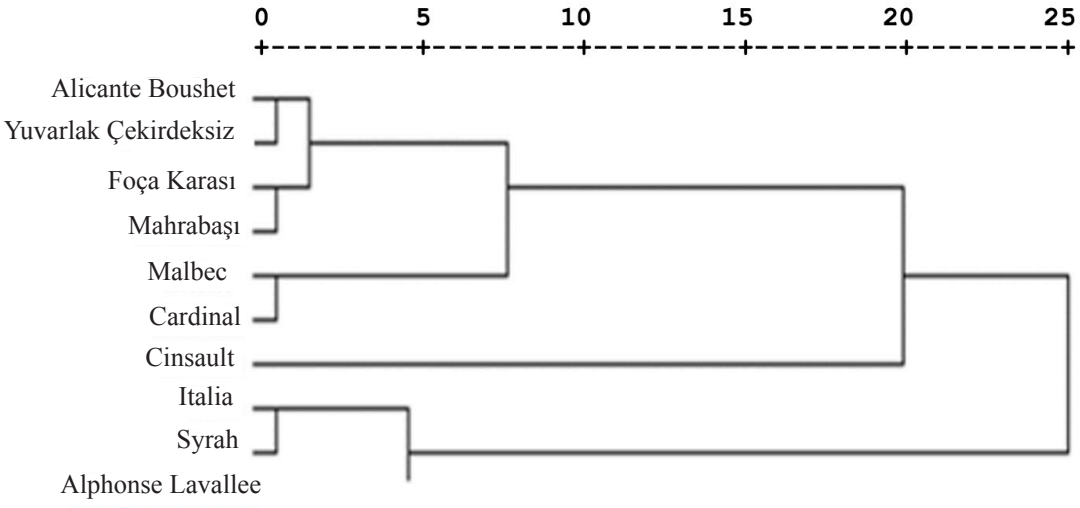

Figure 3. Dendrogram of hierarchical cluster analysis obtained by Ward's clustering method

Pollen width sizes of the "Cardinal" studied has 13.96 µm, and lenght of pollen grains has 29.43 µm. In relation to the results reported by Marasalı et al. (2005) and Gökbayrak and Engin (2016), the "Cardinal" pollens were medium sized, the values obtained in our studies were higher than in both studies. The pollen width and lenght of "Yuvarlak Çekirdeksiz" were 19.09 µm and 26.27 µm, respectively. Roytchev et al. (1994) reported that pollen is same sized (the highest mean values - 24.13 and 24.04 µm) all investigated the seedless grape cultivars.

Vitis is characterized by it's tricolporate pollens. However, there was difference in pollen shape in our study. Prolate-spheroidal pollen was found in the material from "Foça Karası", whereas subprolate pollen was "Mahrabaşı". Perprolate were in "Alicante Bouschet", "Syrah", "Cardinal", "Italia", and prolate were in "Alphonse Lavallee", "Malbec", "Cinsaut", "Yuvarlak Çekirdeksiz". To confirm our findings, pollen shape and P/E ratio change from one sample to another by Marasalı et al. (2005) and Gökbayrak and Engin (2016) for grapes. Roytchev (1997) reported in seedless cultivars, this ratio varies from 1.10 (cv. Seedless Red) to 2.08 (cv. Russalka), being < 2 for most of the cultivars. The eliptical oval shape of pollen grains is typical for most of the seedless grapes.

Reticulated pollen grains in members of *Vitaceae* were described by Erdtman (1952). The exine sculpturing of *Vitis* was reticulate, foveolate-perforate and that lumina size increased towards the poles reported by Faegri and Iversen (1989). Our study showed that in the ten grape cultivars, exine sculpturing was obscurely reticulate under SEM, and scrobiculate and striate at the mesocolpia and distincly reticulate at and around the poles. An increase in lumina size towards the poles, observed by SEM, supports the results for Faegri and Iversen (1989). The results of this study showed that pollen can be used with SEM as a distinctive feature in

Vinifera cultivars.

As a result of this study, we found without furrows some of the examined cultivars (Cabello et al., 1994; Reille, 1966; Roytchev, 1997). These pollens were "Alicante Bouschet", "Syrah", and "Cardinal", cultivars. Areolat is surrounded in exine were determined in cultivars of "Foça Karası", "Mahrabaşı", "Alphonse Lavallee", "Cinsaut", "Malbec", "Italia" and "Yuvarlak Çekirdeksiz" (Figure 2).

The pollen viability was appreciated with TTC test, having verified that without furrows pollen grains presented similar percentages of viability such as, "Cardinal" (84.25 %), "Alicante Bouschet" (75.75 %) and "Syrah" (74.25 %) cultivars. Areolat pollen grain was viable, even though the lowest germination was recorded in "Malbec" (67.00 %), "Italia" (56.75 %), Yuvarlak Çekirdeksiz" (42.25 %), "Foça Karası" (26.50 %),"Alphonse Lavalee" (24.25 %), "Mahrabaşı" (20.25 %) and "Cinsaut" (11.75 %), respectively. However, the *in vitro* germination tests showed different results according to the pollen assayed. High pollen germination with agar in petri dishes was only observed in the "Mahrabaşı" (87.25 %, tricolporated form) (Table 5). Kelen and Demirtaş (2003) analysed in Burdur Dimriti, Sariemin, Tilki Kuyruğu, Razaki, Buzgülü, Siyah Buzgülü, and Siyah Gemre (*Vitis vinifera* L) table grape varieties pollen germination and pollen tube growth were only observed in the tricolporated form, as the acolporated pollen grains did not germinate. These results resemble previous data on *V. vinifera* from other authors that attributed nongermination to the lack of pores (Lombardo et al. 1978; Caporali et al. 2003; Lombardi 2007), on the other hand the low productivity might be owing to the presence of the acolporated pollen or to environmental conditions. Determination of pollen characteristics is extremely important, especially for grape varieties that will be subject to breeding studies. This work is of interest to the grape breeder for the purpose of avoid reduction in the productivity because the entity of grapevines producing acolporated pollen.

Pollen morphological features such as pore structure and ornamentation view are the most valuable variables for separating the grape species. The results of Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering projection for species are quite common. The results from cluster analysis show that the examined members of the ten grape cultivars that fall into two main groups coincide with pollen morphological features. "Alicante Bouschet", "Yuvarlak Çekirdeksiz", "Foça Karası", "Mahrabaşı", "Malbec", "Cardinal", "Cinsault" were included in the first group while "İtalia", "Syrah" and "Alphonse Lavallee", were collected in the second group. First and second groups divided into different sub-groups (Figure 3).

Conclusion

Morphological characteristics of pollens showed significant differences among the ten grape cultivars.

Pollen width ranged from 10.12 µm to 22.44 µm, pollen length ranged from 16.26 µm to 29.91 µm, the length and width of pores are 112.40 µm to 397.45 µm and 97.06 µm to 245.68 µm, respectively.

According to the P/E ratio the ten cultivars are divided in four groups: I - with perprolate (P/E > 2); II - with prolate (P/E 1.34-2.00); III - with subprolate (P/E 1.15-1.33); and IV – prolate-spheroidal (P/E 1.01-1.14). Especially "Mahrabaşı" (subprolate) and "Foça Karası" grape varieties (prolate-spheroidal) were separated from other varieties with their P/E feature.

High pollen germination with the agar in petri dishes was only observed in the "Mahrabaşı" (87.25 %, tricolporated form). To pollen grain viability with the TTC test, the highest germination was recorded in "Cardinal" (84.25 %), "Alicante Bouschet" (75.75 %) and "Syrah" (74.25 %) cultivars were without furrows pollen grains.

Some differences in size, polarity and ornamentation were observed among some of the studied cultivars in some cases among the ten grape cultivar. There were differences in pollen ornamentation in the cultivars studied. In this regard "Alicante Bouschet", "Syrah" and "Cardinal", cultivars were without furrows. On the other hand, areolat pollen determined in some cultivars were, such as "Foça Karası", "Mahrabaşı", "Yuvarlak Çekirdeksiz", "Alphonse Lavallee", "Cinsaut", "Malbec" and "Italia". Palynology of *Vitis vinifera* L., is an adequate and complementary observation for identification.

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

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgments

The authors thank Ege University, Central Research Test and Analysis Laboratory Application and Research Center (EGE MATAL) for help on SEM operation.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.5 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):287-293 (2021)

Recombinant production and characterization of *Aspergillus niger* prolyl endopeptidase enzyme for gluten-free food production

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Abstract

Gluten is a protein group found in wheat, barley, rye, and oats, known as cereals. When this vegetable protein is introduced into the body, celiac disease can occurs. The use of bacterial and fungal oligopeptidase to ensure the cleavage of gluten into non-toxic fragments are considered a promising alternative for celiac disease. In this study, the *Aspergillus niger* Prolyl EndoPeptidase (AN-PEP) enzyme was cloned into pET22b vector and recombinantly produced in *BL21 (DE3) pLysE* cells. PEP enzyme expressed as inclusion body and was recovered by refolding. And N-terminal His-tagged recombinant protein was purified by nickel affinity chromatography. 280 mg AN-PEP enzyme from 1L bacterial culture was purified at very high yield, and this protein was 90% purity. As a result; It has been determined that the recombinantly produced PEP enzyme can digest gluten. This study shows that recombinantly produced AN-PEP (rAN-PEP) has great potential to use in the production processes of gluten-free foods.

Keywords: Gluten, Celiac disease, *Aspergillus niger*

Introduction

Celiac disease, (celiac sprue, gluten-sensitive enteropathy), is a chronic inflammatory disease that causes destruction of the villi in the small intestinal mucosa and is stimulated by the intake of gliadins in wheat, rye sequins and hordeins in barley (Di Sabatino & Corazza, 2009; Edens et al., 2005). Celiac disease has a very high prevalence in children and adults (seen in rates approaching 5-10% of the population) (Elli et al., 2015; Ortiz et al., 2017). The only current treatment of the disease is a "gluten-free diet" (Comino et al., 2016; Moreno et al., 2017; Rodrigues et al., 2018). The celiac patients need to eliminate gluten from their diet completely. Mammalian digestive enzymes can not easily digest gluten due to protease-resistant domains in gluten (Helmerhorst et al., 2010). Therefore, gluten-digesting enzymes are needed in food processing to produce gluten-free foods in the food industry. Another approach is the enzymatic hydrolysis of gliadins with oral enzyme supplements in celiac patients (Ciacci et al., 2015; Wei et al., 2020). Prolyl endopeptidases obtained from *Myxococcus xanthus*, *Sphingomonas capsulata* and *Flavobacterium meningosepticum* microorganisms have shown significant potential in pharmacological use (Shan et al., 2004). Aspergillopepsin obtained from the combination of *Aspergillus niger* and *Aspergillus oryzae* is seen to digest gluten in vitro environmental conditions (Ehren et al., 2009). The combination of prolyl endopeptidase from *Sphingomonas* capsulata and barley cysteine endoprotease EP-B2 can also successfully digest gluten fragments in the stomach in clinical trials (Gass et al., 2007; Tye-Din et al., 2010). KumaMax, a synthetic enzyme, has similar results in clinical trials

Cite this article as:
Senol, B., Kapan, O., Imamoglu, R., Gokce, I. (2021). Recombinant production and characterization of *Aspergillus niger* prolyl endopeptidase enzyme for gluten-free food production. Int. J. Agric. Environ. Food Sci., 5(3), 287-293
Doi: https://doi.org/10.31015/jaefs.2021.3.5
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Received: 10 February 2021 **Accepted:** 04 June 2021 **Published Online:** 09 August 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 287-293
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

(Krishnareddy et al., 2017). Prolyl endopeptidase, also known as AN-PEP, obtained from *Aspergillus niger*, is an enzyme that digests gluten (Edens et al., 2005; Lopez & Edens, 2005; Stepniak et al., 2006).

E. coli, which is frequently used in recombinant protein production, has advantages such as low cost and rapid production of recombinant proteins (Vallejo & Rinas, 2004). In this study, the AN-PEP enzyme was produced by using recombinant DNA technology, which is one of the modern biotechnological methods. The PEP enzyme gene of *Aspergillus niger* organism was determined using bioinformatics tools, and codon optimization was made. The expression of the PEP gene was performed in E. coli BL21 (DE3) plySE strain. Since the produced PEP protein was found in the form of inclusion bodies in the cell, it was provided to be folded again with the refolding protocol. N-terminally His tagged recombinant protein AN-PEP was purified by nickel affinity chromatography. Then, the activity of the purified AN-PEP enzyme was determined.

Materials and Methods

Construction of AN-PEP expression plasmid

The DNA sequence of the PEP enzyme was taken from NCBI (National Center for Biotechnology Information) (Accession number: AX458699.1). The codon optimization was performed for the E. coli K12 strain in the gene sequence from NCBI using bioinformatics tools via using jCat codon optimization program (http://www.jcat.de/). The codons found in the nucleotide sequence of the PEP gene were replaced by codons expressed in E. coli K12 strain with higher frequency. Additionally, N terminal 6 His tag nucleotide sequence has been added to allow easy purification of the produced protein. The pET22b vector was chosen as the cloning vector. The gene sequence regulated by bioinformatics tools was synthesized by Biomatik company.

Expression of AN-PEP enzyme in E. coli BL21(DE3) pLysE cells

The expression of AN-PEP protein was carried out similar to the protocol detailed in our previous study (Kuduğ et al. 2019; Kaplan et al. 2021). E. coli BL21 (DE3) pLysE strain was used in protein expression studies. E. coli BL21 (DE3) pLysE cells containing the pET22b-ANPEP plasmid were transferred to 50 ml LB media and incubated in a shaker incubator at 37ºC at 240 rpm. The cells were induced with IPTG when OD600: 0.7. Then, samples were taken from the cells incubated for 4 hours to be analyzed in SDS PAGE.

The recovering from inclusion body of rAN-PEP Enzyme

Two protocols were used to recover the rAN-PEP enzyme from the inclusion body.

Protocol 1

The cells suspended with Tris-HCl buffer (50 mM Tris, 0.1 mM DTT, pH: 8.0) were lysed by sonicator. The lysed cells were centrifuged at 16,000xg for 15 minutes, and the pellet was washed twice with resuspension buffer (50 mM Tris-HCl (pH: 8.0), 2.5% triton X-100, 20% sucrose) (5ml/g). The pellet was incubated for 5 hours with 50 mM Tris-HCl buffer (pH: 8.0) containing 8 M urea after centrifugation at 16,000xg for 15 min. A cold refolding buffer (100 mM Tris-HCl, 10 mM DTT, 20% glycerol (pH: 8.0)) was added dropwise to the supernatant. This supernatant was stirred at 4°C for 16 hours. After the completion of the mixing process, centrifugation was carried out for 15 minutes at 16,000xg. The supernatant was taken and dialyzed in 20 mM Tris HCl (pH: 8.0) containing 100 mM NaCl. The purification of the PEP enzyme from the supernatant obtained was done with a column containing Ni-NTA agarose (Melissis et al., 2010).

Protocol 2

The cells suspended with 20 mM Tris-HCl, 0.5 M NaCl (pH: 8) were lysed by sonication and centrifuged at 16,000xg for 20 minutes. The pellet was dissolved by adding 0.5 M NaCl, 2% Triton-X, 2 M urea 20 mM Tris-HCl pH: 8 solution on the pellet and this process was repeated twice. The solution was centrifuged at 16,000xg for 20 minutes. The pellet was mixed with 6 M guanidine hydrochloride, 20 mm Tris-HCl, 0.5 M NaCl, 5 mM imidazole, 1 mM 2- mercaptoethanol pH: 8 buffer at room temperature for 30 and centrifuged at 16,000xg for 20 minutes. Refolding and purification processes of the obtained supernatant in the column containing Ni-NTA resin. Refolding was achieved by generating urea gradient (20 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, 1 mM 2-mercaptoethanol, and 6M-0M Urea Buffer) from the column. Urea gradient was provided by passing through the column at a rate of 0.1-1 ml/min, with 5 ml of buffer containing 6M, 5M, 4M, 3M, 2M, 1M, and 0M urea, respectively. Protein was finally eluted from the column with 20 ml of 20 mM Tris-HCl, 0.5 M NaCl, 300 mM imidazole, 1 mM 2-mercaptoethanol pH: 8.0 buffer (Palmer & Wingfield, 2012).

AN-PEP Enzyme Activity Assays

It was determined using the methodology reported by Edens et al.(Edens et al., 2005). The substrate (Z-Gly-Pro-pNA, benzyloxycarbonyl-glycine-proline-p-nitroanilide) was first dissolved in 40% 1.4 dioxane at 60°C to prepare 250 µM solution. PEP activity was determined by spectrophotometric monitoring of the release of pNa from the Z-Gly-Pro-pNA substrate at a wavelength of 410 nm (Edens et al., 2005). To determine the pH value which the enzyme showed optimum activity, Mcllavine buffer, between pH 2.2-8.0, and Tris-HCl buffer at pH 9-10 were used. To determine the effect of temperature on enzyme activity, activity measurements were made at temperatures between 25ºC-70ºC. Based on the highest absorbance value, % relative enzyme activity was determined. To determine the thermal stability of the enzyme, the enzyme was incubated for 30, 60, and 90 minutes at 30ºC, 40ºC, 50ºC, 60°C temperatures. Then, the stability of the enzyme was determined by determining the activity at optimum pH and temperature conditions. To determine the effect of substrate concentration on enzyme activity, enzyme activity at optimum conditions was defined against the amount of substrate that varies. Measurement results were interpreted using the Michaelis-Menten kinetic model and Lineweaver-Burk models. For the enzyme-substrate specificity, the kinetic models used were evaluated by calculating Km and Vmax values.

The experimental steps of this study are illustrated as a summary in Figure 1.

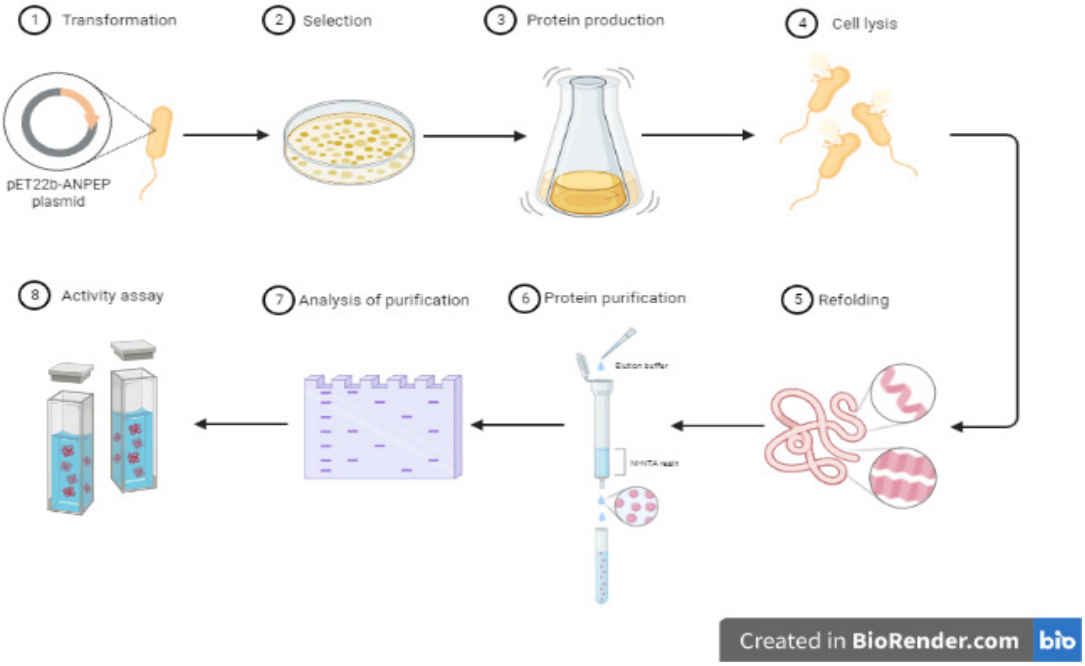

Figure 1. Experimental steps for recombinant production of AN-PEP protein (Created in BioRender.com)

Results and Discussion

Expression of AN-PEP enzyme in *E. coli BL21(DE3) pLysE*

E. coli BL21 (DE3) pLysE cells were transformed with the pET22b-ANPEP, plasmid mapped in Figure 2.

E. coli cells incubated in a shaker incubator at 37°C at 240 rpm were induced with IPTG when OD_{600}: 0.7. It was analyzed whether AN-PEP protein was expressed by taking samples from the culture before and after induction with IPTG. As expected, a protein band of around 58 kDa was observed after IPTG induction (Figure 3).

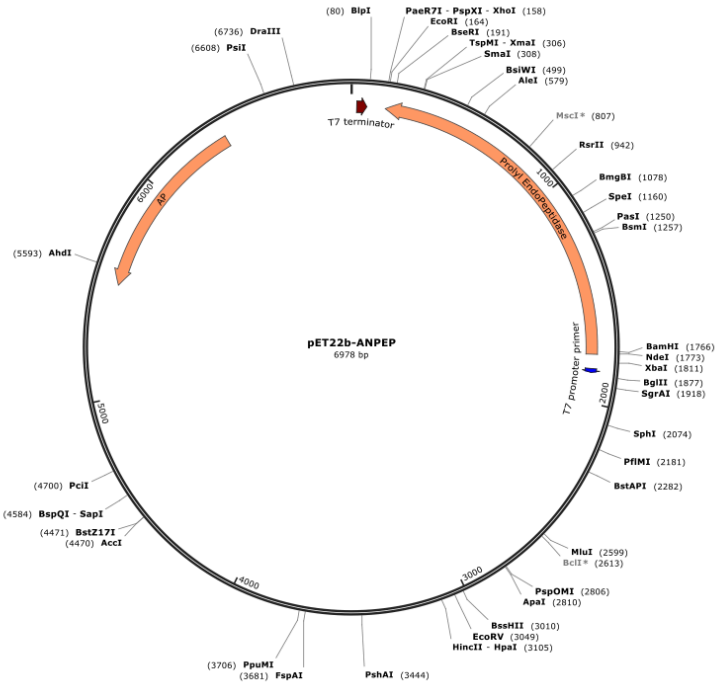

Figure 2. Plasmid map of the pET22b-ANPEP construct

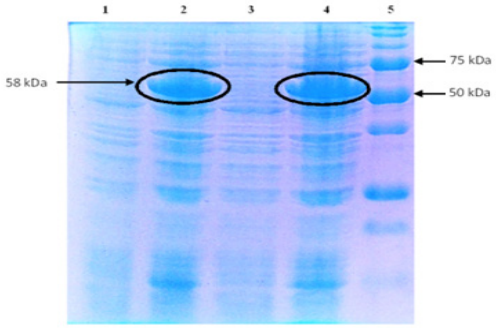

Figure 3. Analysis of recombinant expression of AN-PEP protein in 12% SDS-PAGE 1, 3. *E. coli BL21 (DE3) pLysE* cells containing the pET22b-ANPEP plasmid before induction with IPTG 2, 4. *E. coli BL21 (DE3) pLysE* cells containing the pET22b-ANPEP plasmid, after induction with IPTG. 5. Protein marker (SeeBlue® Plus2 Pre-stained Protein Standard)

Recovering from the inclusion body of rAN-PEP Enzyme

The rAN-PEP enzyme, which was highly expressed in *E. coli*, was in the form of the inclusion body. Two protocols was applied for the recovery of protein from the inclusion body. Although *protocol 1* took a relatively long time, the rAN-PEP enzyme could be obtained quite purely and efficiently (Figure 4).

A relatively shorter protocol was used to recover the rAN-PEP enzyme from the inclusion body, in which the refolding procedure was performed on the column. Compared with *protocol 1*, relatively less protein was obtained in *protocol 2*. However, the rAN-PEP enzyme was very pure (Figure 5)

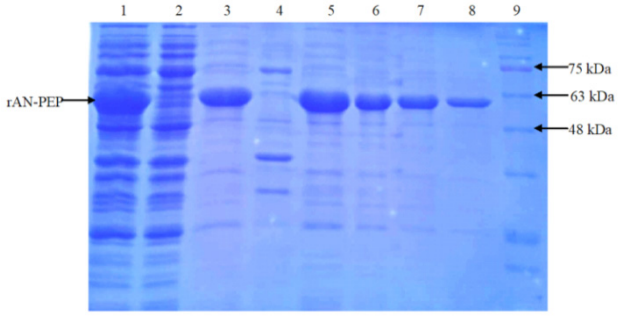

Figure 4. Analysis of rAN-PEP enzyme the recovery from inclusion body in 12% SDS-PAGE (*Protocol 1*) 1. *E. coli* cells lysed with a sonicator. 2, 3. Supernatant and pellet obtained after centrifugation at 16.000xg for 15 minutes, respectively. 4, 5. Supernatant and pellet obtained after two washes with resuspension buffer, respectively. 6. Supernatant after incubation with 8 M urea for 5 hours 7. The supernatant obtained after the dialysis procedure. 8. The obtained filtrate by passing the elution buffer containing 300 mM imidazole from the column. 9. Protein marker (NZYColour Protein Marker II)

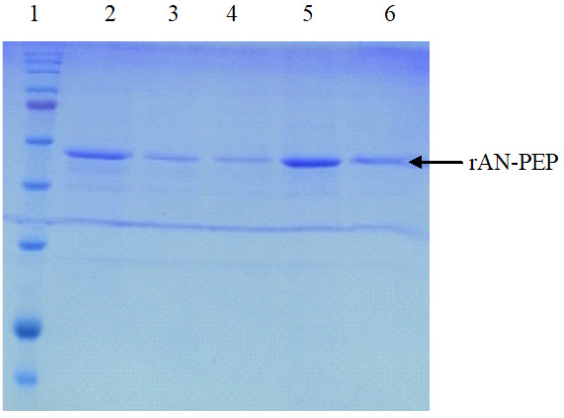

Figure 5. Analysis of rAN-PEP enzyme recovery from the inclusion body in 12% SDS-PAGE (*Protocol 2*) 1. Protein marker (Page Ruler Plus Prestained Protein Ladder) 2-6, the obtained filtrate by passing the elution buffer containing 300 mM imidazole from the column.

AN-PEP activity assays

Activity tests of the protein recovered from the inclusion body were carried out using the Z-Gly-Pro-pNA substrate following the protocol of Edens et al. (Edens et al., 2005). The optimum pH of the enzyme was determined as 6. The enzyme was found to have a wide range of activities, such as pH: 2.2-8. The optimum working temperature of the enzyme was found to be 30°C, but it was also observed to show activity in the range of 25-40°C. In thermal stability experiments performed on rAN-PEP enzyme, it was observed that it preserves its activity by 30% after 30 minutes incubation at 60°C (Figure 6).

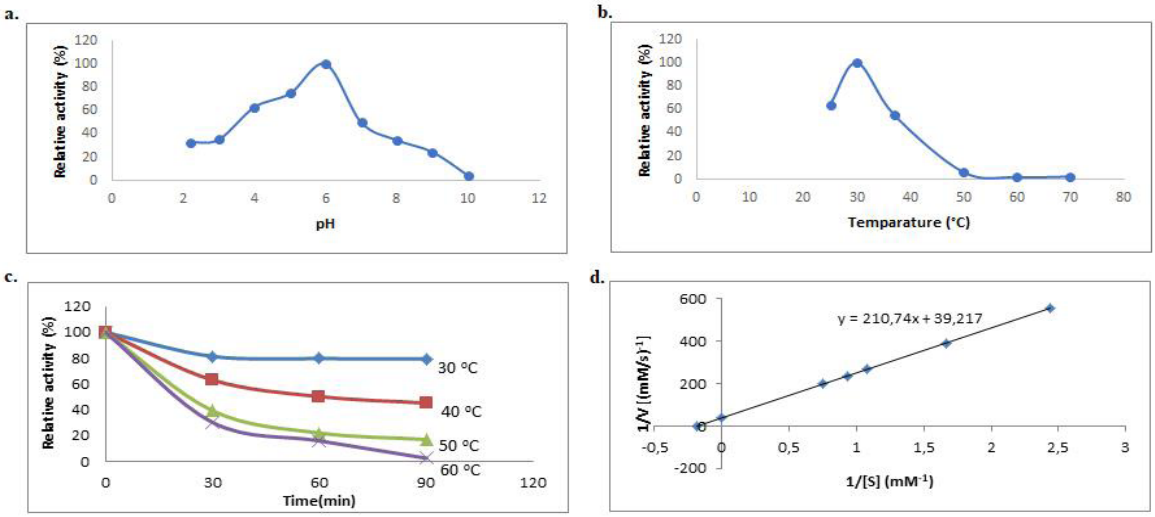

Figure 6. Characterization of the rAN-PEP (a) Relative activity of the rAN-PEP enzyme in the pH: 2.2-10 range (b) Relative activity of the rAN-PEP enzyme in the 25ºC-70ºC temperature range (c) Thermal stability of the rAN-PEP enzyme (Activity was determined at optimum temperature and pH) (d) Lineweaver-Burk model of the rAN-PEP enzyme (Activity was determined at optimum temperature and pH value.)

Conclusion

Prolyl endopeptidase is proline-specific endoprotease, also known as EC 3. 4. 21.26 and belongs to the serine protease family, the peptides can break down the inner proline residues (Shan et al., 2004). Today, many studies have been conducted to reduce or modify gluten to eliminate celiac immunoreactivity. Some researchers (Gessendorfer, 2011; Loponen et al., 2009; Schwalb, 2012) have worked with enzymes that degrade gluten in plants such as Triticum sp., Carica papaya, Hordeum vulgare, and Secale cereale. Gluten-degrading enzymes from bacteria such as *Myxococcus Xanthus*, *Bacillus* sp., *Sphingomonas capsulata*, *Rothia mucilaginosa*, *Bifidobacterium* sp., *Flavobacterium meningosepticum*, *Pseudomonas aeruginosa* and *Lactobacillus* sp. has been discovered, and its effectiveness has been studied in digesting gluten (Shan et al., 2004; Siegel et al., 2006; Wei et al., 2015; Zamakhchari et al., 2011). Gluten-degrading enzymes of fungi such as *Aspergillus niger* and Aspergillus flavus var oryzae have also been frequently studied gluten (Edens et al., 2005; Lopez & Edens, 2005; Stepniak et al., 2006). The AN-PEP enzyme does not completely digest into amino acids but instead breaks down the peptide bond in the proline amino acid on the carboxyl side of the peptide (Edens et al., 2005). The optimum pH value of this enzyme with activity between pH 2.5 and 7.5 was determined as pH:4.2. This enzyme digests gluten and gliadin with high efficiency (Lopez & Edens, 2005; Stepniak et al., 2006). Studies have shown that AN-PEP is capable of degrading gluten in all starches with a gluten content of up to 20mg/kg. The AN-PEP is obtained by fermentation of Aspergillus niger (Jiang et al., 2021;Walter, 2015).

In this study, AN-PEP enzyme, which has been obtained with Aspergillus niger fermentation so far, was produced recombinantly in E. coli. The production of proteins in E. coli provides a low cost, fast, and highly efficient production opportunity (Vallejo & Rinas, 2004). The AN-PEP enzyme was produced in E. coli at a high level and in the form of an inclusion body. When the proteins are produced in inclusion body form, it allows the protein to be obtained very pure and without degradation by proteases (Babaeipour et al., 2010). The rAN-PEP enzyme was recovered from inclusion bodies in a very pure and active form. We applied two different procedures for recovery from inclusion bodies and compared their efficiency. A very high amount of protein, such as 280 mg, was obtained from a 1 L bacterial culture. The optimum pH value of the rAN-PEP enzyme was 6, and the optimum working temperature was 30°C. This enzyme shows activity in a wide pH range such as pH: 2.2-8. Besides, even after incubation of this enzyme at 60°C for 30 minutes, it shows around 30% activity at pH: 6.

There are differences between the AN-PEP enzyme obtained by Aspergillus niger fermentation and the rAN-PEP enzyme produced in this study. The optimum pH value of the natural AN-PEP enzyme is 4.2, and the optimum temperature is 50°C (Edens et al., 2005). This difference may be due to

the lack of glycosylation in E. coli. Because, various post-translational modifications such as glycosylation occur in the natural AN-PEP enzyme. Sebela et al. reported that the AN-PEP enzyme contains high-mannose type N-glycans and is partially phosphorylated. It was also stated by Sebela et al. that AN-PEP enzyme with N-glycolysis was 63kDa, and its non-glycolysis form appeared to be around 58 kDa in SDS-PAGE (Sebela et al., 2009). Indeed, our results confirm this situation. Although glycolysis did not occur on the rAN-PEP enzyme, it showed high activity (Km: 5.37 Mm, Vmax: 0.025 mM/s). However, there is a shift in optimum pH and temperature values. Glycolysis and phosphorylation appear to be highly effective in AN-PEP enzyme activity. With this study, we presented a new methodology on the recombinant production and purification of the AN-PEP enzyme in E. coli. As a result, the rAN-PEP enzyme we produce in this study can be used in gluten-free food production.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

Funding

This work was supported by the Tokat Gaziosmanpaşa University Research Fund under Grant number:2018/32.

Data availability

Not applicable.

Consent for publication

Not applicable.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.6 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):294-301 (2021)

Optimization of Small Castor Seed (Ricinus Communis) Oil Extraction Yield Using Response Surface Methodology

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Abstract

The process conditions that affect the percentage yield of oil from small castor seed was optimized for maximum extraction. In this study, surface response methodology (RSM) that employed a two-factor, five-level factorial central composite design (CCD) was used. Thirteen (13) experiments with different combinations of reaction time (x_1) and reaction temperature (x_2) ranging from 1hr (60mins) to 10hrs (600mins) and 60oC to 100oC respectively were also performed. A quadratic model that is polynomial in nature was obtained to predict the percentage oil yield for the small castor seed. Within the experimental variables range, the optimal conditions were found to be 8.68hrs (520.92mins) and 94.14oC respectively. These values were fitted into the quadratic polynomial model that gave rise to the optimum value of small castor seed oil yield to be 55.76% with a p-value less than 0.05. The coefficient of determination R2 was obtained as 0.9530 representing 95.30% of variation in the original data. Our result also gave an adjusted R2 value of 91.95% and predicted R2 value of 66.59% which indicate that the model explains 67% variation in predicting original observations. The result of this study showed clearly the percentage yield of oil that could be extracted from small seeded varieties of castor seed and the optimum yield value possible at a certain reaction time and temperature. The result also established the reliability of response surface methodology to model and optimize the expression of oil from small seeded varieties of castor seed.

Keywords: Response surface methodology, Central composite design, Castor seed oil, Optimization, Oil yield

Introduction

Some concern has been expressed over the measure of oil that could be gotten from various varieties of castor seed. This is as a result of the various varieties and sizes of castor seed we have available. This research gap has necessitated this study to know the percentage oil yield from small seeded varieties of castor seed and as well optimize the yield to achieve the maximum oil extraction at a particular reaction temperature and time. Oil extraction yield is referred to as the amount of oil that can be derived from an oil seed. The yield is usually being represented as a percentage (%). In arid and semi-arid regions where little or no maintenance is applied, Castor bean (Ricinus Communis) usually produces up to 350-650 kg of oil per hectare. The oil from the seed is the principal product of the castor bean plant. This oil can be extracted either by the use of cold-pressing (mechanical) or by using soxhlet extraction method with solvent. The content of the oil ranges from 35% to 55% of the weight of the beans depending on the variety (Kulkarni and Sawant, 2003; Oluwole *et al.,* 2014). It is instructive to note that castor seed oil has many industrial uses. Some of these uses are found in internal combustion engines, in processing and manufacturing of products like rubber, inks, flypaper, linoleums, artificial leather, varnish and medical preparations as well as being a plasticizer in plastic production.

Cite this article as:
Omotehinse, S.A., Ovuworie, G. (2021). Optimization of Small Castor Seed (Ricinus Communis) Oil Extraction Yield Using Response Surface Methodology. Int. J. Agric. Environ. Food Sci., 5(3), 294-301
Doi: https://doi.org/10.31015/jaefs.2021.3.6
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Received: 24 July 2020 **Accepted:** 06 July 2021 **Published Online:** 09 August 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 294-301
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

It can also be used as a sulfonated oil to produce Turkey red oil which is a compound that is widely engaged in the dyeing and cloth printing industry. The latest advances in castor seed oil dehydration makes it a substitute to sunflower oil or tung oil as a drying oil. Apart from the various uses of castor seed oil, recent investigations have shown that the pulps can also be used as fertilizers (Lima et al., 2011; Mello et al., 2018).

Research interest in the percentage oil extraction yield from castor seed and its application has built up over the years. Notable among them are Abdulrasheed *et al.* (2015) who characterized and investigated the use of castor seed oil extract in soap manufacture. Yusuf *et al.* (2015) extracted oil from castor seed in Katsina, Nigeria and went ahead to checked the physicochemical properties of the oil extracted to assess its industrial potentials. The result showed oil extraction yield to be 39.43% while the physicochemical characterization of the purified oil revealed low acid value of 2.07, low iodine value of 84.18, low peroxide value of 38.00, but relatively high specific gravity of 0.959, hydroxyl of 163.64 and saponification of 175.31 values. These values compare favourably well with the general standards and specification of the American standard for testing and materials (ASTM) for grade castor oil (WHC, 2012) suggesting that the oil has good industrial potential. Nangbes *et al.* (2013) Extracted and Characterized Castor (Ricinus Communis) Seed Oil native to Kapil-Lankan area of Pankshin Local Government Area of Plateau State, Nigeria. The outcome of this analysis revealed percentage oil extraction yield of 48.32. The result that show case the commercial value of the seed in Nigeria. The result also confirms the castor seed oil to be a good quality oil that can be useful as food additives in food industry as well as in industrial application for the manufacturing of products like soaps, paint, varnishes, cosmetics etc. Shridhar *et al.* (2010) optimized the dilution level as well as the agitation time for castor seed oil yield using response surface methodology technique. The author also investigated the percentage recovery of castor seed oil in relation to dilution level (x_1) and agitation time (x_2). The maximum extraction was found to be 48.75% while the optimal value for dilution and agitation time were established as 7.3 and 2.38 hr respectively. Other outstanding research in this area includes: Akpan *et al.,* (2006), Kyari (2008), Montoya *et al.* (2011), Mosquera-Artamonov *et al.* (2016). The aim of this study is geared towards optimizing small castor seed oil extraction yield using response surface methodology. To achieve this, central composite design (CCD) in response surface methodology (RSM) that is seen as a proficient statistical technique used for optimizing multiple variables was adopted to maximize the best reaction conditions with least possible number of experiments.

Materials and Methods

Materials and equipment

The castor seeds used in this research include the small castor oil bean seeds- Ricinus Communis obtained from a farmland in Ikokogbe, Omhen in Ewossa Community, Igueben Local Government Area of Edo State, Nigeria (9.0820° N, 8.6753° E). The seeds were sown in plots having a size of 3 x 4m, 1metre between plants and 1metre between rows. The experiment was laid out in a completely randomized design replicated two times. Germination was observed at the emergence of the cotyledons above the soil surface and monitored until the seed matured. The harvested ripe and dried castor fruits were carefully cleaned and dried in the sun for about 5 days at ambient conditions. This was done until the capsules of the fruit split open to release encased seeds. Seed pod removal as well as tray-winnowing followed to separate the shells from the beans. The castor beans were then oven dried to a constant weight of 100g per sample at 80^0C for 9hrs with a moisture content of 0.32%. Prior to extraction, mortar and pestle was used to grind the dried beans to a paste. All the chemicals and reagents used was analytical grade obtained from Sigma Aldrich without further purification. Reagents were prepared using distilled water while the laboratory apparatuses washed with detergent, cleaned with distilled water and oven-dry before use.

Method

Extraction

Soxhlet extraction method was used. A Soxhlet extractor is a piece of laboratory apparatus designed in 1879 by Franz von Soxhlet. The typical soxhlet extraction set up for the small castor seed oil is shown in Figure 1. Soxhlet extractor with three main sections was used. The first is a percolator that circulate the solvents. The second is a thimble made of thick filter paper that help retain the solid to be extracted. The third is a siphon mechanism which empties the thimble. The compound to be extracted is positioned inside the thimble while the thimble is placed in the main chamber. The solvent being used for the extraction is placed in the distillation flask with the flask positioned on the heating element. The soxhlet extractor is however put on the flask and the reflux condenser placed on the extractor. Extraction solvent is taken in the round bottom flask and heated by using heating source like heating mantle. The heating temperature is built on the solvent employed to extraction. Due to heat the solvent in the bottom flask vaporizes into the condenser and then drip back to the sample thimble. When liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again which is the end of the process indicated by the clear solution in the siphon tube.

Thirteen (13) experimental runs were carried out to dig out maximum oil yield in small castor seed where optimum process conditions were sustained. The controllable factors considered are reaction time as well as reaction temperature. The reaction time varies from 1hr (60mins) to 10hrs (600mins), and the reaction temperature ranges from 60^OC to 100^OC.

The percentage yield of the oil was determined by using the expression described in equation 1.

$$\%\,\text{Oil yield} = \frac{y_1 - y_2}{y_1} \times 100 \qquad (1)$$

where, y_1 and y_2 represent the weights of small castor beans prior to and after extraction.

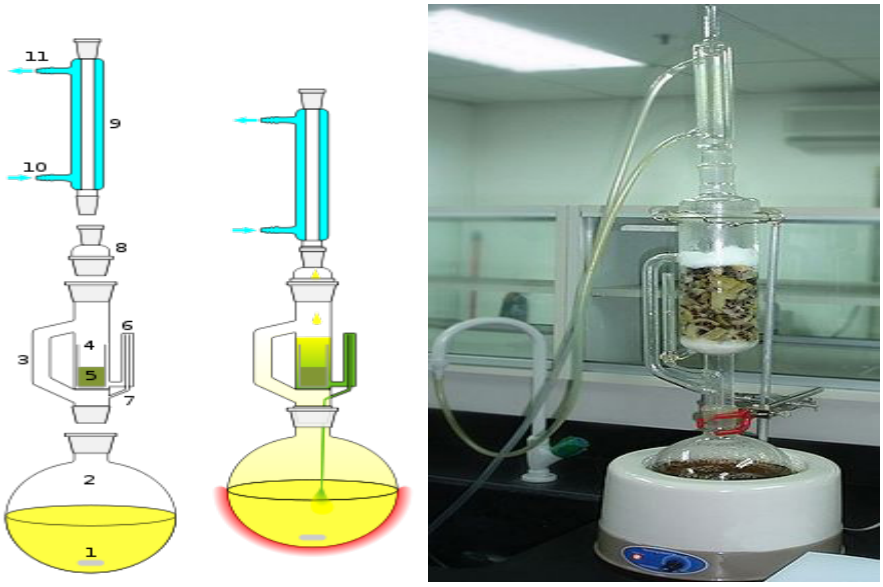

Figure 1. A schematic representation of a Soxhlet extractor
1- The Stirrer bar, 2 - The Still pot, 3 - The Distillation path, 4 - The Thimble, 5 -The Solid to be extracted, 6 - The Siphon top, 7 - The Siphon exit, 8 - The Expansion adapter, 9 - The Condenser, 10 - The Cooling channel.

Experimental Design and Data Analysis

Two-factor, five-level factorial of Central Composite Design (CCD) in surface response methodology (RSM) was employed with replicates at the centre point and star points. Reaction time (x_1) and reaction temperature (x_2) are the variables used in this study with each at low (-1) and high (+1) coded levels. The initial levels selected as the center points were based on the actual levels of the variables. Thirteen (13) experimental trials which consist of 4 trials for axial points – two for each variable, 4 trials for factorial design as well as 5 trials for replication of the central points were executed. The oil yield responses Y (%) adopted in this study refers to the average of triplicate representing yield for the small castor seed. Table 1 illustrate the CCD experimental conditions for the extraction process.

Table 1. CCD Experimental Conditions for Small Castor Seed oil Extraction Process

Independent variable	Unit	Symbol	Levels		
			-1	0	+1
Reaction time	Mins	x_1	60	330	600
Reaction temperature		x_2	60	80	100

The analysis of the above experimental data was carried out based on the response surface regression system to accommodate the second-order polynomial equation. The level of significance of the coefficients was less than 0.05. Statistical software package design-expert® (version 8.0.6; stat-ease, Inc., Minneapolis, USA) was used to determine the regression coefficient which help to predict the process response as a function of the independent variables as well as their interaction that aid the understanding of the system behavior. The relationship that exist between the response and the process variables was calculated mathematically using the quadratic polynomial expression in equation 2.

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{j>1}^{n} \beta_{ij} x_i x_j \quad (2)$$

Y is the percentage oil yield (response), X_i and X_j are the coded independent variables. β_0 - constant, β_i - linear term coefficient, β_{ii} - the quadratic term coefficient, β_{ij} - interaction (cross-term) coefficient while n denote the number of process variables studied and optimized. Analysis of variance was used to estimate the effects of process variables including their interaction effects on the maximum yield of oil in the response surface regression procedure. Regression coefficient R^2 was also used to estimate the best of fit and goodness of the model. The fitted quadratic polynomial equation generated from regression analysis helped to obtain the response surface and contour plots by holding one of the independent variables at central value (0) and changing the other two.

Results and Discussion

The percentage (%) yield of oil for the small seeded varieties of castor seed was determined and the results are presented in Table 2. The in depth analysis involving the interaction of reaction time and reaction temperature was carried out on the percentage (%) oil yield. The Design-Expert (Stat-Ease, Inc., Minneapolis USA) software was employed for regression analysis and graphical analysis of the data obtained. The

optimum values of the reaction time and reaction temperature were reached by solving the regression equation. This was also achieved by analyzing the response surface and the contour plots. Table 2 shows the coded and actual design matrix as well as the results for the combination of the variables for extraction process.

Figure 2 represent the correlation that stuck between the predicted % oil yield and the actual (experimental) % oil yield plot for small castor seed oil. The 45° straight line shows a perfect fit.

Analysis of Variance Table result for percentage oil yield of small castor seed is depicted in Table 3.

Table 2. Coded and Actual values and Experimental responses for CCD experimental combination of variables for small castor seed oil extraction process.

Run	Coded values		Actual values		$Yield_{Small\ seed}$ (%)	
	X_1	X_2	Reaction time (mins)	Reaction temperature (C^0)	Observed values	Predicted values
1	-1.000	1.000	139.08	94.14	44.14	44.29
2	-1.414	0.000	60.00	80.00	41.36	42.43
3	0.000	0.000	330.00	80.00	50.80	50.82
4	1.000	-1.000	520.92	65.86	53.90	52.98
5	0.000	1.414	330.00	100.00	52.60	51.54
6	0.000	0.000	330.00	80.00	50.83	50.82
7	1.000	1.000	520.92	94.14	53.90	55.02
8	1.414	0.000	600.00	80.00	53.90	53.60
9	-1.000	-1.000	139.08	65.86	49.81	47.92
10	0.000	-1.414	330.00	60.00	50.83	52.66
11	0.000	0.000	330.00	80.00	50.83	50.82
12	0.000	0.000	330.00	80.00	50.83	50.82
13	0.000	0.000	330.00	80.00	50.83	50.82

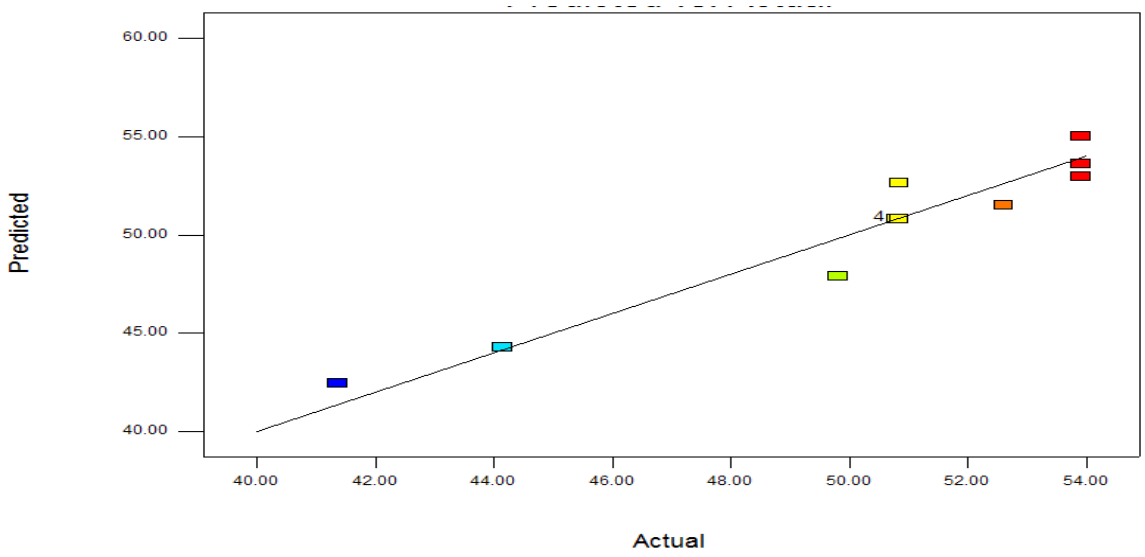

Figure 2. Predicted % oil yield vs the actual (experimental) % oil yield for small castor seed

 DOI: 10.31015/jaefs.2021.3.6

Table 3. Analysis of Variance Result for Small Castor Seed Percentage oil yield

Source	Sum of Squares	df	Mean Square	F value a = 0.0	p-value Prob > F
Model	152.490	5	30.50	18.72	0.0006 significant
A–Reaction time	124.70	1	124.70	76.52	<0.0001
B-Reaction temperature	1.25	1	1.25	0.77	0.4095
AB	8.04	1	8.04	4.93	0.0618
A^2	13.75	1	13.75	8.44	0.0228
B^2	2.82	1	2.82	1.73	0.2298
Residual	11.41	7	1.63		
Lack of fit	11.41	3	3.80	21123.01	<0.0001 significant
Pure Error	7.200E-004	4	1.800E-004		
Cor total	163.90	12			

R-sq. = 93.50 % R-sq. (adjusted) = 88.07% R-sq. (predicted) = 50.51%

Table 3 show that the model F-value of 18.72 is small as well as the corresponding P-value of 0.0006. The implication is that the model employed is highly significant. It also implies that the chances of having "Model F-Value" this large could be 0.06%. The values of "Prob > F" less than 0.500 show that the model terms are significant which infers that A, A^2 are significant model terms. If the values are greater than 0.1000, it means that the model terms are not significant. It was observed from the Table also that effect of reaction temperature is insignificant on the response variable (yield) studied. The response variable had a quadratic response, where both reaction time and reaction temperature (AB) exhibited an effect, with a quadratic relationship. Adequacy precision was used to measure the signal to noise ratio involving the average prediction error and the design points. The adequacy precision of 14.524 was obtained in this study. This result show adequate signal because a ratio that is greater than 4 is required for the model to be adequate. And since the ratio obtained in this study is greater than 4, the model developed could be used to guide the design space. The ''Lack of fit F-value" of 21123.01 shown in Table 3 denotes that the ''Lack of Fit" is significant. We have only 0.01% chance in this case for the Lack of Fit F-value to occur due to noise. Second order polynomial regression model was obtained from the experimental data to predict optimum oil yield from the small castor seed using design expert software. The empirical model in-terms of coded variables for percentage oil yield (Y, %) for small castor seed is expressed in Equation 3.

$$Y_{Small\ seed} = 50.82 + 3.95x_1 - 0.40x_2 + 1.42x_1x_2 - 1.41x_1^2 + 0.64x_2^2 \quad (3)$$

where, Y is the yield (response variable)

x_1 and x_2 are the reaction time and reaction temperature respectively.

The negative sign indicates the antagonistic relationship whereas the positive sign point to the synergistic relationship. The terms with positive coefficient have positive effect on the yield (i.e. increase in these terms leads to corresponding increase in small castor seed oil yield). Reaction time (), interaction term () and the quadratic term ^{2}) has significant influence on the yield of small castor seed oil. On the other hand, terms with negative coefficient (and ^{2}) do not significantly influence the yield of small castor seed oil.

The coefficient of determination R^2 for small castor seed oil extraction was obtained to be 0.9350. The result point to the model been effective in describing 93.50% of variation in the original data. The value of 0.8807 was obtained for the respective adjusted R^2. The R^2_{pre} value gotten through cross-validation advocated that the model is capable of explaining about 51% variation in predicting novel observations. Figure 3 (a-d) shows residuals based on the empirical model developed for the small castor seed.

To fully comprehend the relationship between the variables studied, the response surface curves was plotted as it also helped us to evaluate the optimum level of both the reaction time and the reaction temperature for maximum response. The contour plots for small seed oil extraction is shown in Figure 4 while the response surface plot displaying the interactive effect of reaction time and reaction temperature is shown in Figure 5.

The achieved maximum desirability of 0.935 obtained point out that it is possible to reach maximum castor oil yield target. The maximum small castor seed oil yield of 55.02% was attained at the reaction time of 520.92 mins (8.68 hrs) and reaction temperature of 94.14 respectively.

Conclusion

This study has been able to revealed the percentage yield of oil that could be extracted from small seeded varieties of castor seed as well as the optimum yield value possible at a certain reaction time and reaction temperature. The reliability of central composite design in response surface methodology was also demonstrated in determining the process conditions such as reaction time and reaction temperature leading to optimum oil extraction yield of small castor seed. This study discovered the range of percentage oil extraction yield in small seeded varieties of castor seed to be 41.36 - 53.90. The

outcome of the study compared favourably well with 48% oil extraction yield obtained by Abitogun *et al.* (2009), 48.75% discovered by Shridhar *et al.* (2010), 50.16% reported by Muzenda *et al.* (2012) as well as 48.32% obtained by Nangbes *et al.* (2013). Others include Akande *et al*. (2012) and Yusuf *et al.* (2015). The optimal conditions to achieve the maximum oil extraction yield of 55.02% in small seeded varieties of castor seed was reached at a reaction time of 520.92 mins (8.68 hrs) and reaction temperature of 94.14. The result also suggest that the response surface methodology adopted could effectively be utilized in any reaction process that involves so many experimental factors to study their various effects, the optimum values and their interaction.

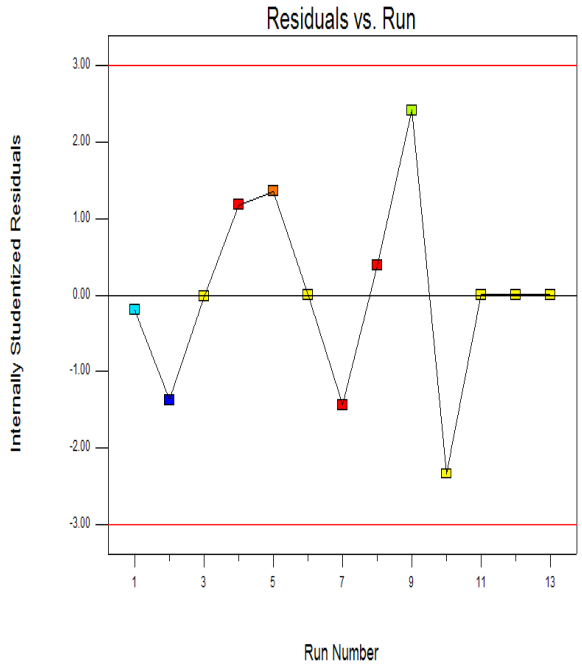

Figure 3(a). Residuals based on the run order for small castor seed oil yield

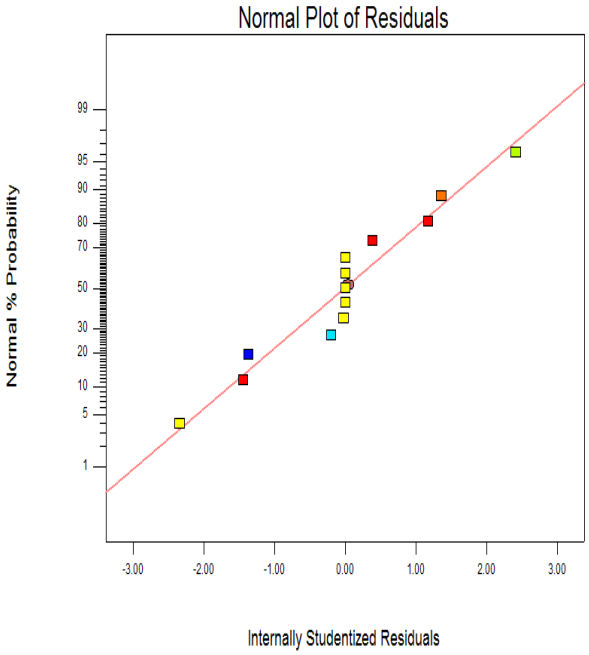

Figure 3(b). Normal probability plot of raw residuals for small castor seed oil yield

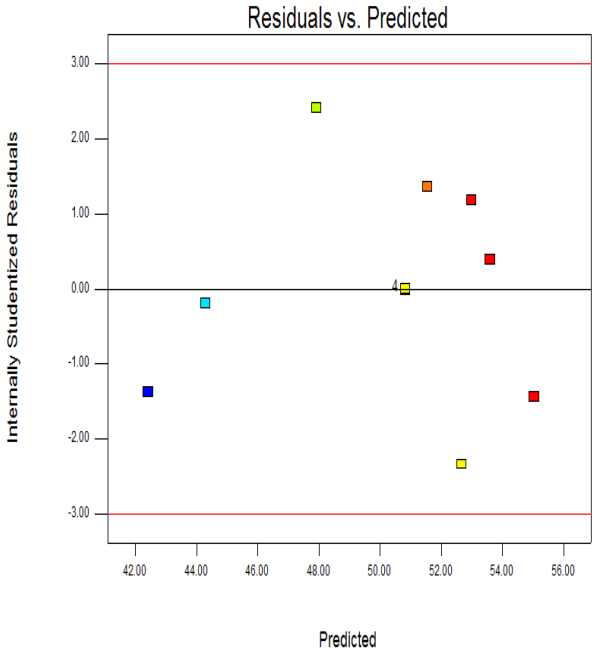

Figure 3(c). Residuals based on predicted order for small castor seed oil yield

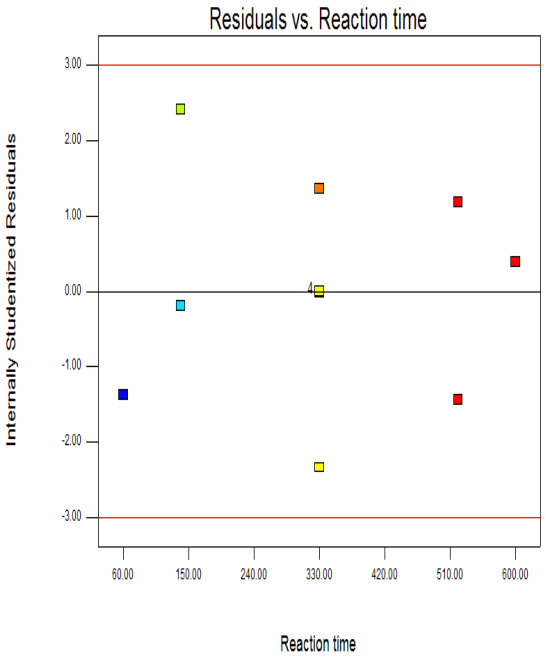

Figure 3(d). Residuals based on experimental order for small castor seed oil yield

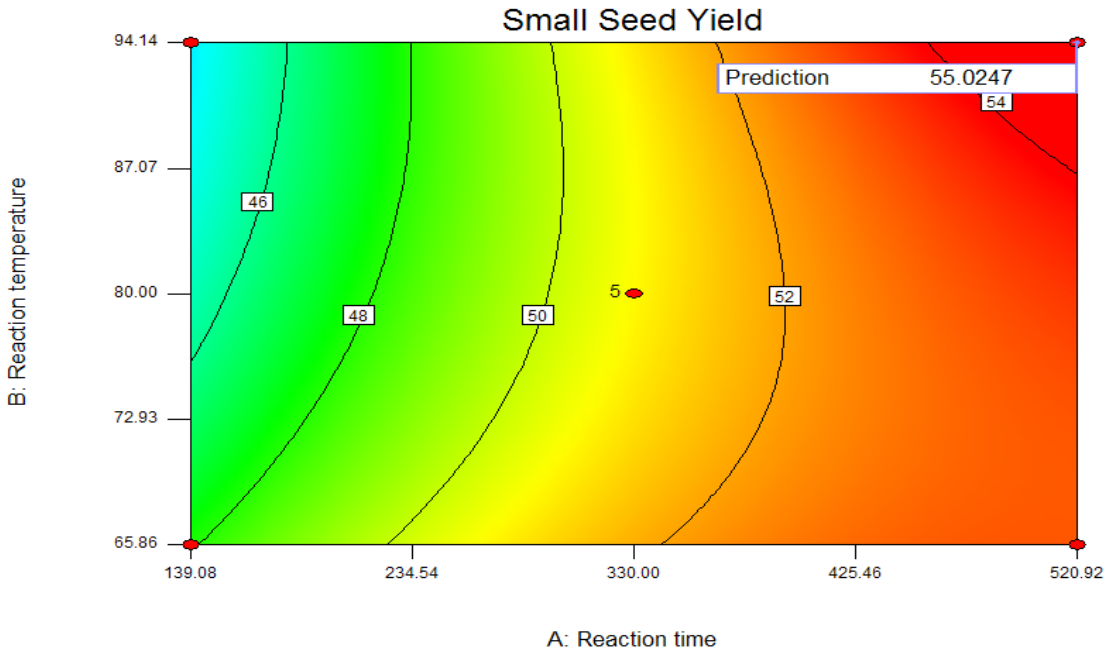

Figure 4. Isoresponse contour plots displaying the influence of reaction time and reaction temperature and their interaction effect on the % yield of small castor seed oil.

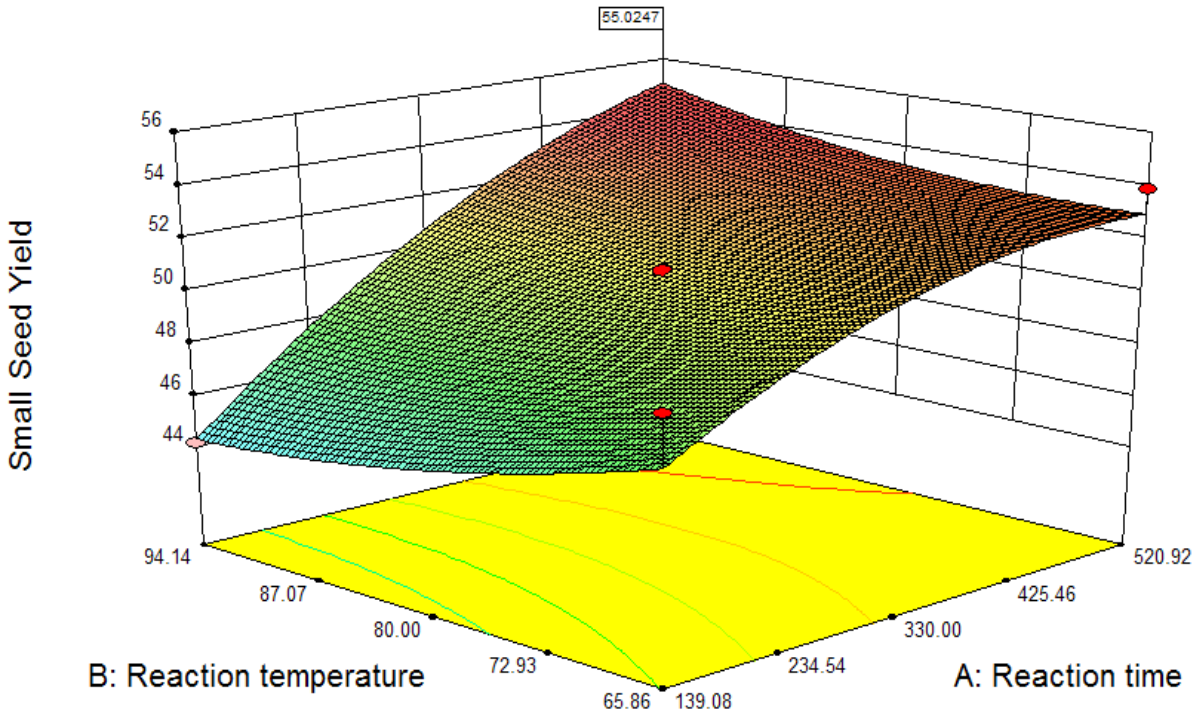

Figure 5. Response surface plots displaying the influence of reaction time and reaction temperature and their interaction effect on the % yield of small castor seed oil.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2021.3.7

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 5 (3):302-309 (2021)

Use of chia seed on regular and low-fat crackers, their antioxidant properties, and *in-vitro* bioaccessibility

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Abstract

Although having functional properties, fat is known to be adversely effective in case of high consumption. High fat consumption causes health disorders such as obesity, cardiovascular diseases and high blood pressure, insulin balance disorders and cancer. For this reason, it is important to reduce fat consumption and create food formulations rich in bioactive components. In the scope of this study, *CS* (Chia seed) was replaced with wheat flour 10%, 20%, and 30% (w/w) and the fat amount was decreased in 25%, 50%, 75% (w/w) ratios for formulating low-fat crackers, and the antioxidative potential of the samples was evaluated. Extractable, hydrolysable, bioaccessible phenolic fractions of samples were analyzed in terms of $TEAC_{ABTS}$, $TEAC_{CUPRAC}$, $TEAC_{DPPH}$ and Total Phenolic Content (TPC) (Folin Ciocalteu's method). *CS* replacement was determined to be more effective than a fat reduction on AC and TPC results of samples. By 25, 50 and 100% fat reduction of extractable, hydrolysable and bioaccessible phenolic fractions, $TEAC_{ABTS}$ values increased respectively as 5.87%, 9.33% and 12.11%. 75% fat reduced-30% *CS* supplemented sample was 91.0% higher than 100% fat including-30% *CS* supplemented sample and 143.4% higher than the control sample in terms of $TEAC_{ABTS}$ for bioaccessible phenolic fractions. The dietary fiber, protein content and fatty acid composition are thought to be effective in the potential of *CS*. It is proved that *CS* could be expressed as a convenient pseudo-cereal for functional food formulations.

Keywords: Chia,Low-fat, Antioxidant activity, Total phenolic content, *in-vitro* bioaccessibility

Introduction

Fat is the most important component that affects the texture, mouthfeel, aroma, and all properties of foods (Marangoni et al., 2014). However, excessive energy intake resulting from the high fat intake is associated with obesity, cancer, high blood cholesterol, and coronary heart diseases (Chowdhury et al., 2014; Siri-Tarino et al., 2015). Reducing the amount of fat in the daily diet has become very important for public health (Borneo et al.,2010; EFSA, 2010). Consumers want to reduce the amount of fat without damaging the quality criteria of the food.

Crackers are popular snack foods in the human diet and often described as thin, hard-baked, crunchy wafers or biscuits (Sedej et al., 2011; Isik and Topkaya, 2016). In order to achieve the desired features in crackers, low moisture and high amount of shortening should be used in the dough (Lee and Inglett, 2006). For fat reduction or displacement in crackers; protein and carbohydrate-based fat substitutes (Laguna et al., 2014),

Cite this article as:
Dundar, A.N., Yildiz, E., Parlak, O., Aydin, E. (2021). Use of chia seed on regular and low-fat crackers, their antioxidant properties, and in-vitro bioaccessibility. Int. J. Agric. Environ. Food Sci., 5(3), 302-309
Doi: https://doi.org/10.31015/jaefs.2021.3.7
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Received: 12 February 2021 **Accepted:** 28 April 2021 **Published Online:** 09 August 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 302-309
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

the hydrogenated or saturated fat substitutes with vegetable oils, and recently stabilized shortenings which are consisting of newly developed water-in-oil emulsions are started to use (Tarancón et al., 2012).

Chia (*Salvia hispanica*) is an annual herbaceous plant native to Mexico and Central America, it has attracted the attention of consumers in Turkey and used various foods such as biscuits, pasta, bread and yoghurt in recent years (CC, 2009). *CS* is a good source of antioxidants, depending on the presence of polyphenols (Taga et al.,1984; Martı´nez-Cruz and Paredes-Lo´pez, 2014).

CS can be used as a fat substitute due to its fat and protein content, in addition to this they can be used as an enricher and functional properties developer in the cracker production. For this aim, *CS* was replaced with wheat flour at the levels of 10%, 20%, and 30% (w/w) and the fat amount was decreased in 25%, 50%, 75% (w/w) ratios for formulating low-fat crackers, and the antioxidative potential of the samples was evaluated.

Materials and Methods

Materials

The wheat flour obtained from Toru Flour Milling Co. Ltd. (Bandırma/Balıkesir/Turkey) and rest of the ingredients such as CS (Chia Seed), salt, baker's yeast, sodium bicarbonate ($NaHCO_3$), ammonium bicarbonate (NH_4HCO_3) and shortening were purchased from local stores in Bursa (Turkey) for production cracker production.

Methods

Cracker preparation

For the production of crackers, method described by Lee and Inglett (2006) applied with slight modifications. The control sample was prepared with 100% wheat flour and 100% fat (shortening). In rest of the cracker samples, *CS* was replaced with wheat flour at levels of 10%, 20%, and 30% (w/w) and the fat amount was decreased in 25%, 50%, 75% (w/w) ratios for evaluating low-fat crackers (Table 1.). For the production, firstly, dry ingredients were mixed for 30 secs in a mixing bowl, homogeneously. First part of the water and shortening were mixed in a separate container then added into the mixture and kneaded (5SS model 5SS, Kitchen-Aid, USA) for 120 sec. For the baker's yeast activation, the rest of the water was used. The cracker dough with all ingredients was kneaded for more 4 min. Then, the dough was proofed for 2 h at 35 °C. Lamination machine (TMM Inc., Turkey) was utilized for reducing the thickness of the proofed dough to 1.5 mm. The spread dough was docked and cut into 5×5 cm cracker size by the cutter-docker device. Afterwards, crackers were baked at 180 °C for 7 min in a convection oven (FKE 006 model, Inoksan, Turkey). Then, baked crackers were cooled down to room temperature (~30 min). The cracker samples were grounded and stored at -18 °C hermetically, till sample preparation of antioxidant capacity (AC) and total phenolic content (TPC) analysis.

Table 1. Formulation of crackers incorporated with chia seed

Fat Ratio	CS* Ratio	Sample Code	Wheat Flour (g)	CS* (g)	Shortening (g)	Water (g)	$NaHCO_3$ (g)	NH_4HCO_3 (g)	Salt (g)
100%	%0	C_{Aa}	100.00	0.00	13.00	40.00	0.50	2.00	1.60
	%10	C_{Ab}	90.00	10.00	13.00	40.00	0.50	2.00	1.60
	%20	C_{Ac}	80.00	20.00	13.00	40.00	0.50	2.00	1.60
	%30	C_{Ad}	70.00	30.00	13.00	40.00	0.50	2.00	1.60
75 %	%0	C_{Ba}	100.00	0.00	9.75	40.00	0.50	2.00	1.60
	%10	C_{Bb}	90.00	10.00	9.75	40.00	0.50	2.00	1.60
	%20	C_{Bc}	80.00	20.00	9.75	40.00	0.50	2.00	1.60
	%30	C_{Bd}	70.00	30.00	9.75	40.00	0.50	2.00	1.60
50%	%0	C_{Ca}	100.00	0.00	6.50	40.00	0.50	2.00	1.60
	%10	C_{Cb}	90.00	10.00	6.50	40.00	0.50	2.00	1.60
	%20	C_{Cc}	80.00	20.00	6.50	40.00	0.50	2.00	1.60
	%30	C_{Cd}	70.00	30.00	6.50	40.00	0.50	2.00	1.60
25%	%0	C_{Da}	100.00	0.00	3.25	40.00	0.50	2.00	1.60
	%10	C_{Db}	90.00	10.00	3.25	40.00	0.50	2.00	1.60
	%20	C_{Dc}	80.00	20.00	3.25	40.00	0.50	2.00	1.60
	%30	C_{Dd}	70.00	30.00	3.25	40.00	0.50	2.00	1.60

***CS:**Chia Seed; **C:**Cracker Sample; **A-D** for fat reduction; **a-d** for CS replacement*

Sample preparation

The AC and TPC of cracker samples were evaluated in terms of extractable, hydrolysable, bioaccessible fractions of phenolics (Vitali et al.,2009; Bouayed et al.,2012). For the extraction procedure, 2 grams of sample was mixed with HCl$_{conc}$/metanol/water (1:80:10, v/v) for 20 °C in shaking water-

bath (250 rpm, 2 h; Thermo Fisher Scientific Inc., USA) and centrifuged (3500 rpm, 4 °C, 10 min; 3 K 30 model, Sigma, Germany). The supernatant was taken as extractable fraction, the residue was subjected with methanol/H_2SO_4 (10:1) and shaken in shaking water-bath (250 rpm, 85 °C, 20 h) and centrifuged (3500 rpm, 4 °C, 10 min). The supernatant was taken as hydrolysable fraction. For bioaccessible fraction, the in-vitro digestion procedure that include the enzymatic extraction was mimic for the cracker samples (Bouayed et al.,2012) was applied to cracker samples. 2 grams of cracker sample was treated with the pepsin enzyme solution (40 mg/mL in 0.1 M HCl; Merck, USA) in shaking water-bath (250 rpm, 37 °C, 2 h). Then the extraction was continued with the intestinal digestion procedure with using a porcine pancreatic enzyme solution (2 mg/mL; Sigma-Aldrich, Germany) and porcine bile solution (12 mg/mL; Sigma-Aldrich) at 37 °C (250 rpm, 2 h), at the end of the time centrifuged (3500 rpm, 15 °C, 10 min). The extracts were kept at -18 °C and utilized in AC and TPC assays.

Determination of total phenolic contents (TPC)

TPC of cracker extracts was evaluated by the Folin-Ciocalteu method with Apak et al. (2007a) procedures. The absorbance of the extracts was determined spectrophotometrically, gallic acid (Sigma-Aldrich, Germany) was utilized as standard and the results were expressed as mg gallic acid equivalents (GAE) per 100 g sample. The bioaccessibilities (%) of AC and TPC results were calculated according to Anson et al. (2009).

Antioxidant capacity

Different methods are used to determine the antioxidant capacity. Three methods commonly used to assess antioxidant capacity in vitro are the 2,2-azinobis (3-ethylbenzothiazoline6-sulfonic acid) (ABTS) test, DPPH, and the copper ion-reducing antioxidant capacity (CUPRAC) test.

The AC analysis determined according to methods of ABTS (Apak et al.,2007a), CUPRAC (Apak et al.,2007b) and DPPH (Brand-Williams et al., 1995) assays. The absorbance of the extracts was determined spectrophotometrically (Jenway, 6405 UV/Vis). Trolox equivalent (TE) calibration curve obtained for assays in between range of 0.02-0.08 µmol Trolox (Sigma-Aldrich, Germany) and the results were expressed as µmol Trolox equivalent (TE)/g sample.

For ABTS radical solution, 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12–16 h before use to obtain an absorbance at 734 nm. ABTS radical solution of blue-green color was diluted with ethanol (96%) at a ratio of 1:10. The stage was used adding 1 mL of the ABTS solution to (x) mL of extract and (4.0–x) mL of ethanol, and the absorbance measured at 734 nm after 6 min by using UV/Vis spectrometer (Optizen 3220 UV, Mecasys).

For CUPRAC assay briefly, One mL of 1 9 10^{-2} M CuCl2, 1 mL 7.5 9 10^{-3} M neocuproine, 1 mL ammonium acetate buffer solutions, x mL extract of samples and (4–x) mL of water were added and mixed. The final mixture at 4.0 mL total volume was let to stand at room temperature and after 30 min, the absorbance at 450 nm (Optizen 3220 UV, Mecasys) was recorded against a reagent blank.

The initial absorbance of the DPPH in methanol was measured at 515 nm and did not change throughout the period analysis. Briefly, all diluted extracts (0.1 mL) were added to the test tube and 3.9 mL of 6 x 10^{-5} M methenolic solution of DPPH was added and mixed. This mixture was left in the dark for 30 minutes and then the measurement was carried out.

Statistical evaluation

The obtained data was evaluated statistically by variance analysis with JMP IN 7.0.0 (Statistical Discovery from SAS 2005. Institute Inc.). The LSD (Least Significant Differences) test was used for determination of the statistical difference between the obtained mean values in terms of CS addition and fat reduction in cracker samples.

Results and Discussion

Phenolic compounds are present in three different forms such as free, extractable-conjugated and, non-extractable-bound forms in food. The bound forms of phenolic compounds can only be physically trapped in the structure of macro components without binding to the food matrix or various cellular structures (Gökmen et al.,2009; Nayak et al., 2015; Karabulut and Yemiş, 2019).

Bioaccessiblity of phenolic compounds in foods depends on components release from the food matrix, its absorption and its passage to the blood circulation system during digestion or intestinal fermentation. Especially, polyphenols in some foods bind to macromolecules such as proteins, carbohydrates and lipids in the cell wall structure and greatly affect the bioaccessibility in the gastrointestinal system. Because of being difficult to digest, bound phenolic compounds can reach the colon without alteration in the gastrointestinal tract (Arranz et al., 2010; Pastoriza et al., 2011; Karabulut and Yemiş, 2019).

The AC and TPC of cracker samples were evaluated in terms of extractable, hydrolysable, bioaccessible fractions of phenolics and given in Figure 1-4. Hydrolysable fractions of crackers were the highest results comparing to extractable and bioaccessible fractions. According to the extraction procedure, it is thought that the values of the hydrolysable fractions were increased due to the higher temperature and longer extraction duration of the cracker samples. Also, the bioaccessible fractions were shown higher results than the extractable fractions. The amount of released bioactive components were probably increased with the enzymatic treatment of *in-vitro* gastric and intestinal digestion with relatively higher extraction temperature (37 °C).

Evaluating the hydrolysable fractions for $TEAC_{ABTS}$, by the fat reduction, there were 7.70, 14.82 and 23.62% increase observed in antioxidative potential of crackers. For the C_D group of samples with 75% fat reduction, by the 10, 20, 30% *CS*-supplementation, there were 24.22, 54.32 and 82.36% increase for $TEAC_{ABTS}$ in C_{Db}, C_{Dc}, C_{Dd} samples. For the extractable fractions, a higher increase of antioxidative potential was observed as 48.24 , 91.45, 180.40% for $TEAC_{ABTS}$ in the same samples.

It is an important parameter to determine the *in-vitro* bioaccessibility of the food bioactive components, for revealing their health potential and improving existing food formulations and processes. Evaluation of the effect of *CS*

on bakery product as a nutritional perspective, Pigni et al. (2020) produced supplemented wheat pasta with obtained by-product of the *CS* oil extraction and evaluated their *in-vitro* bioaccessibility of polyphenols. They did not determine the significant increase in oral or gastrointestinal digestion steps but by the intestinal digestion markable increase was observed in terms of TEAC (Trolox Equivalent Antioxidant Capacity). It can be expressed as the evidence of the bioactive compounds release in intestinal digestion step.

In terms of *CS* replacement, evaluating the C_A group of samples according to *CS* supplementation in 100% fat production, there are 9.1%, 20.0%, and 38.9% increases in TPC of bioaccessible phenolic fractions of C_{Ab}, C_{Ac}, and C_{Ad} respectively for 10%, 20% and 30% *CS* replacement. Withal, AC results were reflected higher increment in the same way of evaluation. There are 30.3%, 105.0% 121.5% increase for C_{Ad} comparing to C_{Aa} in terms of $TEAC_{DPPH}$, $TEAC_{CUPRAC}$, $TEAC_{ABTS}$. In terms of fat reduction, the highest increase was determined in AC content determined according to ABTS and CUPRAC methods. By 25, 50 and 100% fat reduction (Comparing to C_B, C_C and C_D with C_A group of samples) in extractable, hydrolysable and bioaccessible phenolic fractions, the AC results were increased respectively as 5.87%, 9.33%, 12.11% according to $TEAC_{ABTS}$ and respectively 5.87%, 9.33%, 12.11% increase were determined in $TEAC_{CUPRAC}$. *CS* replacement was determined to be more effective than fat reduction on AC and TPC results of cracker samples. 75% fat reduced-30% *CS* supplemented C_{Dd} sample is 91.0% higher than C_{Da} and 143.4% higher than C_{Aa} in terms of $TEAC_{ABTS}$ for bioaccessible phenolic fractions.

CS is a good source for bakery products with high protein (21.78%) and dietary fibre (38.70%) contents, including fatty acid varieties (Dundar et al., 2020) together with its bioactive potential. As a part of this study, by *CS* replacement; dietary fiber, protein, and ash contents were increased for cracker samples; and the fatty acid content was were enriched in terms of linoleic, oleic, α-linolenic acids. C_{Dd} was also expressed as the healthiest sample according to $C_{18:1}/C_{18:0}$ ratio (Dundar et al., 2020). In this study, C_{Dd} is also determined as the richest sample in terms of bioactive potential (Fig. 1-4) as a result of AC and TPC analysis.

Enes et al. (2020) published a systematic review according to electronic databases by following Prisma recommendations for understanding *CS* potential on health. Oxidative stress decreasing effect of *CS* associated with increasing the AC and providing antioxidant enzymes; decreasing the lipid peroxidation and amount of reactive oxygen species (ROS). Its antioxidant properties and bioactive potential comes form including the carotenoids, phospholipids, tocopherols, and phenolic acids such as chlorogenic acid, caffeic acid, kaempferol, quercetin, together with fatty acid content (especially α-linolenic acid: $C_{18:3}$) (Enes et al., 2020; Da Silva et al., 2016). Chlorogenic acid and caffeic acid are known with stronger inhibition effect on lipid peroxidation than the well-known antioxidative compounds such as vitamin E and C (Valdivia-López and Tecante, 2015).

Demin et al. (2020) evaluated the nutritional value of cracker samples based on *CS*, rye and buckwheat flours with extra virgin olive oil. *CS* amount provided a significant increase (p<0.05) in crude fiber, total ash and mineral (Cu, Fe, Mg, and Zn) contents of cracker samples. Especially with fatty acid content, *CS* was expressed as a convenient pseudo-cereal for functional food formulations by low amount of saturated fatty acids (<11%) and high amount of polyunsaturated fatty acids (> 89%). In the present study, *CS* supplemented cracker samples, comparing C_{Ad} with C_{Aa}; $C_{18:3}$ content increased from 0.05 to 5.52 g/100g (Also proportional increase of *CS* in content by decreasing fat in total dough amount). Evaluating the *CS* and fat effect together, $C_{18:3}$ content was determined 7.21 g/100g in C_{Dd} sample (Dundar et al., 2020). Therefore, the fatty acid content was found as one of the reasons of higher bioactive potential.

Jethwani et al. (2020) prepared bars with mango, apple and guava supplemented with chia seeds. When the antioxidant activity and total phenolic components of these bars were examined; while the antioxidant (DPPH) values of the control bars were 76.38%, mango, apple and guava supplemented with chia seeds were found 80.09%, 78.43%, 78.14%, respectively. The antioxidant activity ABTS (mmolTE / 55g) values of the control bars were found 38.17 mmolTE / 55g, while the bars with chia seed mango, apple and gouava were 45.49 mmolTE / 55g, 42.30 mmolTE / 55g, 39.6 mmolTE / 55g, respectively. The results for total phenols reveals the significant increase ((p <0.01) in the selected antioxidant rich bars as compared to the control bar. The control bar was seen to have a total phenols content of 64.50 mgGAE / 55g. A highest total phenol among the selected bars was found to be in guava chiaseed bar (97.01 mgGAE / 55g) while lowest was observed in apple chiaseed bar (69.93 mgGAE / 55g).

Antonini et al. (2019) made beef burgers that contain chia seeds and goji puree (2.5 and/or 5%), and total phenolics and antioxidant capacity were determined. The ORAC and DPPH methods revealed a higher antioxidant capacity when goji puree and chia seeds where added, respectively, thus highlighting the different ability of polyphenols to scavenge free radicals.

Sharma et al. (2020) prepared six different formulations of muffins. One formulation was prepared with wheat flour (control), and in other five formulations, wheat flour was replaced with chia seeds at different levels (5%,10%,15%,20%,25%). Total antioxidant activity was found between 0.39 (mg TE/100g) and 0.78 (mg TE/100g). It was determined the lowest total antioxidant activity in control.

Mesías et al. (2016) determined significant and progressive increase in the AC ($TEAC_{ABTS}$, $TEAC_{DPPH}$, $TEAC_{ORAC}$ and $TEAC_{FRAP}$) by means of *CS* increase in formulations. In other respects, increasing amounts of *CS,* in chips (Coorey et al., 2012) and bread (Costantini et al., 2014) formulations, higher AC potentials were observed according to analyze results.

Together with the recent studies, using chia (*Salvia hispanica*) in the functional food formulations could be expressed to supportive. *In-vitro* studies have shown the health potential of *CS* with different properties, and further *in-vivo* studies could be recommended to conduct for understanding the health properties with the mechanism.

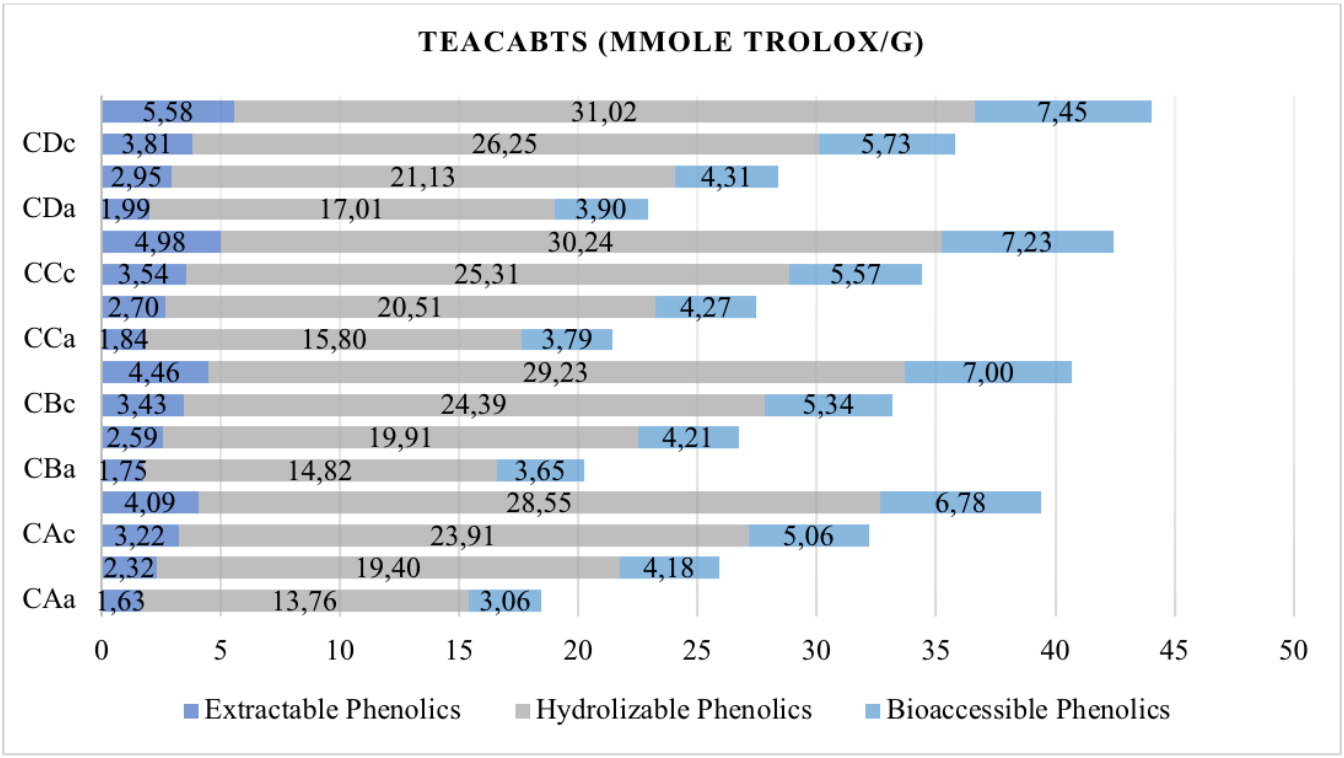

Figure 1. $TEAC_{ABTS}$ results of cracker samples
*CS:Chia Seed; A:100% fat; B:%75 fat; C:%50 fat; D:%25 fat, a:0% CS; b:10% CS; c:20% CS; d:30% CS in cracker samples

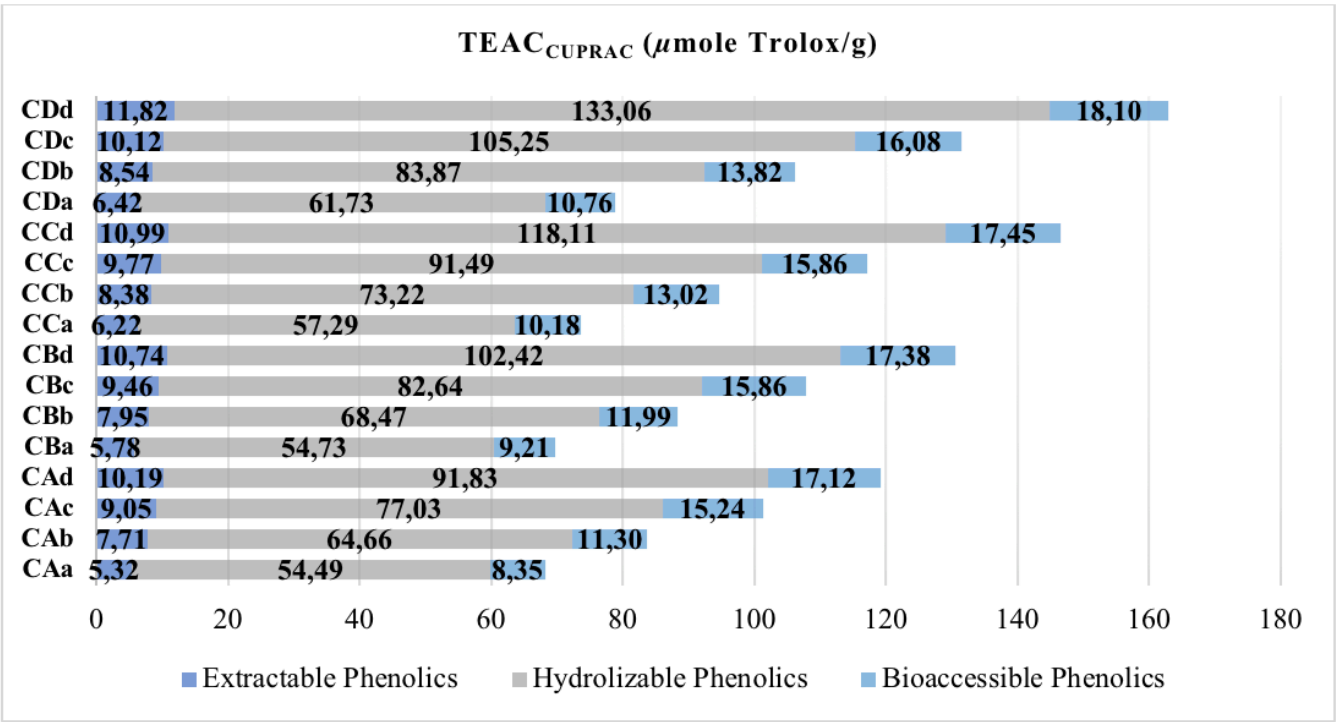

Figure 2. $TEAC_{CUPRAC}$ results of cracker samples
*CS:Chia Seed; A:100% fat; B:%75 fat; C:%50 fat; D:%25 fat, a:0% CS; b:10% CS; c:20% CS; d:30% CS in cracker samples

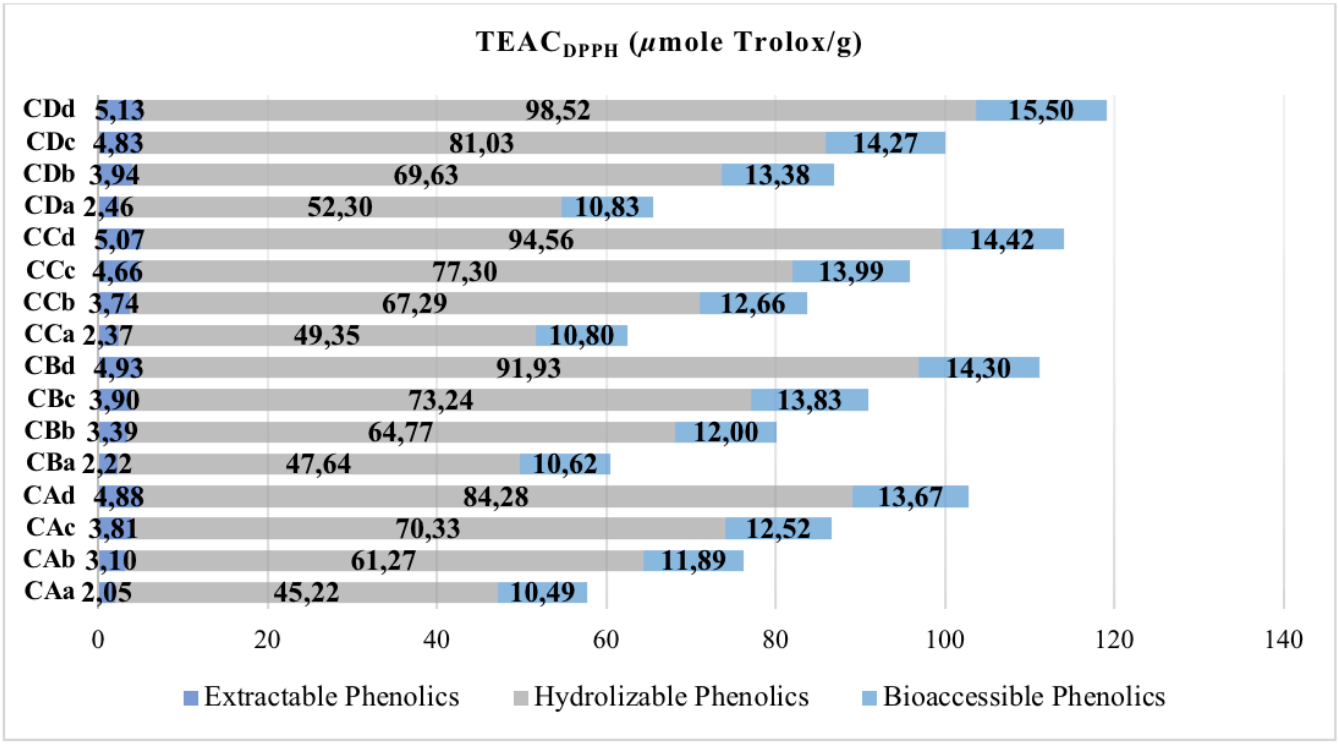

Figure 3. TEAC$_{DPPH}$ results of cracker samples
*CS:Chia Seed; A:100% fat; B:%75 fat; C:%50 fat; D:%25 fat, a:0% CS; b:10% CS; c:20% CS; d:30% CS in cracker samples

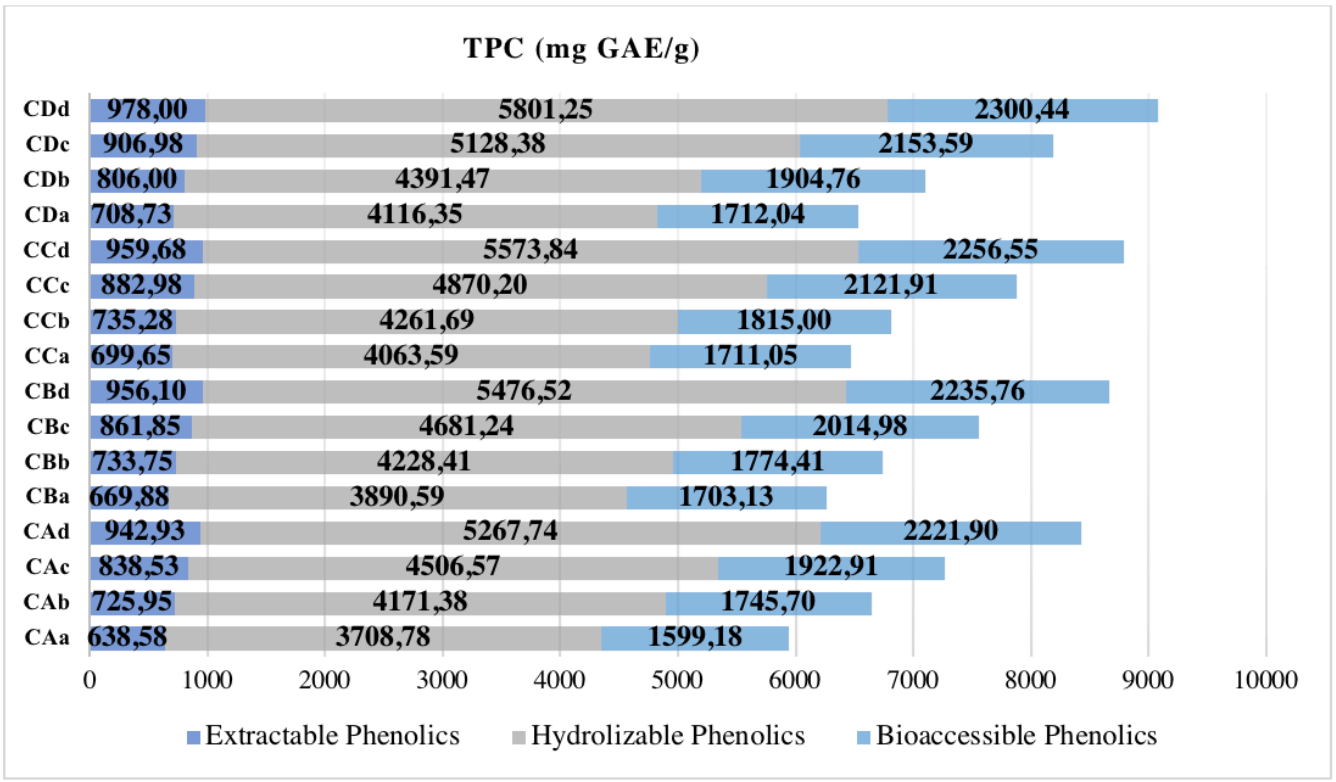

Figure 4. Total Phenolic Compound analysis results of cracker samples
*CS:Chia Seed; A:100% fat; B:%75 fat; C:%50 fat; D:%25 fat, a:0% CS; b:10% CS; c:20% CS; d:30% CS in cracker samples

Conclusion

In addition to providing energy, the fat has a nourishing function because of providing intake of essential fatty acids and fat-soluble vitamins. The effects of fat on the bakery products are important in regard of the mouthfeel, texture and taste properties. Although having functional properties, fat is known to be adversely effective on health in case of high consumption. Reducing the amount of fat in the daily diet, which has become a public health issue, is a problem and concern for many consumers. Cracker is one of the common bakery products because of being satisfying, nutritious, and delicious. For creating a functional and nutritious formulation, *CS* was replaced with wheat flour at the levels of 10%, 20%, and 30% (w/w) and the fat amount was decreased in 25%, 50%, 75% (w/w) ratios for production of low-fat crackers. For determination of the bioactive potential of the crackers, extractable, hydrolysable, and bioaccessible phenolic fractions of samples were analyzed in terms of $TEAC_{ABTS}$, $TEAC_{CUPRAC}$, $TEAC_{DPPH}$ and total phenolic content. Hydrolysable fractions of crackers were the highest results comparing to extractable and bioaccessible fractions. *CS* supplementation is remarkably more effective on bioactive compounds of cracker samples comparing with the fat reduction. In terms of *CS* replacement, evaluating the C_A samples according to *CS* supplementation in 100% flour production, there are 9.1%, 20.0%, and 38.9% increases in TPC of bioaccessible phenolic fractions of C_{Ab}, C_{Ac}, and C_{Ad} respectively for 10%, 20% and 30% *CS* replacement. *CS* could be expressed as a convenient pseudo-cereal for functional food formulations by increasing the bioactive potential and also providing the quality parameters. The results of our study showed that the addition of chia seeds plays an important role, especially in increasing antioxidant capacity and phenolic compounds. Thanks to this feature, it is recommended as a guide for future studies in order to increase the functionality of commercial products.

Compliance with Ethical Standards

Conflict of interest

The authors report no declarations of interest.

Author contribution

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

Ethical approval

Not applicable.

Funding

This work was supported by the Scientific Research Council of Bursa Technical University, Turkey (Project No: 171N06).

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

Authors are thankful to the Scientific Research Council of Bursa Technical University, Turkey for their financial supports.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.8 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):310-322 (2021)

Electric Energy Potential that can be Produced Using Cattle Manure in the Isparta Region

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Abstract

Today, in order to meet the increasing energy needs, sustainable and renewable energy production methods are needed in addition to the existing energy sources. Especially taking into consideration the husbandry potential of our country, biogas and energy production through the use of animal waste have emerged as an efficient alternative of production. In this study, the amount of electrical energy that can be produced using cattle manure in the Isparta region was calculated. The husbandry data used in these calculations were obtained from the database of the Turkish Statistical Institute. The potential amount of manure that can be obtained from all of the cattle in the Isparta region was determined as 1,117,002.08 tons. The amount of existing potential manure that can be collected and used for electricity generation was determined as 558,501.03 tons. The amount of biogas to be obtained as a result of the use of animal manure in biogas plants was determined as 12,815,682.93 m^3. The amount of electrical energy that can be produced by using the obtained biogas was calculated as 60,233.71 MW. This electrical energy, obtained as a result of the calculations, will be able to meet 6.00% of the electricity needs of Isparta Province annually.

Keywords: Cattle, Isparta, Biogas, Manure, Electrical energy

Introduction

Biogas is a gas mixture obtained by biomethanization processes in the presence of different microorganisms in an oxygen-free environment of organic substances by anaerobic degradation. Biogas contains methane (CH_4), carbon dioxide (CO_2), nitrogen (N_2), hydrogen sulfide (H_2S), ammonia (NH_3), hydrogen (H_2), Carbon Monoxide (CO) (Anonim, 2014). Because of this property, biogas is a flammable gas mixture consisting of many organic wastes such as Forest Products, domestic and industrial wastes, agricultural wastes and animal manure and is defined as converted energy (Senol et al., 2017; Yetis et al., 2019).

Reliable and cheap energy sources are the most important element of the renewable environment. The probability of experiencing energy crises in the world is great. One of the most important energy sources of our country which is a country of Agriculture and animal husbandry is biogas. The revealing of this potential and the correct assessment of resources is of great importance to the national economy (Aslan et al., 2016; Atilgan et al., 2021).

Failure to evaluate animal wastes, which have high energy production potential, is a significant loss for our national energy resources. Construction and dissemination of biogas plants in accordance with regional conditions and production capacity will prevent environmental pollution and It will develop producers in terms of socioeconomic and cultural aspects in rural (Dagtekin et al., 2019).

Countries require a great deal of energy to maintain their basic activities. In the last 30 years, the increasing energy demand has also increased the need for different and new

Cite this article as:
Yilmaz, H.I., Gonbe, Y. (2021). Electric Energy Potential that can be Produced Using Cattle Manure in the Isparta Region. Int. J. Agric. Environ. Food Sci., 5(3), 310-322
Doi: https://doi.org/10.31015/jaefs.2021.3.8
Orcid: Halil Ibrahim Yilmaz: 0000-0002-5427-3608 and Yalcin Gonbe: 0000-0001-8185-5032
Received: 31 March 2021 **Accepted:** 20 June 2021 **Published Online:** 16 August 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 310-322
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

energy production sources (Dalpaz et al., 2020).

Used for electricity and heat generation, biogas is a practical way to efficiently recycle animal and vegetable waste. Animal and plant waste has significant potential for energy production due to its low costs (Ardebili, 2020).

The positive effect of converting animal and plant waste for energy production should not be ignored. Biogas plants in developed countries are used effectively and efficiently, and these facilities recover investment costs in a short period of time (Tolay et al., 2008).

The renewable and continuous production of biogas depends on various factors, namely economic, environmental, and social ones. Due to the use of waste, biogas production has a positive effect in terms of both reducing both foreign-source dependency, and energy and carbon dioxide emissions. Furthermore, the elimination of organic wastes that harm human health and cause environmental problems by converting them into energy further increases the importance of biogas technology (Yagli and Koc, 2019).

Energy production through the utilization of animal and plant waste is considered one of the best methods used for the development of energy production in many developed and developing countries (Khalil, 2019).

Biogas has an important place among renewable and continuous energy sources in many countries around the world. While production is widely conducted in family-type biogas plants in countries such as China and India on the Asian continent, industrial production is widespread in countries such as Finland, Germany, and Austria, due to the plenitude of cooperatives, and animal waste is generally used in these biogas plants (Salihoglu et al., 2019).

It is known that there are about 50 million biogas plants in the world. Approximately 43 million of the existing facilities are located in China, whereas 4.5 million are in India, but these facilities are primitive, have old technology, and are used for heating and cooking purposes. In the United States, approximately 1 billion kWh of electricity is produced annually from 265 biogas plants used for the utilization of agricultural waste (Ar, 2018).

In the European Union (EU) countries, since the early 2000s, the amount of energy produced using sustainable and renewable sources has continued to increase (Sturmer et al., 2021).

Energy production using biogas is expected to play an important role in the energy policy of the EU in the future (Meyer et al., 2018).

When the EU countries were analyzed in terms of the number of biogas production facilities, Germany ranked first with 10,971 biogas plants. Italy, which ranked second, has 1655 biogas plants, while France, which ranked third, has 742 biogas plants. Turkey ranked sixteenth with its 100 existing biogas plants. It is possible to say that Turkey lags behind many developed or developing countries when compared to continental European countries, according to the current number of biogas plants (Figure 1).

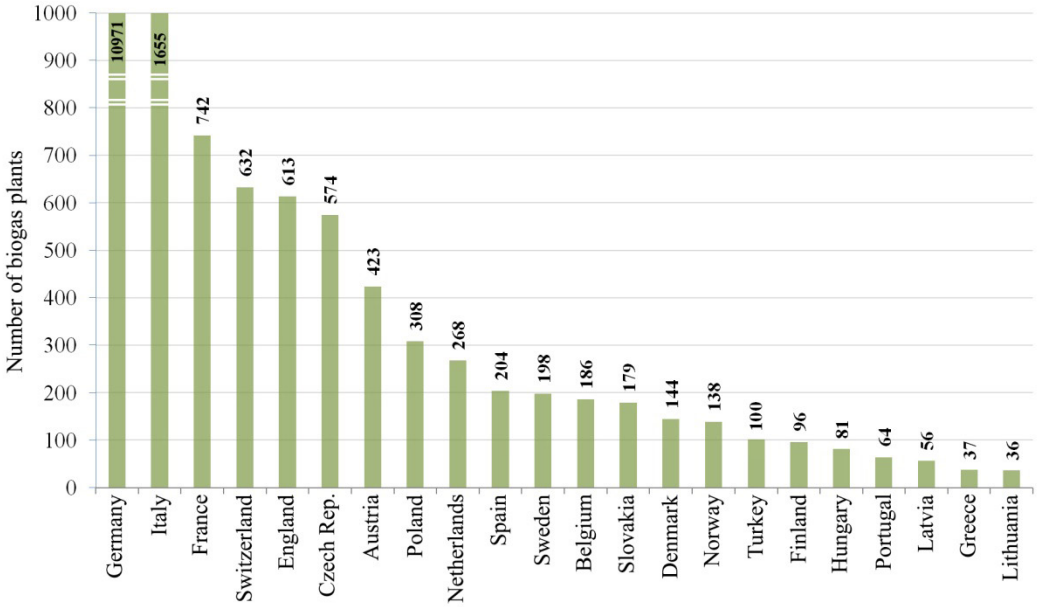

Figure 1. Number of biogas plants in countries on the European Continent (Anonim, 2018; EBA, 2018)

Biogas and biomass power plants operating in our country are examined, it is seen that the Marmara region ranks first in terms of the number of facilities and installed power. The total number of facilities in the Marmara region is 28 and the total installed capacity of these facilities is 197.89 MWe. In addition, the Marmara region alone accounts for 37.28% of Turkey's total installed capacity. The second-ranked Central Anatolia region has 20 facilities, and the total installed power of these facilities is 113.82 MWe. The Central Anatolia region accounts for 21.44% of Turkey's total installed capacity. The Mediterranean Region where Isparta is also included, ranked third in terms of the number of facilities and installed power. The number of facilities in the Mediterranean Region is 19, and the total installed power of these facilities is 78.18 MWe. The installed capacity of the existing facilities in the Mediterranean region to 14.73% of the total installed capacity (Table 1).

Table 1. Biogas and biomass power plants in Turkey by region (Anonim, 2018)

Regions	Number of biogas plants	Installed power (MWe)	Ratio (%)
Marmara	28	197.89	37.28
Central Anatolia	20	113.82	21.44
Mediterranean	19	78.18	14.73
Black Sea	10	57.55	10.84
Aegean	13	44.45	8.37
Eastern Anatolia	6	24.51	4.62
Southeast Anatolia	4	14.38	2.71
Total	100	530.78	100.00

Mediterranean region is one of Turkey's seven geographical regions. It stretches along the Mediterranean Sea coast in the south of Anatolia. The Aegean region is located in the west and northwest of the Mediterranean region. To the North is the Central Anatolia region and to the East is the Southeastern Anatolia region. It borders Syria in the Southeast. The provinces located in the Mediterranean region are Adana, Antalya, Burdur, Hatay, Kahramanmaraş, Mersin, Isparta and Osmaniye (Figure 2).

When the provinces in the Mediterranean region is evaluated according to the number of bovine animals it has, Adana province ranks first with the 248,527 cattle. Adana province has 18.47% of the total animal number of the Mediterranean Region. Burdur province, which ranks second, has 212,727 cattle and its share in the total cattle is 15.81%. Kahramanmaraş, which is in the third place, has 206,836 cattle and its ratio in the region is 15.37%. Isparta province ranks fifth after Antalya in the care of bovine animals in the Mediterranean region. The presence of cattle in the province of Isparta is 145,820 which corresponds to 10.84% of the total presence of cattle in the Mediterranean region (Table 2 and Figure 3).

Figure 2. Mediterranean Region and provinces (HGM, 2021)

According to Turkey's energy production and consumption scenarios, a rapid increase in both production and consumption amounts is expected over the next decade. Our main energy resources are lignite, hydroelectric and biomass energies. In addition to that about 3/4 of the energy used is imported (Aybek et al., 2015). It has become a requirement to use sustainable and renewable energy sources effectively to solve this problem. However, the use of animal waste as manure without any processing causes environmental pollution and also decreases productivity in agriculture. Taking into account the energy needs in Turkey and the environmental problems caused by animal waste, biogas plants should be established to solve these two problems effectively (Tuncez, 2018).

Bovine farming is one of the agricultural activities that contribute greatly to the city economy in the province of Isparta. The most accurate evaluation of the manure obtained with cattle breeding appears as a problem that needs to be solved. The use of manure obtained for this purpose as organic fertilizer for use in agricultural activities or use in energy production by converting it to biogas are the most appropriate alternatives. Evaluation of animal manure as biogas contains two solutions, both in terms of its evaluation in energy production and in terms of allowing the remaining organic fertilizer to be used in crop production at the end of this production.

Table 2. Bovine presence of Mediterranean Region provinces (TUIK, 2019)

Provinces	Number of bovine animal (piece)	Ratio (%)
Adana	248,527	18.47
Burdur	212,727	15.81
Kahramanmaraş	206,836	15.37
Antalya	188,579	14.01
Isparta	145,820	10.84
Hatay	140,246	10.42
Mersin	125,100	9.30
Osmaniye	77,737	5.78
Total	1,345,572	100.00

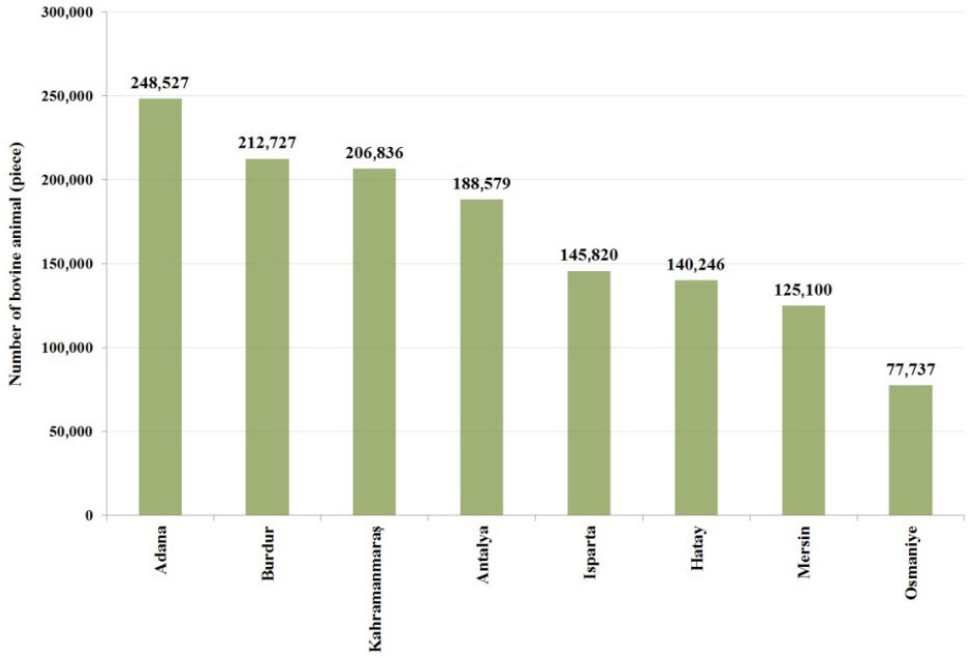

Figure 3. Presence of bovine in the Mediterranean Region provinces (TUIK, 2019)

In this study, the amount of biogas and electrical energy that can be obtained from the manure of cattle in Isparta was determined. The contribution of this amount of electrical energy that can be obtained to the electricity consumption of Isparta province has been questioned. The use of electrical energy which will be obtained from the waste of cattle use in agricultural production is also important in terms of reducing energy usage costs on agricultural activities.

Materials and Methods

Isparta Province is located at 30°20′ and 31°33′ E, and 37°18′ and 38°30′ N. Its surface area is 8933 km^2 and its altitude is 1050 m. Located in the Region of Lakes in the Mediterranean Region, Isparta Province is under the influence of a cold-semi continental climate (DIE, 1999). The research area is shown in Figure 4.

The material of the study comprised the presence of cattle in Isparta Province. The entire bovine animals was covered by the research on the basis of the full counting. Data on the existing bovine animals in Isparta Province were obtained from the Turkish Statistical Institute (TURKSTAT) database. The cattle farming data obtained were sorted and evaluated according to the age groups of the animals. Dairy cattle bred for the purpose of milk production comprised calves between 0 and 12 months of age, heifers between 12 and 24 months of age, and cows (milking) +24 months of age. Beef cattle bred for meat production comprised calves between 0 and 12 months of age, and heifers between 12 and 24 months of age. In Turkey, bull breeding is not preferred due to the use of artificial insemination methods in dairy cattle breeding. For this reason, the presence of bulls was excluded from the scope of the study. Data on the presence of cattle in Isparta Province and its districts are given in Table 3 and Figure 5.

Figure 4. Isparta Province and its districts (Anonim, 2021a)

Table 3. Presence of cattle in Isparta Province and its districts (TUIK, 2019)

Districts	Dairy cattle			Beef cattle		Number of cattle (piece)	Ratio (%)
	Calf (<12 month)	Heifer (12-24 month)	Cow (+24 month)	Calf (<12 month)	Heifer (12-24 month)		
Yalvaç	3,810	4,078	15,683	4,096	3,531	31,198	21.39
Şarkikaraağaç	5,531	5,812	9,929	5,735	2,325	29,332	20.12
Merkez	2,421	2,730	8,900	2,230	2,775	19,056	13.07
Eğirdir	2,163	2,762	6,893	1,732	1,169	14,719	10.09
Sütçüler	1,628	1,406	4,866	1,562	934	10,396	7.13
Keçiborlu	1,166	1,266	3,569	1,779	1,348	9,128	6.26
Aksu	1,123	1,826	2,870	885	1,249	7,953	5.45
Gelendost	1,145	711	3,096	1,216	367	6,535	4.48
Atabey	837	1,051	2,337	859	891	5,975	4.10
Gönen	935	650	2,240	795	373	4,993	3.42
Senirkent	508	515	2,100	520	261	3,904	2.68
Yenişarbademli	211	352	653	165	124	1,505	1.03
Uluborlu	137	116	514	137	222	1,126	0.77
Total	21,615	23,275	63,650	21,711	15,569	145,820	100.00

In dairy cattle, calves <12 months of age had an average live weight of 150 kg in Turkey and the daily amount of manure produced was 8.62 kg. The average live weight of heifers between 12 and 24 months of age was 350 kg, and the daily amount of manure produced was 20.41 kg. The average live weight of cows >24 months of age was around 450 kg, and the daily amount of manure produced was 28.12 kg. In beef cattle, calves <12 months of age had an average live weight of 200 kg in Turkey and the daily amount of manure produced was 11.79 kg. The average live weight of heifers between 12 and 24 months of age was 350 kg, and the daily amount of manure produced was 22.68 kg. (Anonymous, 2006; Anonymous, 2016; MWPS, 2004) (Table 4)

The ratio at which these manure values obtained Turkey can be collected and used as biogas was 50% (Aybek et al., 2015; Ekinci et al.,2010; Kulcu, 2002) (Table 4).

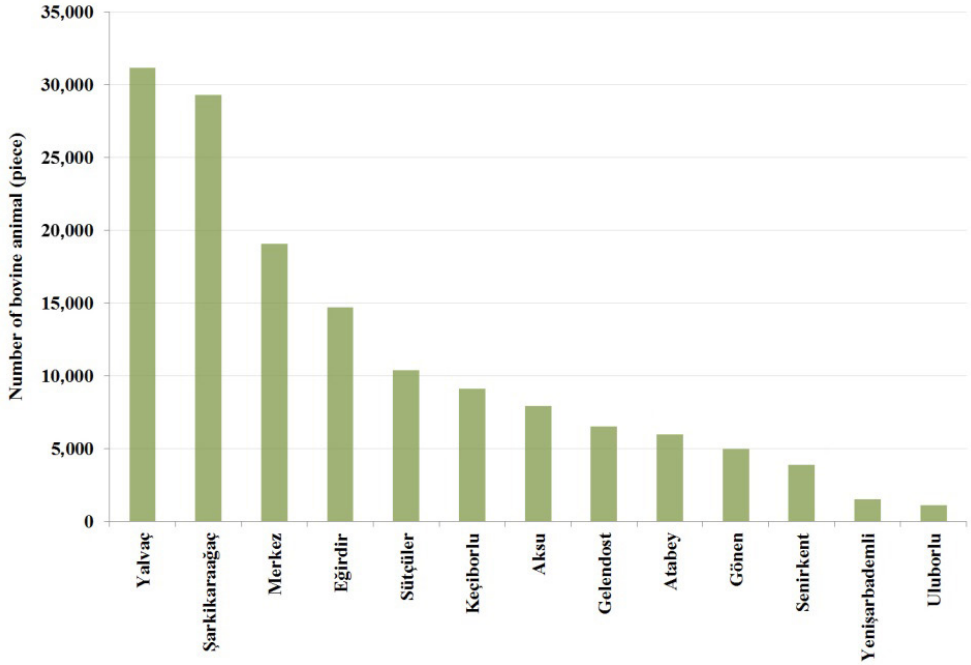

Figure 5. Presence of bovine animals in Isparta Province districts (TUIK, 2019)

Table 4. Daily amount of manure produced by the cattle

Dairy and beef cattle		Age group (month)	Average live weight (kg)	Manure production (kg day^{-1})	The ratio of collectable animal manure (%)
Dairy cattle	Calf	<12	150	8.62	50
	Heifer	12-24	350	20.41	50
	Cow	>24	450	28.12	50
Beef cattle	Calf	<12	200	11.79	50
	Heifer	12-24	350	22.68	50

When the biogas yields of the manure to be obtained from the various cattle were evaluated, the organic dry matter ratio of dairy cattle manure was 85%, and the biogas production efficiency per ton was determined as 20.2 m^3. The organic dry matter ratio of the manure obtained from beef cattle was 85%, and the biogas yield was 34.0 m^3 per ton (Table 5).

It was found that 1 m^3 of biogas obtained using animal and plant wastes is equal to 0.25 m^3 of propane gas. Moreover, 1 m^3 of biogas corresponded to 0.66 liter of diesel, 0.75 liter of gasoline, and 4.70 kWh of electrical energy. Similarly, it was determined that 1 m^3 of biogas corresponded to 4700–5700 kcal heat energy (Table 6).

Table 5. Biogas production quantities according to the various animal groups (Anonim, 2021b)

Animals groups	Organic dry matter ratio (%)	Biogas production efficiency (m^3 ton^{-1})	Methane (CH_4) (%)
Dairy cattle manure	85.0	20.2	55.0
Beef cattle manure	85.0	34.0	55.0
Horse manure	75.0	63.0	55.0
Chicken manure	75.0	100.0	65.0
Sheep manure	80.0	108.0	55.0

Table 6. Energy equivalents of 1 m^3 of biogas (Bugutekin, 2007; Celikkaya, 2016)

Energy equivalents	Units of measurement	Equivalent amount of energy
Propane	m^3	0.25
Diesel	liter	0.66
Gasoline	liter	0.75
Electricity	kWh	4.70
Heat energy	Kcal	4700-5700

Results and Discussion

When the amount of manure produced by dairy cattle in Isparta Province and its districts were evaluated, the amount of manure to be obtained from calves in the Yalvaç district was calculated as 11,987.40 tons. The total amount of manure to be obtained from heifers was found to be 30,379.67 tons, whereas the amount of manure to be obtained from cows was 160,967.18 tons, and the total amount of manure in Yalvaç was 203,334.25 tons. Barely 101,667.13 tons of this manure can be collected and will be used for biogas production.

The amount of manure to be obtained from calves in dairy cattle enterprises in the Şarkikaraağaç district was 17,402.19 tons. The amount of manure to be obtained from heifers was 43,297.37 tons, whereas the amount to be obtained from cows was 101,909.27 tons, which all totaled 162,608.82 tons. The amount of manure that can be used for biogas production was calculated as 81,304.41 tons.

The amount of manure to be obtained from calves in dairy cattle enterprises in the Merkez district was 7,617.19 tons. The amount of manure to be obtained from heifers was 20,337.54 tons, and the amount of manure to be obtained from cows was 91,347.82 tons, which all totaled 119,302.56 tons. The amount of manure that can be used for biogas production was found to be 59,651.28 tons.

The amount of manure produced in dairy cattle enterprises in the Yalvaç, Şarkikaraağaç, and Merkez districts of Isparta Province that can be used as biogas corresponded to 54.23% of the amount of manure that can be used for biogas in Isparta Province.

In the entire Isparta Province, the amount of manure to be obtained from calves in dairy cattle enterprises was calculated as 68,007.27 tons. The amount of manure to be obtained from heifers was 173,390.60 tons, and the amount of manure to be obtained from cows was 653,290.87 tons, totaling 894,688.75 tons. The amount of manure that can be used for biogas production was determined to be 447,344.37 tons (Table 7).

Table 7. Annual amount of manure produced by the dairy cattle

Districts	Manure production (tons $year^{-1}$)			Total manure production (tons $year^{-1}$)	The amount of manure to be used in biogas production (tons $year^{-1}$)	Ratio (%)
	Calf (<12 month)	Heifer (12-24 month)	Cow (+24 month)			
Yalvaç	11,987.40	30,379.67	160,967.18	203,334.25	101,667.13	22.73
Şarkikaraağaç	17,402.19	43,297.37	101,909.27	162,608.82	81,304.41	18.17
Merkez	7,617.19	20,337.54	91,347.82	119,302.56	59,651.28	13.33
Eğirdir	6,805.45	20,575.93	70,748.37	98,129.75	49,064.88	10.97
Sütçüler	5,122.18	10,474.21	49,943.65	65,540.04	32,770.02	7.33
Keçiborlu	3,668.59	9,431.26	36,631.50	49,731.34	24,865.67	5.56
Aksu	3,533.29	13,603.06	29,457.11	46,593.46	23,296.73	5.21
Gelendost	3,602.51	5,296.70	31,776.72	40,675.94	20,337.97	4.55
Atabey	2,633.45	7,829.58	23,986.50	34,449.54	17,224.77	3.85
Gönen	2,941.79	4,842.27	22,990.91	30,774.98	15,387.49	3.44
Senirkent	1,598.32	3,836.57	21,553.98	26,988.87	13,494.44	3.02
Yenişarbademli	663.87	2,622.28	6,702.26	9,988.41	4,994.20	1.12
Uluborlu	431.04	864.16	5,275.59	6,570.80	3,285.40	0.73
Total	68,007.27	173,390.60	653,290.87	894,688.75	447,344.37	100.00

When the amounts of manure to be obtained from beef cattle grown in Isparta Province and its districts were evaluated, the amount of manure to be obtained from calves in the Yalvaç district was calculated as 17,626.52 tons, and the amount of manure to be obtained from the heifers was calculated as 29,230.32 tons. The amount of manure to be obtained in beef cattle in the Yalvaç district was 46,856.85 tons in total, and the portion of this manure that can be used for biogas production was 23,428.42 tons.

In the Şarkikaraağaç district, the amount of manure to be obtained from calves in beef cattle enterprises was 24,679.71 tons. The total amount of manure to be produced was 43,926.53

tons, with 19,246.82 tons of manure to be obtained from the heifers. The amount of manure that can be used for biogas production was determined to be 21,963.26 tons.

In the Merkez district, the amount of manure to be obtained from calves in beef cattle enterprises was 9,596.47 tons, and the amount of manure to be obtained from the heifers was 22,972.01 tons, which all totaled 32,568.48 tons. The amount of manure that can be used for biogas production was calculated as 16,284.24 tons.

The amount of manure to be obtained from beef cattle enterprises in the Yalvaç, Şarkikaraağaç, and Merkez districts of Isparta Province that can be used as biogas corresponded to 55.49% of the total amount of manure that can be utilized for biogas in Isparta Province.

In the entire Isparta Province, the amount of manure to be obtained from calves in beef cattle enterprises was 93,430.03 tons, and the amount of manure to be obtained from the heifers was 128,883.30 tons, which all totaled 222,313.33 tons. The amount of manure that can be used for biogas production was 111,156.66 tons (Table 8).

Table 8. Annual amount of manure produced by beef cattle

Districts	Manure production (tons year^{-1})		Total manure production (tons year^{-1})	The amount of manure to be used in biogas production (tons year^{-1})	Ratio (%)
	Calf (<12 month)	Heifer (12-24 month)			
Yalvaç	17,626.52	29,230.32	46,856.85	23,428.42	21.08
Şarkikaraağaç	24,679.71	19,246.82	43,926.53	21,963.26	19.76
Merkez	9,596.47	22,972.01	32,568.48	16,284.24	14.65
Eğirdir	7,453.40	9,677.22	17,130.62	8,565.31	7.71
Sütçüler	6,721.83	7,731.84	14,453.67	7,226.84	6.50
Keçiborlu	7,655.66	11,159.01	18,814.67	9,407.34	8.46
Aksu	3,808.46	10,339.47	14,147.94	7,073.97	6.36
Gelendost	5,232.87	3,038.10	8,270.97	4,135.49	3.72
Atabey	3,696.58	7,375.88	11,072.45	5,536.23	4.98
Gönen	3,421.16	3,087.77	6,508.93	3,254.47	2.93
Senirkent	2,237.74	2,160.61	4,398.35	2,199.18	1.98
Yenişarbademli	710.05	1,026.50	1,736.55	868.27	0.78
Uluborlu	589.56	1,837.76	2,427.32	1,213.66	1.09
Total	93,430.03	128,883.30	222,313.33	111,156.66	100.00

When Table 7 and Table 8 were evaluated together, the amount of manure to be obtained from dairy cattle was calculated as 894,688.75 tons, the amount of manure to be obtained from fattening beef cattle was calculated as 222,313.33 tons and in total 1,117,002.08 tons. The amount of manure that can be used for biogas production was found to be including 447,344.37 tons in dairy cattle, 111,156.66 tons in beef cattle and 558,501.03 in total.

When the amount of biogas to be produced from the manure of cattle in Isparta Province and its districts was analyzed, the amount of biogas that can be produced by utilizing the manure obtained from dairy cattle in the Yalvaç district was calculated as 2,053,675.94 m^3 and the amount of biogas that can be produced by utilizing beef cattle manure was 796,566.38 m^3. It was observed that a total of 2,850,242.31 m^3 of biogas production is possible in the Yalvaç district.

In the Şarkikaraağaç district, the amount of biogas that can be produced by utilizing manure obtained from dairy cattle was 1,642,349.10 m^3, and the amount of biogas that can be produced by utilizing beef cattle manure was 746,750.96 m^3 It was determined that a total of 2,389,100.06 m^3 of biogas production is possible in the Şarkikaraağaç district.

In the Merkez district, the amount of biogas that can be produced by utilizing manure obtained from dairy cattle was 1,204,955.82 m^3, and the amount of biogas that can be produced by utilizing beef cattle manure was 553,664.08 m^3. It was calculated that a total of 1,758,619.91 m^3 of biogas production is possible in the Merkez district.

The amount of biogas obtained as a result of the conversion of manure collected from dairy and beef cattle enterprises into biogas in the Yalvaç, Şarkikaraağaç, and Merkez districts of Isparta Province corresponded to 54.60% of the total biogas production in Isparta Province.

The amount to be obtained as a result of converting the manure procured from dairy cattle enterprises into biogas in Isparta Province was 9,036,356.36 m^3. The amount to be obtained as a result of converting the manure procured from beef cattle enterprises into biogas was 3,779,326.57 m^3 and totally 12,815,682.93 m^3 in Isparta Province (Table 9 and Figure 6).

As a result of converting the biogas obtained from the cattle manure in Isparta Province and its districts into electrical energy, it was calculated that 13.396.14 MWe of electrical energy production is possible in the Yalvaç district. The electrical energy that can be produced from biogas in the Şarkikaraağaç district was 11,228.77 MWe, and the electrical energy that can be produced from biogas in the Merkez district was 8,265.51 MW. The electricity generation based on biogas in Isparta Province was 60,233.71 MWe (Table 10, Figure 7 and Figure 8).

As a result of the calculations, it was calculated that 5.30%–6.69% of the monthly electricity consumption of Isparta Province can be met by the electricity generated from manure obtained from cattle in Isparta Province and its districts. On an annual basis, it was calculated that 5.98% of the electricity needs of Isparta Province can be met by converting biogas from cattle manure into electrical energy (Table 11).

Table 9. Amount of biogas to be produced from cattle manure

Districts	Dairy cattle			Beef cattle			Total biogas production (m^3 $year^{-1}$)	Ratio (%)
	The amount of manure to be used in biogas production (tons $year^{-1}$)	Biogas yield (m^3 ton^{-1})	Biogas production (m^3 $year^{-1}$)	The amount of manure to be used in biogas production (tons $year^{-1}$)	Biogas yield (m^3 ton^{-1})	Biogas production (m^3 $year^{-1}$)		
Yalvaç	101,667.13	20.2	2,053,675.94	23,428.42	34.0	796,566.38	2,850,242.31	22.24
Şarkikaraağaç	81,304.41	20.2	1,642,349.10	21,963.26	34.0	746,750.96	2,389,100.06	18.64
Merkez	59,651.28	20.2	1,204,955.82	16,284.24	34.0	553,664.08	1,758,619.91	13.72
Eğirdir	49,064.88	20.2	991,110.51	8,565.31	34.0	291,220.51	1,282,331.02	10.01
Sütçüler	32,770.02	20.2	661,954.35	7,226.84	34.0	245,712.42	907,666.77	7.08
Keçiborlu	24,865.67	20.2	502,286.58	9,407.34	34.0	319,849.45	822,136.03	6.42
Aksu	23,296.73	20.2	470,593.96	7,073.97	34.0	240,514.92	711,108.89	5.55
Gelendost	20,337.97	20.2	410,826.99	4,135.49	34.0	140,606.54	551,433.53	4.30
Atabey	17,224.77	20.2	347,940.31	5,536.23	34.0	188,231.72	536,172.03	4.18
Gönen	15,387.49	20.2	310,827.25	3,254.47	34.0	110,651.84	421,479.09	3.29
Senirkent	13,494.44	20.2	272,587.59	2,199.18	34.0	74,771.99	347,359.58	2.71
Yenişarbademli	4,994.20	20.2	100,882.92	868.27	34.0	29,521.34	130,404.26	1.02
Uluborlu	3,285.40	20.2	66,365.04	1,213.66	34.0	41,264.43	107,629.47	0.84
Total	447,344.37		9,036,356.36	111,156.66		3,779,326.57	12,815,682.93	100.00

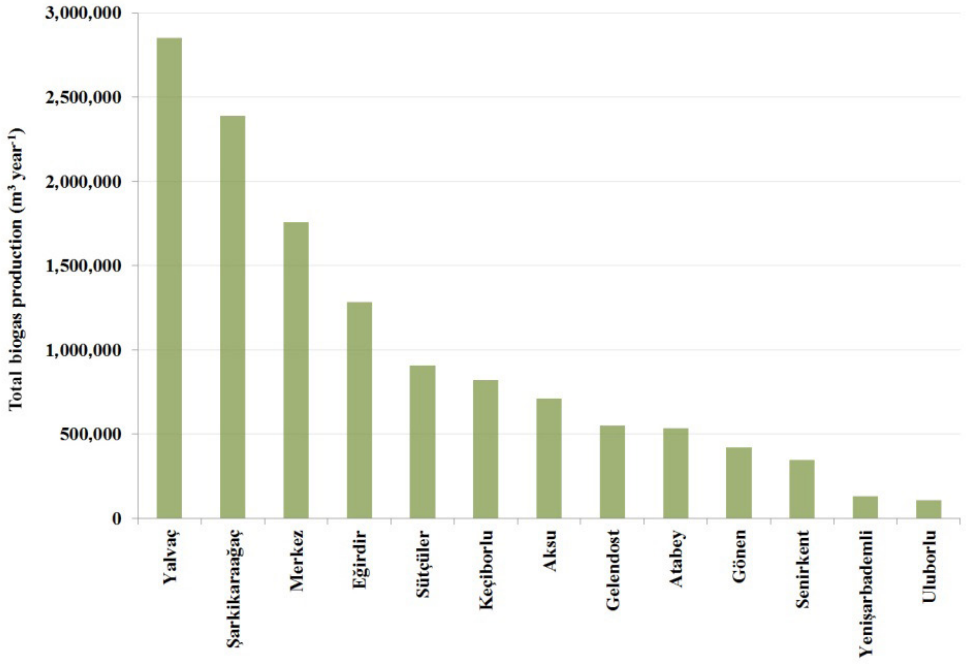

Figure 6. Amount of biogas to be produced from cattle manure in Isparta Province districts

Table 10. Amount of electrical energy to be generated from biogas

Districts	Total biogas production (m^3 $year^{-1}$)	Electricity generation from 1 m^3 biogas (kW $year^{-1}$)	Electricity generation from biogas (kW $year^{-1}$)	Electricity generation from biogas (MWe $year^{-1}$)	Ratio (%)
Yalvaç	2,850,242.31	4.7	13,396,138.88	13,396.14	22.24
Şarkikaraağaç	2,389,100.06	4.7	11,228,770.27	11,228.77	18.64
Merkez	1,758,619.91	4.7	8,265,513.56	8,265.51	13.72
Eğirdir	1,282,331.02	4.7	6,026,955.78	6,026.96	10.01
Sütçüler	907,666.77	4.7	4,266,033.82	4,266.03	7.08
Keçiborlu	822,136.03	4.7	3,864,039.34	3,864.04	6.42
Aksu	711,108.89	4.7	3,342,211.76	3,342.21	5.55
Gelendost	551,433.53	4.7	2,591,737.59	2,591.74	4.30
Atabey	536,172.03	4.7	2,520,008.53	2,520.01	4.18
Gönen	421,479.09	4.7	1,980,951.72	1,980.95	3.29
Senirkent	347,359.58	4.7	1,632,590.01	1,632.59	2.71
Yenişarbademli	130,404.26	4.7	612,900.01	612.90	1.02
Uluborlu	107,629.47	4.7	505,858.49	505.86	0.84
Total	12,815,682.93		60,233,709.76	60,233.71	100.00

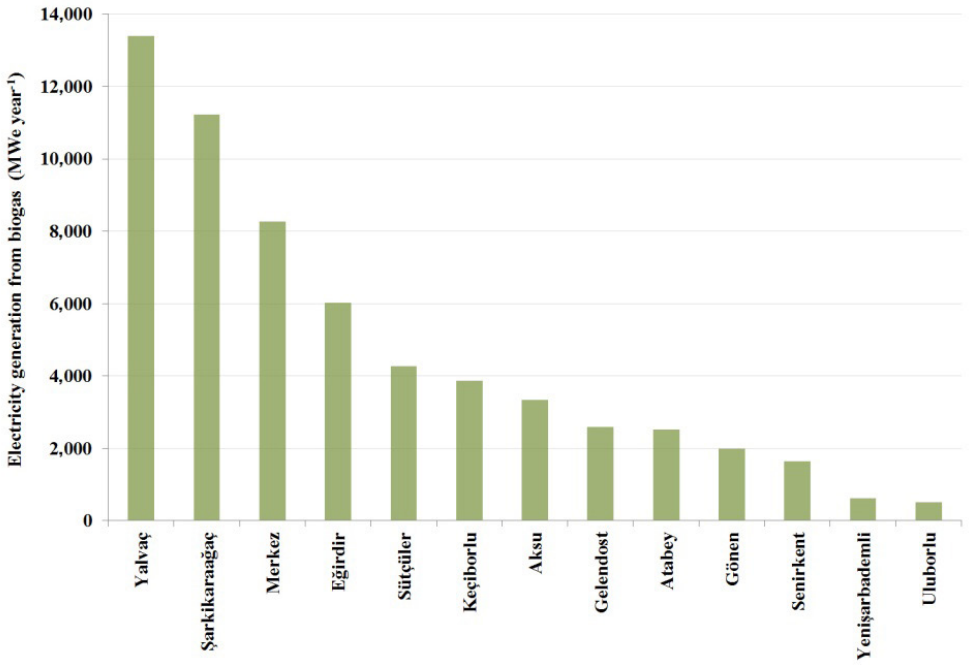

Figure 7. Amount of electrical energy to be generated from biogas in Isparta Province districts

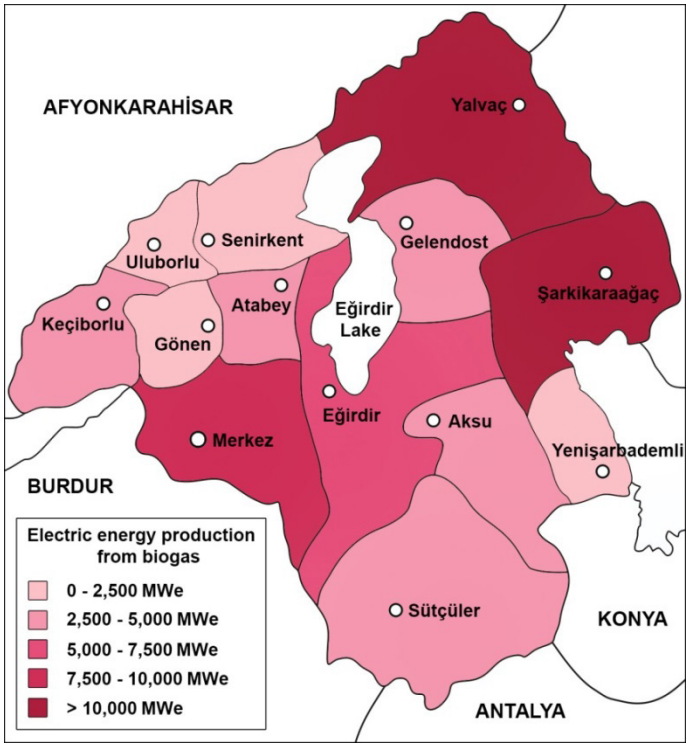

Figure 8. Amount of electrical energy to be generated from biogas by the districts

Table 11. Ratio of the amount of electrical energy to be produced to the electricity consumption

Months	Electricity consumption in Isparta* (MWh month^{-1})	Electricity generation from biogas (MWh month^{-1})	Ratio of production to consumption (%)
January	85,984.20	5,019.48	5.84
February	77,314.13	5,019.48	6.49
March	81,486.98	5,019.48	6.16
April	79,224.84	5,019.48	6.34
May	80,006.21	5,019.48	6.27
June	75,045.98	5,019.48	6.69
July	94,688.74	5,019.48	5.30
August	91,192.81	5,019.48	5.50
September	85,704.44	5,019.48	5.86
October	88,359.41	5,019.48	5.68
November	83,896.57	5,019.48	5.98
December	84,306.22	5,019.48	5.95
Total	1,007,210.53	60,233.71	6.00

*Monthly electricity consumption in Isparta (EPDK, 2019)

Conclusion

As of 2019, the amount of dairy cattle is 108,540 head, the amount of beef cattle is 37,280 head and total bovine animals are 145,820 head in Isparta province. The amount of manure to be obtained from existing dairy cattle is 894,688.75 tons and 222,313.33 tons from beef cattle in total 1,117,002.08 tons. The portion of this manure that can be collected and converted to biogas is 447,344.37 tons in dairy cattle, 111,156.66 tons in beef cattle and in total is 558,501.03 tons. The amount of biogas to be obtained by converting dairy cattle manure to biogas is 9,036,356.36 m^3, the amount of biogas to be obtained by converting beef cattle manure to biogas is 3,779,326.57 m^3 and in totally 12,815,682.93 m^3. The amount of electrical energy to be produced from biogas is 60,233.71 MWe.

The fact that Isparta province can meet 6.00% of its current electricity consumption if the existing cattle potential in Isparta Province is used for the production of electrical energy requires major projects to be conducted in the regional plan. It is necessary to determine the wastes that are obtained through animal and plant production in each province in Turkey, and make the necessary investments for their utilization. In this way, the use of electrical energy that can be produced by utilizing wastes obtained via animal and plant production in the agricultural sector will provide financial relief for the agricultural sector, as well.

Moreover, by converting animal and plant wastes into electrical energy, the negative effects that emerge due to these wastes will be eliminated. Many negative environmental factors, such as the risk of disease, bad odors, and the contamination of groundwater that emerge as a result of the accumulation of manure, will be prevented.

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.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

This study has been prepared by benefiting from the master thesis submitted by Yalcin Gonbe to Graduate Education Institute of Isparta University of Applied Sciences.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.9 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):323-328 (2021)

Economics of production and marketing of mandarin in Parbat and Baglung districts of Nepal

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Abstract

This study was conducted in mandarin growing areas of Parbat and Baglung districts of Gandaki Province where total of 67 representative farmers and 20 traders were selected as sample, in which 36 farmers were from Parbat and 31 farmers were from Baglung district. The average cost of Mandarin production per ropani (19.66 ropani= 1 hactare) was NRs. 17220 highest contributor being labor cost with 43.82 percent of share. Average return and profit of Mandarin farming per ropani was NRs. 48,978 and NRs. 31,757 respectively with B:C ratio of 2.93. Average return, profit and B:C ratio was found to be significantly higher in Parbat than Baglung. The Cobb-Douglas production function of Mandarin farming shows, output of Mandarin farming is significantly and positively affected by expenditure on labor, land rent, and cost of nutrients with the increasing return to scale (1.097). Producer-Pre-harvest contractor–Wholesaler-Retailor-Consumer was most prominent marketing channel with 55 percent share of total sold volume. Inadequate irrigation facility was the most prominent production problem, while poor marketing facility and access to market information were the most prominent marketing problems faced by farmers. Difficulty in transportation was recognized as the most prominent marketing problems faced by mandarin traders. Average marketing margin and producers' share were NRs. 45.95 and 57.41 percent respectively both of which were highest in Parbat. In Parbat, harvesting is carried in January and February, which serves as off-season production in domestic markets, which ensures higher per unit price for respective district's output among two districts.

Keywords: Mandarin, Benefit cost ratio, Production function, Marketing channel, Producers' share

Introduction

Mandarin is the one of the highly grown perennial fruit crop in the country. It is well liked for its taste and rich vitamin C content and consumed mainly as fresh fruit (Shrestha, 1996). It contributes almost 17 percent to the total volume of fruit production and 0.97 to the AGDPs of Nepal (MOF, 2019). Mid-hills of Nepal possess immense opportunity and potentiality for mandarin production due to suitable climate and unique topography (Shrestha and Verma, 1998). Also, the economic return from mandarin production is far greater than its contemporary crops i.e. cereal crops in case mid-hills of Nepal (NCRP, 2016). The market for mandarin is expanding and the demand is high (DOC, 2018). Mandarin farming is labor intensive enterprise which works out best because there is availability of cheap labor in the country. Understanding the factors that directly affect the cost and profitability and the economic advantages mandarin farming possess could attract more farmers towards it.

This could uplift the farmer from poverty and lead to sustainable and secure future ahead. Despite these possibilities

Cite this article as:
Baral, S., Marahatta, S., Shrestha, A. (2021). Economics of production and marketing of mandarin in Parbat and Baglung districts of Nepal. Int. J. Agric. Environ. Food Sci., 5(3), 323-328
Doi: https://doi.org/10.31015/jaefs.2021.3.9
Orcid: Samata Baral: 0000-0002-5981-5779 Santosh Marahatta: 0000-0002-5128-4029 Anish Shrestha: 0000-0003-2941-9828
Received: 28 June 2020 **Accepted:** 15 July 2021 **Published Online:** 30 July 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 323-328
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

there was no significant increase in production area in the last few years (AITC, 2019). Also the present productivity of mandarin production (9.43 Mt/ha) is far less than that of world's mandarin productivity i.e.13.03 Mt/ha (FAO, 2017). Thus, with the aim of realizing its opportunity this study was carried out analyze the economics of production and marketing along with problems in Parbat and Baglung districts of Nepal.

Materials and Methods

Study area

This study was conducted in Jaljala rural municipality of Parbat and Kathekhola rural municipality of Baglung of Nepal. They lie in mid-hill region of Western Nepal, which falls under Gandaki province. The sites were selected on the basis of cultivation area and production status within their respective districts.

Sample Size, Sampling Procedure and Selection of Respondents

For the study, total of 67 respondents were randomly selected as sample out of which 36 were from Parbat and 31 were from Baglung. Due to economic and manpower limitation, limited number of respondents were selected for the study.

Source of information

For primary data pre-tested systematic semi-structured questionnaire was used for face to face interview and key informant interview. Secondary information was collected by reviewing various books, reports, article and publication from Government of Nepal and other concerned agencies.

Data Collection and Analysis

The field survey and key informant interview for the study was conducted in March, 2019. Information obtained from the interview was crosschecked through key informants (Shrestha et. al., 2018). Collected data were coded, tabulated, summarized and analyzed for determination and interpretation. Microsoft Excel and STATA12 were used for analysis purpose.

Cost of production

Only operating cost were incorporated in the study, price of orchard establishment was not included in the estimation of total cost of production.

Total Cost = Cost of (Land rent + Nutrients + Inter culture + Protection measures + Labor)

Benefit Cost Analysis

Benfit cost analysis is the simplest method to analyze economic performance of any enterprise. It measures the amount of return per unit of input cost (Shrestha, 2017), which was estimated using following formula;

B:C ratio = Total Return/Total Cost

Profit analysis

Total cost of production is the sum of quantity of input multiplied by their respective prices, and total return is the multiplication of quantity of output and unit price of output. So the profit analysis can be done by subtracting total cost from total return (Debertin, 2012).

$\Pi = TR - TC$

$\Pi = \sum P_y \times Y - \sum P_{xi} \times X$

Where,

Π = net profit TR = total return

TC = total cost Y = quantity of output

Xi = quantity of i^{th} input Py = price of output

Pxi = price of i^{th} input

Marketing Margin and Producer's Share

Marketing margin is the difference between the price paid by the consumer and price actually received by the farmers for their produce. Producers' share is the percentage share of the producers on consumes' rupees. Lower marketing margin and higher producers' share is the indication of efficient and healthy marketing system (Shrestha et al., 2018).Marketing Margin (MM) = Retailer Price (P_r) – Farm Gate Price (P_f)

$$\text{Producer's Share (Ps)} = \frac{\text{Farm gate price (Pf)}}{\text{Retailer price (Pr)}} \text{ x100}$$

Factors Affecting Mandarin Production

In order to estimate the factors affecting Mandarin production general form of Cobb-Douglas type production function were applied. This form of production function were used by the likes of Islam et al. (2012) and Poudel et al. (2016) for resource use analysis of agricultural production. The estimating model for the coefficients of Mandarin production is, the following:

$Yg = a\, L^{b1} N^{b2}\, I^{b3} P^{b4}\, Lr^{b5} eu$

In log-log_form, the above model can be expressed as follows;

$\ln Yg = \ln a + b1 \ln L + b2 \ln N + b3 \ln I + b4 \ln P + b5 \ln Lr$

Where, Yg= Mandarin production (NRs),

L = Land rent (NRs)

N = Cost of nutrients (FYM + Chemical fertilizer)

I = Cost of inter-culture

P = Cost of protection measures

Lr = Cost of labor (included animal cost)

The intercept has been denoted by 'a' and 'bi' are the associated slope coefficient of the variable Xi, where i = 1…5.

Indexing / Scaling

Indexing technique was used to rank production and marketing problems faced by farmers. The index of importance of such problems was estimated by using the following formula (Shrestha, 2017);

$I_{imp} = \sum (S_i\, f_i)/N$ Where,

I_{imp} = Index of importance S_i = Scale value

f_i = Frequency of importance given by the respondents

N = Total number of respondents

Matrix Ranking

Matrix ranking is a tool or system used to analyze, compare and prioritize the problems, options or any related situation among them and establishes superiority of one above another. Matrix ranking was done by creating a matrix using sets of problems, which were identified using tools like literature review, key informant interview and focus group discussion, needed to compare and performing pair wise comparisons (Mahesh et al., 2017). Then, scoring and ranking of problem is done.

Results and Discussion

Average land holding and Mandarin cultivation area

The average land holding size of the overall sampled household was 17.99 ropani (0.92 ha) which is higher than

the national average of 0.65 ha (CBS, 2011). Such higher land holding is mainly due to the nature of farming area in such hilly district, as it comprises not only plain farming land, rather more sloped small hilly and forest area as well. The average number of mandarin plants per households is about 270 plants.

Cost of Mandarin production per ropani

Average operating cost of mandarin cultivation per ropani was estimated to be NRs. 17,220. Cost of labor was the highest contributor of the total cost with share of 43.82% which is similar to the findings of Bheel and Burak (2013) and Diliprao (2014). Details of cost of mandarin production per ropani along with their share of total cost are presented in Table 1.

Table 1. Cost of mandarin production in Parbat and Baglung districts of Nepal

Particulars	Cost	Percent Share
Land rent	4000	23.23
Machinery/animal	1180	6.85
Plant nutrient	4024	23.37
Plant protection	471	2.73
Labor cost	7545	43.82
Total	17220	100

Cost, Return and Profit of Mandarin Production

The average operating cost, return and profit of households was NRs. 17220, NRs. 48978 and NRs. 31757 respectively. Kafle (2007) found lower cost of cultivation which may be because the cost of labor, inputs and other cost have increased a lot since the time of that research. The return and profit from mandarin cultivation is significantly higher in Parbat than Baglung. In Parbat, harvesting is carried in January and February, which serves as off-season production in domestic markets, which ensures higher per unit price for respective district's output among two districts. Other details about average cost, return and profit among different district are presented in Table 2.

Benefit cost analysis of Mandarin cultivation

Average B:C ratio per ropani was estimated to be 2.93, which indicates highly profitable enterprise. That is double of what Bhat et al. (2011) reported in case Jammu, India, but similar result was reported in Lamjung by Pokhrel (2011).

Factors affecting mandarin production

Five explanatory variables namely human labor cost, intercultural cost, land rent, cost of nutrients and plant protection cost were considered to show their effects on production of mandarin. Cost of nutrient was significant at 1% level of significance. Land rent were significant at 5 % level of significance while Labor cost was significant at 10% level of significance. Kafle (2007) reported that cost of labor and expenditure on nutrient had positive impact on output of Mandarin production. Similarly, Shrestha (2015) also reported that expenditure on nutrients had positive impact on output of Mandarin farming.

The sum of the coefficients of factors of production was 1.097, which indicates production function of mandarin is in increasing return to scale. The coefficient of multiple determinations (R^2) of the model was 0.72, which indicates 72% of the total variations in the output occurred due the explanatory variables. The value of adjusted R square was 0.69, which indicates after taking into account the degree of freedom (df) 69% of the variation in output explained by explanatory variables of the model. Other details of resource use efficiency or factors contribution on mandarin production or return are shown in Table 4.

Table 2. Average cost of production, return and profit of Mandarin cultivation (NRs.) per ropani.

Description	Parbat (n=36)	Baglung (n=31)	Overall (n=67)	Mean difference	t-value
Cost	18060±5572	16381±5735	17220±5669	1679	1.150ns
Return	58655±34124	39302±13946	48978±27626	19353	2.875***
Profit	40594±31568	22921±13931	31757±25780	17673	2.805***

*** and ns indicates 1 % level of significance and non-significant respectively

Table 3. Benefit cost analysis (B:C Ratio) of Mandarin cultivation in Parbat and Baglung districts

	BC ratio			
District	Mean	Standard Deviation	Mean Difference	t-value
Parbat (n=36)	3.29	1.44	0.711	2.171**
Baglung (n=31)	2.58	1.05		
Overall (n=67)	2.93	1.31		

** indicates significance at 5 % level

Table 4. Estimated values of coefficients and related statistics of Cobb-Douglas production function of Mandarin

Factors	Coefficient (bi)	Std. Error	t-value	P> | t |
Constant	1.311	1.4333	0.91	0.364
Human labor cost	0.3860	0.1964	1.97	0.054*
Intercultural cost	-0.01232	0.02242	-0.55	0.585
Land rent	0.33456	0.14925	2.24	0.029**
Cost of nutrients	0.38515	0.1231	3.31	0.003***
Cost of plant protection	0.00395	0.02218	0.18	0.859
F-value	31.75			0.001***
R square	0.72			
Adjusted R-square	0.69			
Return to scale	1.097			

Note: ***, ** and * indicates 1%, 5% and 10% level of significance respectively.

Production problems

Inadequate irrigation facility was recognized by farmers as the most important problems with the index score of 0.86. Pokhrel (2011) and Poorwal (2012) supports the findings of this research. Roy et al. (2018) also stated that lack of irrigation facility and lower productivity are the main production problems of Mandarin. But, Myat Oo (2013) suggested disease and and insect infestation was the main production problem, while NHPC (2017) stated that poor quality planting material was the most important production problem of Mandarin.

Marketing problems faced by farmers

Poor market facility and information was recognized as the most important marketing problems faced by farmers with the index score of 0.72. Kafle and Rana (2003) and Myat Oo (2013) also supported the findings of this research. But, Diliprao (2014) stated that fluctuation and lower price was the most critical problem that Mandarin growers of Amravati, India were facing.

Marketing Problems Faced by Traders

Difficulty in transportation was ranked the most important problem with the matric score of 4. Similar results were found by Kumar et al. (2017) and Porwal (2012). But, Pokhrel (2011) stated that small scale production by farmers was the main marketing problem of traders.

Table 5. Major production problems of Mandarin cultivation faced by farmers.

Description	Parbat (n=36)		Baglung (n=31)		Total (n=67)	
	Index score	Rank	Index score	Rank	Index score	Rank
Disease and Insect infestation	0.71	III	0.43	IV	0.58	III
Poor quality planting material	0.34	IV	0.27	V	0.31	V
Inadequate technical assistance	0.79	II	0.75	II	0.77	II
Inadequate irrigation facility	0.86	I	0.88	I	0.87	I
Unavailability of sufficient labor	0.31	V	0.67	III	0.47	IV

Table 6. Major marketing problems of mandarin faced by farmers.

Description	Parbat (n=36)		Baglung (n=31)		Total (n=67)	
	Index score	Rank	Index score	Rank	Index score	Rank
Lower price	0.52	IV	0.61	II	0.56	II
Poor market facility and information	0.66	III	0.79	I	0.72	I
Lack of storage and processing facility	0.82	I	0.20	v	0.44	III
Poor transportation facility	0.77	II	0.61	III	0.44	IV
High post - harvest loss	0.23	V	0.22	IV	0.22	V

Table 7. Marketing problems faced by Traders during marketing of Mandarin

	Seasonal supply	Lack of storage facility	High marketing cost	Difficulty in transportation	Lack of Collection center
Seasonal supply	*	Lack of storage facility	High marketing cost	Difficulty in transportation	Lack of Collection center
Lack of storage facility		*	Lack of storage facility	Difficulty in transportation	Lack of storage facility
High marketing cost			*	Difficulty in transportation	Lack of Collection center
Difficulty in transportation				*	Difficulty in transportation
Lack of Collection center					*
Score	0	3	1	4	2
Rank	V	II	IV	I	III

Marketing Channels of Mandarin

Among the four marketing channel of the study area, Channel II was found to be the most prominent with 55 percent share of total marketed volume of produces. Similar findings were found by Kumar et al. (2017) and Pokhrel (2011), but Bhatt et al. (2011) identified channel-IV as the main marketing channel and Porwal (2012) identified similar to channel-I as the main marketing channel of mandarin.

Those marketing channels are as follows;

Channel-I: Producer – Collector – Retailer – Consumer

Channel-II: Producer – Pre-harvest contractor -Wholesaler – Retailer – Consumer

Channel-III: Producer – Retailer - Consumer

Channel-IV: Producer – Consumer

Marketing margin and Producers' share

Average marketing margin and producers' share was estimated to be NRs. 45.95 per Kg and 57.41 percent respectively. Kafle (2007) and Bhat et al. (2011) found somewhat similar results to the finding of this research. But, Shrestha (2015) reported lower producers' share i.e. 33.30 percent, which almost half of this finding.

Table 8. Share of Mandarin marketed by the sampled household through various channel

S.N.	Marketing channels	Parbat	Baglung	Overall
1	Channel-I	70	0	35
2	Channel-II	20	90	55
3	Channel-III	5	5	5
4	Channel-IV	5	5	5

Table 9. Marketing margin and producer share of Mandarin in the study area

Study Area	Farm gate price	Retail price	Market Margin	Producers' share
Parbat	88	140	52	59.09
Baglung	50.17	90	39.9	55.74
Total	69.08	115	45.95	57.41

Conclusion

All the above analysis and result shows us that mandarin farming is indeed very profitable business especially for the mid-hills of Nepal. However, there was no significant increase in production area in the last few years and the present productivity of mandarin is far less .There are various production and marketing problems that are acting as hindrance in the way to achieve economic stability through mandarin farming. Government intervention to support branding, export facilitation and market diversification of mandarin could lead to involvement of more and more farmers in mandarin farming rather than opting for foreign employment. Better production and marketing system must be developed so that more and more farmers can be attracted toward mandarin farming and can be used as a means to fight against poverty.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

The contribution of the authors to the present study is equal.

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

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.10 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):329-334 (2021)

Preliminary Study on Investigation of the Use of Effective Microorganism Applications in Walnut

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Abstract

It is observed that beneficial microorganisms are used effectively in agricultural practices that are important for human and environmental health. These microorganisms, which are known biological agents and microbial fertilizers, are important in terms of plant development, quality and reduction of input, thanks to the increase in the amount of nutrients in plants. In this study, it was goaled to determine the effects of EM.FPE and EM.5 as foliar sprays and EM.A as soil application on shoot length, some fruit parameters and yield and leaf nutrient elements in Chandler walnut variety. In the experiment, the longest shoots were measured with 55 cm in EM.A application. Similarly, the highest values in nut weight (12.85 g) and yield (2.28 kg tree^{-1}) were obtained in the same application. All applications positively affected kernel browning. These were found to increase leaf macro and micro nutrient content, except Cu and Fe.

Keywords: *Juglans regia* L., Microorganisms, Fruit properties, Nutrient, Yield

Introduction

The world population is increasing rapidly in spite of the decrease in agricultural areas and natural resources. Accordingly, increasing food demand can be achieved with increased yield. This situation depends on adequate intake of nutrients, in general (Zaman et al., 2014). Excessive use of chemical inputs that affect the product quantity and quality causes a decrease in soil fertility, economic and environmental damages. Today, the awareness of human and environmental health comes to the forefront, and it is important to use microbial fertilizers as an alternative to chemical fertilizers.

Microorganisms have been used in human, animal and environmental health, food processing, food safety and quality, genetic engineering and biotechnology for many years (Higa and Parr, 1994). The physiological effects of microorganisms promoting plant growth have attracted attention since the beginning of the 20th century (Bona et al., 2016; Parewa et al., 2014; Ruzzi and Aroca, 2015). These microorganisms are produced biologically active substances, which have an effect on growth and development (Çakmakçı, 2005). In perennial plants such as fruit trees, the effects of these applications were determined on vegetative development, fruit characteristics, yield, nutrient content and disease resistance (Demirci and Hancıoğlu, 2005; Ertürk et al., 2012; Güneş et al., 2015; İpek et al., 2014; Karakurt et al., 2011; Karlıdağ et al., 2013; Thakur et al., 2015).

Intake of some nutrients in the soil is limited or difficult, so that foliar applications are even more important for plant nutrition. Applications that benefit to the plant in a shorter period and have a direct effect on the amount of product are widely used. In fruit species, microorganisms are applied to the leaves and flowers as well as soil applications as alone or

Cite this article as:
Bilgin, N.A., Yagmur, B., Misirli, A. (2021). Preliminary Study on Investigation of the Use of Effective Microorganism Applications in Walnut. J. Agric. Environ. Food Sci., 5(3), 329-334
Doi: https://doi.org/10.31015/jaefs.2021.3.10
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Received: 23 May 2020 **Accepted:** 29 July 2021 **Published Online:** 11August 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 329-334
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

combination (Atılgan et al., 2019; Eşitken et al., 2010).

Microorganisms that are important for sustainable agriculture and environment are expressed as "Effective Microorganisms (EM)" (Higa and Parr 1994). "EM" technology is not a single type of microorganism; it represents various microorganisms. This group includes photosynthetic and lactic acid bacteria, yeasts, fungi and effective enzymes. "EM" is used in agriculture for various purposes (Acarsoy Bilgin, 2019; Anonymous, 2020; İşçi et al., 2019).

Recently, it is pointed out that microorganisms can be useful in different plant species, climate and soil conditions (Çakmakçı, 2005). In this regard, there are a very limited number of researches on walnuts, although many fruit species have been studied. In our country, new plantations have been established with Chandler walnuts, which have high quality fruit characteristics. In this trial planned in the mentioned walnut variety, it was aimed to determine the use of effective microbial fertilizers on shoot length, some fruit parameters, yield and leaf nutrient elements.

Materials and Methods

The current study was conducted in Manisa/Demirci location in 2018 year. Chandler walnut variety (6-years-old) was used as plant material. This variety is foliar and blooms in the late period. Fruits ripening in the middle season are large, oval and smooth. The shell breaks more quickly and easily (Özçağıran et al., 2014).

EM.FPE (2 cc lt^{-1}) and EM.5 (2 cc lt^{-1}) as foliar sprays and EM.A (2 cc lt^{-1}) as soil application were treated to plant material. These microbial fertilizers belong to Agriton Company. The applications were carried out 3 times such as before the blooming male flowers, after the blooming male flowers and hazelnut-sized fruits for each microorganism. The content of the applied microbial fertilizers is given below (Table 1).

Table 1. Microbial fertilizers and their contents.

Fertilizers	Content	Application method
EM.FPE	Lactic acid bacteria (*Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus rhamnous*), Yeast (*Saccharomyces cerevisiae*) Phototrophic bacteria (*Rhodopseudomonas palustris*)	Foliar
EM.5	Lactic acid bacteria (*Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus rhamnous, Lactobacillus casei*), Yeast (*Saccharomyces cerevisiae*) Phototrophic bacteria (*Rhodopseudomonas palustris*)	Foliar
EM.A	Lactic acid bacteria (*Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus rhamnous, Lactobacillus casei, Lactobacillus delbrueckii*), Yeast (*Saccharomyces cerevisiae*) Phototrophic bacteria (*Rhodopseudomonas palustris*) Others	Soil

The shoot length (cm) was measured in terms of morphological features. The harvested fruits were separated from green peels and dried in the shade. Average nut and kernel weight were determined on precision electronic scale (0.01 g) then the kernel ratio (%) was calculated. The widths, height, length of the nut and shell thickness were stated using a digital caliper sensitive to 0.01 mm (Şen, 1980). Fruit color was measured by a CR400 model Minolta Colorimeter in CIE L* a* b*. For yield, the total amount of nut was recorded in each tree at harvest time (kg). In fruit which is accepted as 4 parts the shrinkage ratio of each part was determined as % (Aşkın and Gün, 1995; Beyhan, 1993; Şen, 1980). In addition, kernel browning ratio (%) was found. For nutrient analysis, the dried leaf samples were ground. The Kjeldahl method for N; the colorimetric method for P; the flame fotometric method for Ca and K; atomic absorption spectrophotometer for Mg, Fe, Cu, Zn and Mn analyses were used (Kacar and İnal, 2008).

The experiment was carried out according to the design of the random blocks, with 3 replications and 3 trees per replication. 10 samples were evaluated for each replication. The data were subjected to analysis of variance using SPSS 20 statistical package program. Significant differences between averages were defined by Duncan test at the $P<0.05$ significant level.

Results and Discussion

Some properties of Chandler walnut variety according to applications are given in Table 2. According to this, the longest shoots were measured with 55 cm in EM.A application. This was followed by control (47 cm) and EM.5 (42.72 cm), respectively. The shortest shoots were determined in the EM.FPE (36.93 cm) microbial fertilizer. Considering some fruit properties, while there was a statistical difference between applications in terms of nut weight and kernel browning, other features did not change (Table 2). In terms of nut weight, the heaviest fruits were obtained in application EM.A (12.85 g). After that EM.FPE (12.58 g) and EM.5 applications (12.47 g) took place. In contrast, the control group was observed the last row (11.97 g). It was seen that the applications had a positive effect on the kernel browning. Although it was not statistically important, the value of shell thickness, nut width, length and height was found to be lower than the others. Applications led to increased yield per tree. In this way, EM.5 (2.52 kg)

and EM.A (2.28 kg) applications were the first group, while EM.FPE (1.28 kg) and control (0.78 kg) were in the second group.

There are many studies on the effects of plant growth promoting rhizobacteria (PGPR) in fruit species (Aslantaş et al., 2007; Eşitken et al., 2003; Dede, 2013). In this study, in which the effects of microbial fertilizer applications on some properties of Chandler walnut variety were tested, EM.A and EM.5 applications increased the shoot length and this effect was detected in different bacterial applications on sweet cherry (Atılgan et al., 2019) sour cherry (Arıkan and Pırlak, 2016), apple (İpek et al., 2016), strawberry (Parewa et al., 2014), raspberry (İpek et al., 2018) and hazelnut (Ertürk et al., 2011). It was reported that this increase may be caused by the change of hormone level (İpek et al., 2014). In our study, bacterial applications had a significant effect on nut weight. Fruit sizes are not statistical but relatively increased compared to control. It was stated that the effect of *Pseudomonas fluorescens* bacteria varies for nut weight, whereas significant increase for fruit width and length was determined on the same walnut variety. In this respect, alone and combination bacteria applications are important in terms of apple fruit weight and yield (Aslantaş et al., 2007; Eşitken et al., 2006; Karakurt, 2006; Karlıdağ et al., 2007; Pırlak et al., 2007). These treatments led to an increase in yield on quince (Gerçekoğlu et al., 2018) and sour cherry (Arıkan and Pırlak, 2016). Likewise, on strawberry (Ağgün et al., 2018) and raspberry (İpek et al., 2018), the positive effect of root bacteria application was mentioned on these properties. It was emphasized that combined microbial fertilizer application (EM.5 + EM.FPE) differed with regard to fruit weight, width and length and yield compared to control in olives tree. Thus, it was concluded that combined application is more effective (Acarsoy Bilgin, 2019).

Table 2. Shoot length, some fruit properties and yield according to applications

Applications Properties	Control	EM.FPE	EM.5	EM.A
Shoot length (cm)	47.00 ab	36.93 b	42.72 ab	55.00 a
Nut weight (g)	11.97 b	12.58 ab	12.47 ab	12.85 a
Kernel weight (g)	5.98^{ns}	5.99	5.83	5.99
Kernel ratio (%)	49.99^{ns}	47.62	46.73	46.66
Shell thickness (mm)	1.51^{ns}	1.66	1.68	1.70
Nut width (mm)	34.45^{ns}	33.58	33.98	34.48
Nut length (mm)	32.33^{ns}	32.71	32.84	33.11
Nut height (mm)	40.10^{ns}	41.61	40.99	41.24
Kernel L*	53.91^{ns}	55.05	56.07	54.07
Kernel a*	7.32^{ns}	7.43	7.39	7.97
Kernel b*	28.99^{ns}	28.97	28.79	28.51
Shrinkage ratio (%)	11.67^{ns}	8.33	8.33	13.33
Kernel browning (%)	20 a	0 b	0 b	0 b
Yield (kg tree^{-1})	0.78 b	1.28 b	2.52 a	2.28 a

The differences in the means were determined by the Duncan test according to P≤0.05. ns: Not significant

As a result of microbial fertilizer applications, macro and micro nutrient contents determined in Chandler walnut leaves are given in Table 3. It was seen that these applications had an important effect on the nutrient content. The effect of the applications on the N, P, K, Ca and Mg content of the leaf was statistically significant at the level of 5%. EM.FPE application with the highest value in terms of all macro elements took the first place. On the other hand, the nutrient content was the lowest in untreated trees. Leaf N nutrient content ranged from 2.42% (control) to 3.04% (EM.FPE). While all applications formed the same statistical group, control was included in different groups. Compared with limit values (2.47-2.98%), N content was determined to be sufficient except untreated trees (Mills and Jones, 1996). Thus, applications had a positive effect. Leaf P nutrient content varied from 0.09% to 0.14%. EM.FPE application, in which the highest content was determined, ranked the first row and formed a separate statistical group. This was followed by EM.5 and EM.A with the same value (0.11%). Even though applications increase P content, limit values (0.16-0.24%) were not reached (Mills and Jones, 1996). The highest value in terms of leaf K nutrient content belongs to EM.FPE application with 1.88%. This application was in the same group with the others, except untreated trees. Control located at the second group with 1.55%. This element content was found above the limit values (1.32-1.47%) in all applications (Mills and Jones, 1996). Leaf Ca nutrient content was similar to K. Accordingly; the Ca content variation range was determined as 1.43% (control) - 2.60 (EM.FPE). It was noteworthy that the applications exceed the limit values of 1.90-2.01% (Mills and Jones, 1996). Leaf Mg nutrient content was determined with the highest values in EM.FPE (0.74) and EM.A (0.70%) applications, respectively. EM.5 and control were in the same group. It was seen that Mg content was the limit value (0.51-0.63%) and above in all applications

including control.

Similar to the macro element content of the leaves, the effect of the applications was statistically important on the Fe, Cu, Zn, Mn and B content of the leaf (Table 3). The highest contents were determined in EM.FPE application, except Zn. In contrast, the lowest value was found in the control group for other elements except Fe and Cu. Leaf Fe element content ranged from 74.97 ppm (EM.5) to 138.20 ppm (EM.FPE). Although EM.FPE application limit values (69-129 ppm) are exceeded, other applications are also included in limit values (Mills and Jones, 1996). For Cu content, EM.FPE was in the first place and in a different group with 8.83 ppm. Other applications were in the same group. EM.5 had the lowest value. All applications are seen below the limit values of 10-12 ppm (Mills and Jones, 1996). Leaf Zn content reached the highest value with EM.A (23.58 ppm). This was followed by EM.FPE with 20.67 ppm. Control and EM.5 were in the same group. This content was determined below the limit values (Mills and Jones, 1996). The leaf Mn nutrient content significantly increased compared to control in EM.FPE (447.20 ppm) and EM.A (393.47 ppm) applications, respectively. The lowest Mn content was found in the untreated trees with 129.27 ppm. EM.5 constituted the second group. Microbial fertilizer applications have increased the Mn content too above the limit values (Mills and Jones, 1996). Leaf B content varied between 26.59 ppm and 41.23 ppm. Accordingly, EM.FPE located the first group and the others were the second group.

In this study conducted by us, applications were determined to have a statistically significant effect in terms of nutrient content. Similarly, it was reported that the increase in the content of leaf nutrients was important as a result of different bacterial applications in Heritage raspberry (İpek, 2019) and Italian grape (Erdoğan et al., 2018). Also, confirming our findings, leaf P and Fe content increased by bacterial inoculations on apple (Pırlak et al., 2007), cherry (Eşitken et al., 2006), hazelnut (Ertürk et al., 2011) and strawberry (İpek et al., 2014). These applications were reported to affect the P and K content on pomegranate, in addition (Fayed, 2010). It was stated by İpek (2019) that leaf nutrient content varies according to bacterial strains, fruit species and varieties. It is seen that the use of microbial-based fertilizers has become widespread in recent years (Küçük, 2019). Through bacteria, nutrients are transformed into a form that plants can use, thereby increasing nutrient uptake (Malua and Vassilev, 2014). Consequently, in our study, EM applications contributed positively to nutrient content and fruit properties.

Table 3. Macro and micro nutrient content

Applications Properties	Control	EM.FPE	EM.5	EM.A
N (%)	2.42b	3.04 a	2.76a	2.79a
P (%)	0.09 c	0.14 a	0.11 b	0.11 b
K (%)	1.55 b	1.88 a	1.77 a	1.85 a
Ca (%)	1.43 b	2.60 a	2.31 a	2.40 a
Mg (%)	0.56 b	0.74 a	0.57 b	0.70 a
Fe (ppm)	122.13 ab	138.20 a	74.97 c	112.47 b
Cu (ppm)	7.03 b	8.83 a	6.40 b	7.27 b
Zn (ppm)	16.44 c	20.67 b	17.96 c	23.58 a
Mn (ppm)	129.27 c	447.20 a	277.77 b	393.47 a
B (ppm)	26.59 b	41.23 a	27.14 b	28.78 b

The differences in the means were determined by the Duncan test according to $P \leq 0.05$.

Conclusion

Due to environmental and human health awareness, studies focused on protecting the environment, reducing the use of chemical fertilizers. Sustainable agricultural techniques are of great importance. In this regard, microorganisms used as bio fertilizers have a positive effect on many properties in horticultural plants. In this study, where effective microorganisms were tested, the best results were obtained from EM.A application in terms of shoot length, nut weight, kernel browning and yield. In addition, all applications positively affected nutrient content compared to control, except Cu and Fe. In this context, it is thought that it would be beneficial to plan the applications according to the methods and doses.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgement

The authors thank to Kerem Kıncı and Agriton Company for supplying the plant material and microbial fertilizers.

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International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2021.3.11

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 5 (3):335-342 (2021)

Analysis of Some Factors Affecting the Growth of Castor Shrub and Suitability of its Seed Oil in Industrial Application

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Abstract

Some concern had been shown regarding the limited availability of castor seed to satisfy the rising yearning for its seed oil for use in industrial and domestic applications. This growing demand calls for refocus on backward integration in order to ensure sustained supply chain. This study adopts a factorial analysis that involves the use of Principal Component Analysis (PCA) and Kendall's Coefficient of Concordance (KCC) as statistical procedures to analyze some critical factors affecting the growth of castor shrub and its seed. KCC analyzed the degree of agreement among the fifteen Judges who ranked the thirty-two identified variables affecting the growth of castor shrub and the suitability of its seed oil in industrial application in descending order of importance. The result of the KCC showed an index of concordance in ranking as W=0.61 indicating 61% agreement among the 15 judges. The PCA helped to analyze the Judges responses arranged in form of data matrix that was facilitated by the use of statistiXL software. The PCA result revealed significant parsimony in data reduction from thirty-two to four principal factors creatively labeled: Seed oil particularities, Resource Conversion Efficiency, Plant-cooperation-oriented yield and Soil Condition respectively. The implication of this is that the principal factors that influence the growth of castor shrub and the suitability of its seed oil in industrial application has been identified.

Keywords: Castor Shrub, Backward Integration, Variables, Castor Seed, Growth

Introduction

Some concern had been expressed over the limited availability of castor seed to produce drying oil discovered to be a close substitute to sunflower oil that is currently being used for wood surface coating. Since castor seed has been found to be a good substitute for sunflower drying oil, the need therefore to ensure steady cultivation of the shrub and subsequent sustainable production of the oil has become imperative if for instance nations in Sub Sahara Africa are to improve their economy through manufacturing of surface coatings, polymers, lubricants for aircrafts, cosmetics, food seasoning, perfumery products, ointments, waxes, pharmaceuticals, printing inks, hair dressings, nylon, enamels, electrical insulations and disinfectants. Attention has been focused on using rubber seed as a substitute but the major problem with that policy approach is that it takes years for rubber to grow to maturity and to fructify. Even as of now, planting of castor seed is being done by subsistence farming in isolated areas of Sub-Saharan Africa. Moreover, the potential utilization of this oil is yet to be explored. Previous research has addressed the use of castor seed

Cite this article as:
Omotehinse, S.A. (2021). Analysis of Some Factors Affecting the Growth of Castor Shrub and Suitability of its Seed Oil in Industrial Application. J. Agric. Environ. Food Sci., 5(3), 335-342
Doi: https://doi.org/10.31015/jaefs.2021.3.11
Orcid: Samuel Ayodeji Omotehinse: https://orcid.org/0000-0002-5036-0800
Received: 13 July 2020 **Accepted:** 16 April 2021 **Published Online:** 18 August 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 335-342
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

oil in various industrial applications but are yet to look into the area of backward integration that consider the best possible ways of growing this seed plant so as to generate sustained supply of the seed. According to Pommerening and Muszta (2015) Analyzing and modeling plant growth remains a vital "interdisciplinary field of plant science. The use of relative growth rates, involving the analysis of plant growth relative to plant size, has independently emerged in different research groups and at different times and has provided powerful tools for assessing the growth performance and growth efficiency of plants and plant populations". The authors reviewed and combined "existing methods of analyzing and modeling relative growth rates and applied a combination of methods to Sitka spruce (Picea sitchensis (Bong.) Carr.) stem-analysis data from North Wales (UK) and British Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) yield table data". The results obtained "showed that, by combining the approaches of different plant-growth analysis laboratories and using them simultaneously, we can advance and standardise the concept of relative plant growth". Vanaja et al. (2008) evaluated the "growth and yield responses of castor bean (Ricinus communis L.) to two elevated CO_2 levels (550 and 700 ppm)". The elevated CO_2 levels were found to "significantly increased the total biomass and yield of castor bean while enhanced CO_2 levels per se did not changed the content and quality of the castor oil".

The literature on the use of castor seed for industrial production is plentiful. Early studies on the use of castor seed dates back to mid-20th century when its dehydrating properties were not still well known but was punitively administered to war prisoners during the World war II by Italian Fascist leader Mussolini Benito (1883-1945). Trevino and Trumbo (2002) studied "the utilization of castor oil as a coating application by converting the hydroxyl functionalities of castor oil to β-ketoesters using *t*-butyl acetoacetate". Others include: Berman et al. (2011), Lima *et al.* (2011), Shojaeefard *et al.* (2013), Thakur and Karak (2013), Calegari *et al.* (2017) and Wang *et al.* (2017). Although "studies have shown promising results for the use of castor oil as a technically feasible biodiesel fuel, a major obstacle still exists in its use as a biodiesel in some countries such as Brazil. For example, in Brazil, government policies promoted castor as a biodiesel feedstock in an attempt to bring social benefits to small farmers in the semi-arid region of the country", see for example, Hall et al. (2009) and da Silva (2010). Furthermore, in recent years, "the oil is largely used in the specialty chemical industry worldwide, and the growth of its consumption is limited by insufficient and unreliable feedstock supply rather than by the industry demand" (Severino et al., 2012a and Mubofu, 2016). That said, regarding the models employed, the application has been widely described in literature such as Scott et al. (2003), Kongbonga et al. (2011), Jolliffe (2012), as well as Baran and Newman (2017). Principal component analysis (PCA) "is a useful tool that has been widely used for the multivariate analysis of correlated variables and also to analyze data in various fields, such as in administration, social sciences, engineering, chemistry and biology". In many studies such as that of Igboanugo et al., (2016) attempts was made to conjointly apply Kendall's coefficient of concordance as well as Principal component analysis (PCA) to analyze factors or unique variables that influence quality and productivity in fibre cement roofing sheet. Similar studies include: Meira et al. (2016), Goodarzi et al. (2011), Tomazzoni et al. (2013), Mueller et al. (2013), Karthil et al. (2014). Others are: Placide et al., (2015) and Anderson et al., (2017) respectively.

It is vividly clear from the foregoing review that past research on the use of PCA and KCC to analyze factors that influence castor shrub growth and the suitability of its seed oil in various industrial and domestic application appears limited. The major "advantage of PCA approach adopted is that it provides a correlation matrix that relativizes the interplay among the identified factors". This study is aimed at identifying some variables through literature that militate against or influence the growth of castor shrub and suitability of its seed oil in industrial application. The identified variables will rotate to form a cluster of related variables labeled as factors to give a better understanding of the way the variables inter correlated. In this way, policy framework on how to enhance the growth of the castor shrub and hence increase yield of the castor seed can be forged. It is hoped that the research outcome would guide backward integration so that sustained supply of castor seed can be achieved.

Materials and Methods

Materials

The primary data used in this study were the thirty-two (32) variables artistically selected from literature survey that militate against or influence the growth of castor shrub and suitability of its seed oil in industrial application.

Methods

Kendall's coefficient of concordance (KCC)

A mixed bag of variables, thirty-two in number artistically selected from literature survey, were used to craft set of questionnaires that were administered to knowledgeable respondents. Under this regime, first set of questionnaires were

administered to twenty five (25) selected judges where only fifteen (15) who ranked the variables in descending order of importance were retrieved. The respondents' scores were used to form a data matrix of 15 by 32 in dimension. The degree of concordance among the judges was computed as W. A test statistic called chi square (X^2) helped to assess the significance level of the judges ranking. The X^2- test guided the application of hypotheses:

H_0: Judges ranking are discordant

H_1: Judges ranking are consistent

Decision Rule: if $X^2_{cal} > X^2_{tab}$, we say that we do not have sufficient evidence to accept the null hypothesis, H_0: and hence accept the alternate hypothesis.

The Kendall coefficient of concordance is given by

$$W = \frac{S}{\frac{1}{12}K^2\left(N^3 - N\right)} \qquad (1)$$

where,

$$S = \sum\left(R_j - \frac{\sum R_j}{N}\right)^2$$

R_j = The Column sum of ranks

N = The total number of Variables

S = Variance

K = The number of Judges

The ranking by the judges were polled to obtain a sequence of well-ordered scale items.

Principal Component Analysis (PCA)

The second set of questionnaires that also contains 32 critical variables were administered to one hundred and thirty (130) respondents where only hundred (100) were retrieved for their expert evaluations. The factorial analysis of the respondent's scores collated as data matrix were solved using StatistiXL software. The output of the analysis include the following: Scree plot, factor plot, correlation matrix, eigenvalues, eigenvector, descriptive Statistic, unrotated factor loading, varimax rotated factor loadings, case-wise factor scores, explained variance among others. Factor matrix interpretation was rendered based on the StatistiXL outputs.

From the data matrix the correlation matrix was obtained using Equation (2) as stated below:

$$r_{ij} = \frac{\sum xy}{\sqrt{\left(\sum x^2\right)\left(\sum y^2\right)}} \qquad (2)$$

where, $x = X_{ij} - \bar{X}_{.j}$

$$y = Y_{ij} - \bar{Y}_{.j}$$

$$\bar{X}_{.j} = \frac{\sum_{i=1}^{N} X_{ij}}{N}$$

$$\bar{Y}_{.j} = \frac{\sum_{i=1}^{N} Y_{ij}}{N}$$

$$N = n_j = I = i_{\max}$$

$$J = j_{\max}$$

Results and Discussion

Result of Kendall Coefficient of concordance (KCC)

To calculate the Kendall's coefficient of concordance *(W)*, equation 1 was used as follows:

$$W = \frac{S}{\frac{1}{12}K^2\left(N^3 - N\right)}$$

$$S = \sum\left(R_j - \frac{\sum R_j}{N}\right)^2$$

From Factor Ranking Matrix

From Factor Ranking Matrix

$$\sum R_j = 7{,}923$$

$$\frac{\sum R_j}{N} = \frac{7{,}923}{32} = 247.59$$

$$S = \sum\left(R_j - \frac{\sum R_j}{N}\right)^2 = 376{,}032.75$$

$$\text{Therefore } W = \frac{376{,}032.75}{\frac{1}{12}\times 15^2\left(32^3 - 32\right)} = \frac{376{,}032.75}{613800} = 0.61$$

This result indicates that 61 percent agreement was observed among the 15 judges.

$$\text{Also, } \chi^2_{cal} = K\,(N-1)\,W \qquad (3)$$

where, K = 15, N = 32, W = 0.61

$$\therefore\ \chi^2 = 15(32-1)0.61 = 283.65$$

Test of Hypothesis:

H_0: The ranking of the fifteen (15) judges are discordant.

H_1: The ranking of the fifteen (15) judges are consistent.

Since $X^2_{cal}=283.65 > X^2_{tab}=44.97$, we reject the null hypothesis (H_0) and accept the alternate hypothesis. This infer that the judges ranking of the 32 variables were consistent.

Table 1 depicts the merit order sequentiality of the 32 variables ranked by the fifteen Judges and analyzed with Kendall coefficient of concordance. The R_js determine the ranking order.

Table 1. Merit order sequentiality of 32 variables for castor shrub growth

S/N	R_j	Variables	S/N	R_j	Variables
1	45	Soil Composition	17	276	Intermolecular force
2	61	Adhesion force	18	278	Storage stability
3	78	Raw material	19	290	Type of Alkyd Resin
4	88	Good shelf life	20	293	Chemical Oxidation
5	117	High Cohesive Strength	21	301	Blend of Driers
6	123	Environmental factor	22	314	Right raw materials
7	136	Trans-esterification	23	319	Reaction Temperature
8	138	Surface Coating	24	328	Moisture content
9	159	Soil Nutrient	25	329	Type of solvent
10	164	Capital Intensive	26	351	Solubility
11	174	Type of Fertilizer	27	352	Physicochemical Properties
12	208	Seed dormancy	28	375	Intercropping
13	249	Disease	29	391	Refining
14	252	Mode of Extraction	30	392	Soaking
15	254	Production Process	31	408	Heating
16	255	Dehydration	32	410	Seed Varieties

Result of the Principal Component Analysis (PCA)

The data obtained from the second questionnaire were arranged in matrix form based on the 5–point Resis-Likert scale. The scree plot showing the elbow at (3, 1) is depicted in Figure 1. It is obvious from the scree plot in Figure 1 that at eigenvalue of 1, and component number 3, the curvity tends to flatten out, suggesting that four factors extracted are adequate. This demonstrate a significant parsimony in factor reduction from 32 to mere 4.

The result of the varimax rotated factor matrix is depicted in Table 2.

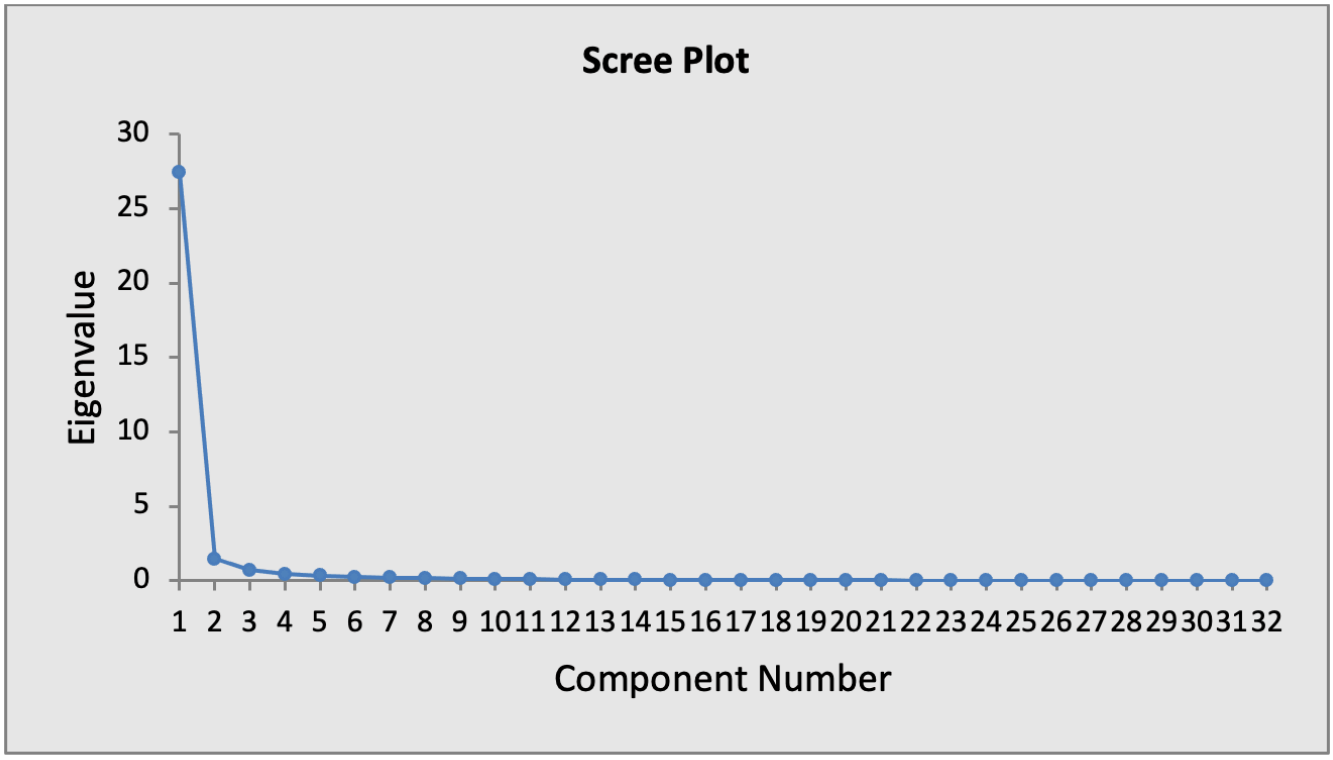

Figure 1. Scree Plot

Table 2. Varimax Rotated Factor Loadings matrix of 32 variables for surface coating manufacture

S/N	Variables	Factor 1	Factor 2	Factor 3	Factor 4
1	Soil Composition	0.406	0.490	0.247	0.716
2	High Cohesive Strength	0.456	0.824	0.207	0.090
3	Environmental Factor	0.791	0.453	0.178	0.256
4	Raw Material	0.462	0.574	0.301	0.317
5	Production Process	0.734	0.540	0.164	0.156
6	Trans-esterification	0.738	0.541	0.246	0.171
7	Seed dormancy	0.460	0.807	0.172	0.183
8	Type of Fertilizer	0.740	0.549	0.166	0.207
9	Soaking	0.388	0.726	0.199	0.407
10	Solubility	0.571	0.516	0.414	0.236
11	Adhesion Force	0.423	0.825	0.172	0.203
12	Good Shelf Life	0.358	0.697	0.315	0.327
13	Physicochemical Properties	0.636	0.393	0.548	0.268
14	Surface Coating	0.815	0.380	0.234	0.263
15	Dehydration	0.830	0.423	0.209	0.124
16	Blend of Driers	0.435	0.812	0.177	0.232
17	Type of Alkyd Resin	0.514	0.616	0.291	0.131
18	Chemical Oxidation	0.734	0.419	0.216	0.178
19	Disease	0.487	0.779	0.245	0.121
20	Reaction Temperature	0.507	0.787	0.205	0.125
21	Soil Nutrient	0.401	0.767	0.213	0.267
22	Intermolecular Force	0.792	0.506	0.159	0.175
23	Moisture Content	0.621	0.450	0.488	0.279
24	Storage Stability	0.497	0.582	0.298	0.186
25	Type of Solvent	0.779	0.482	0.192	0.141
26	Raw Material Formulation	0.392	0.758	0.231	0.326
27	Intercropping	0.648	0.306	0.627	0.220
28	Capital Investment	0.757	0.392	0.233	0.192
29	Refining	0.364	0.636	0.325	0.378
30	Mode of Extraction	0.720	0.400	0.395	0.257
31	Seed Varieties	0.808	0.416	0.223	0.252
32	Heating	0.566	0.728	0.146	0.198

Factor Interpretation

As explained under the methods section, Varimax rotation enhances better redistribution of factors as to promote factor interpretability. The scree plot of Figure 1 indicates that at the elbow (3, 1), Factors 1, 2 and 4 stand out. However, Factor 3 wielded substantial complementary loading which it shared with Factor 1 under physicochemical properties and intercropping (serial numbers 13 and 27 respectively). In this way, the 32 variables clustered under four factors – a significant reduction in variables dimension.

Factor 1: Seed Oil Particularities

This regime clustered 16 variables. All the factor loadings are positive. It is therefore a principal stocky factor providing relevant information about the physicochemical properties of the castor shrub and its seed. The utmost dominant variable, judging by its factor loading of 0.830, is dehydration. This could be due to the fact that castor oil, when consumed, has dehydrating effects because the oil induces diarrhea. Benito Mussolini, the Italian despotic leader, administered it as punishment to political dissenters who, after consuming

certain quantities, began to experience its laxative effects. However at moderate uses, castor oil has alternative medicinal uses. Again, "castor oil is not a drying oil, meaning that it has a low reactivity towards air compared to other drying oils like linseed oil and tung oil. Thus dehydration of castor oil gives linoleic acids, which do have drying properties". Furthermore, one of the useful seed oil particularities is its suitability for the manufacture of surface coatings such as wood varnish and paints. The high factor loading of 0.815 (Surface Coating), next to dehydration (0.830), shows the industrial importance of castor seed oil. The rest factor loadings indicate the importance of the variable under this factor.

Table 3. Clusters 1(Factor 1): Seed Oil Particularities

Factor 1: Seed Oil Particularities		
Variable Number	Variable Description	Factor Loading
3	Environmental Factor	0.791
5	Production Process	0.734
6	Trans-esterification	0.738
8	Type of Fertilizer	0.740
10	Solubility	0.450
13	Physicochemical Properties	0.636
14	Surface Coating	0.815
15	Dehydration	0.830
18	Chemical Oxidation	0.734
22	Intermolecular Force	0.792
23	Moisture Content	0.621
25	Type of Solvent	0.779
27	Intercropping	0.648
28	Capital Investment	0.757
30	Mode of Extraction	0.720
31	Seed Varieties	0.808

Table 4. Clusters 2 (Factor 2): Resource Conversion Efficiency

Factor 2: Resource Conversion Efficiency		
Variable Number	Variable Description	Factor Loading
2	High Cohesive Strength	0.824
4	Raw Material	0.574
7	Seed Dormancy	0.807
9	Soaking	0.726
11	Adhesion Force	0.825
12	Good Shelf Life	0.697
16	Blend of Driers	0.812
17	Type of Alkyd Resin	0.616
19	Disease	0.779
20	Reaction Temperature	0.787
21	Soil Nutrient	0.767
24	Storage Stability	0.582
26	Right Raw Material	0.758
29	Refining	0.636
32	Heating	0.728

Factor 2: Resource Conversion Efficiency

Cluster 2 is creatively labelled Resource Conversion Efficiency. Top three variables include: Adhesion force (0.825), High cohesive strength (0.824) and blend of driers employed (0.812) which principally showed the industrial importance of castor seed oil under the resource conversion efficiency. These variables are critical under this factor. Next to this trio of variable is seed dormancy (0.807). The variable caveats that if the seed stays unused for certain length of time, it might go rancid and thereby affect its physiochemical properties. As earlier stated, other variables under this factor exercise influence according to the level of the factor loading they wield.

Factor 3: Plant-cooperation-oriented yield

The third factor creatively labelled Plant-cooperation-oriented yield suggests that physicochemical properties as well as intercropping wield middling factor loading indicating that intercropping affects the quality of seed of castor plant and thus influence the growth of the seed as well as the physicochemical properties of the seed oil that influences its industrial importance.

Factor 4: Soil Condition

Cluster 4 is creatively labelled Soil Condition.

Table 5. Clusters 3 (Factor 3): Plant-cooperation-oriented yield

Factor 3: Plant-cooperation-oriented yield		
Variable Number	Variable Description	Factor Loading
13	Physicochemical Properties	0.636
27	Intercropping	0.648

Table 6. Clusters 4 (Factor 4): Soil Condition

Factor 2: Soil Condition		
Variable Number	Variable Description	Factor Loading
1	Soil Composition	0.716

Finally, there is soil condition as a lone factor. This suggests that fertilization, depending on the prestine condition of the soil, is an important consideration in castor plant plantation.

Conclusion

This study has been able to identify thirty two (32) important variables affecting the growth of castor shrub and the suitability of its seed oil in industrial application. The Kendall's Coefficient of Concordance (KCC) model used was able to evaluate the level of agreement among the knowledgeable respondents (Expert Judges) that ranked the variables in merit order of sequentiality. The Principal Component Analysis (PCA) model adopted helped to reduce the large sum of the data from thirty-two (32) to four (4). This implies that a considerable parsimony was achieved in terms of data summarization. The PCA analysis show that 4 principal factors creatively labeled: Seed oil particularities, resource conversion efficiency, plant-cooperation-oriented yield and soil condition respectively, represent the principal factors affecting the growth of castor shrub and the suitability of its seed oil in industrial application.

Compliance with Ethical Standards

Conflict of interest

The author solemnly 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

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.12 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):343-351 (2021)

Effect of oregano water on *Pythium* density in soil and damping-off disease on bean plants

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Abstract

Pythium genus includes significant and destructive soil borne plant pathogens causing losses on many plants, especially by root rot and damping-off diseases. Like other soil borne pathogens, control of the diseases caused by this group is rather difficult. Usually fungicides are used against *Pythium* species, but investigations on alternative methods and chemicals gained importance because of the harmful effects of pesticides on human health and environment. Plant extracts and essential oils are among the safer alternative chemicals. Since oregano oil has high fungitoxic effect even in low concentrations, effect of oregano water, by-product of oregano oil, was investigated in this study against *P. deliense*, one of the most common and virulent species in Turkey. Oregano water obtained from *Origanum onites*, diluted with distilled water in 100, 80, 60, 40, 20, 10 and 5% rates was applied to soil and its effects on *P. deliense* population density in soil and severity of damping-off disease on bean plants caused by the pathogen were determined. As a result, all solutions containing oregano water over 10% rate significantly decreased pathogen populations in the soil samples taken every three days after the applications, and the least pathogen density was obtained with undiluted oregano water. Similarly, undiluted oregano water increased emergence rates of the bean seedlings and significantly decreased root and hypocotyl rot severity by suppressing the effect of the pathogen. In addition, application of oregano water supported the shoot and root development of the plants by decreasing the negative effect of the pathogen on plant growth. This study showed that oregano water was an effective alternative against *Pythium* related damping-off disease.

Keywords: *Pythium* spp., Root rot, Control, Oregano hydrosol

Introduction

Pythium genus, belonging to Phylum Oomycota of the Chromista Kingdom, includes species, parasitic on different organisms such as fish, insects, fungi and even on human. There are 355 described species in the genus with many destructive plant pathogens causing root rot and damping-off diseases (Ho, 2018). *Pythium* species are especially common in rhizosphere soil, prefer young and watery plant tissues and cause pre or post-emergence damping-off symptoms on young plants. They can also decrease yield by causing root rot and suppressing growth of older plants. Leaf, stem or fruit rots may also be caused by *Pythium* species (Hendrix and Campbell, 1973). *P. deliense* is among the most common and virulent species of the genus. It is first isolated from tobacco plants showing stem blight symptoms in Sumatra. It is known as a pathogen living mostly in warmer soils with an optimum growth temperature of 36°C (Yu and Ma, 1989). It is also isolated from cabbage (Yu and Ma, 1989), tomato, mung bean, ginger, pawpaw, lettuce, bitter melon and cowpea (Lodha et al., 2004). It is reported as a common pathogen in vegetable and tobacco nurseries and that

Cite this article as:
Demirbas, E., Karaca, G. (2021). Effect of oregano water on Pythium density in soil and damping-off disease on bean plants. J. Agric. Environ. Food Sci., 5(3), 343-351
Doi: https://doi.org/10.31015/jaefs.2021.3.12
Orcid: Emine Demirbaş: 0000-0002-9346-1636 and Gürsel Karaca: 0000-0002-5159-2734
Received: 27 July 2020 **Accepted:** 28 June 2021 **Published Online:** 19 August 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 343-351
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

it also caused severe root rot on wheat and sugarbeet plants in Turkey (Hatat, 1995; Karabuğa and Karaca, 2011).

Human beings have been dealing with agricultural production for centuries and continuously increasing yields by modernizing the techniques. However, monoculture agriculture, wrong irrigation techniques, intensive soil tillage and pesticide and chemical fertilizer usage have some harmful effects. Growers generally prefer chemical control method to reduce the yield losses caused by pests, diseases and weeds. But pesticides have negative side effects on environment and human health (Arslan, 2016). Research showed that pesticides had serious dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine effects on human body depending on the pesticide type, duration and route of exposure (Nicolopoulou-Stamati et al., 2016). In addition, unconscious and excessive pesticide applications cause development of resistant pest and pathogen populations. Alternative methods and chemicals gained importance because of the negative effects of pesticides. Chemicals synthesized by plants are among those alternatives and they are thought as biologically effective, wide spectrum, easily decomposable, economical and safer pesticides (Alvarez-Castellanos, 2001).

Effects of plants on plant pests and pathogens depend on their secondary metabolites and azadirachtin, pyrethrin, rotenone, nicotine, ryania, sabadilla and thymol are some examples (Bayram et al., 2010). Recently significance of phytochemicals and studies on them increased with the development of sustainable and ecological agricultural systems (Boyraz and Koçak, 2006). Oregano comes first among the plants whose secondary metabolites have commonly been studied. It's also one of the medicinal plants with highest production in Turkey. It is reported that 60% of the 52 known *Origanum* species grow in Turkey and this is a strong proof that Turkey is the gene origin of this group of plants (Başer, 2001). Before, internal and external consumption was mainly provided with the naturally grown *Thymus* and *Thymbra* species. However, with the production of *Origanum* species, amount of yield increased in years and Turkey becomes the first country in terms of production (Bayram, 2018). More than 90% of oregano production is made in Turkey and *Origanum onites* has the biggest part in the export. It is the only species commercially cultivated besides natural production (Kapluhan, 2013). Oregano production in Turkey was 11 752 tonnes in 92 959 da area in 2014, while production increased to 15 752 tonnes in 139 061 da area in 2018. According to 2018 data, Denizli province comes first with 14 009 tonnes production, followed by Manisa (828 tonnes), Kütahya (475 tonnes), Uşak (262 tonnes), and Hatay (187 tonnes) provinces, respectively (TUİK, 2019).

Oregano is used as a medicinal plant for years in order to cure different diseases. Besides it is used in agriculture to prevent crops from pests and diseases. It is especially known as a repellent against storage pests (Altundağ and Aslım, 2005). It was reported in some studies that it was not phytotoxic when used as an insecticide or fungicide (Hayta and Arabacı, 2011). Extract or essential oils obtained from oregano species were found to have antifungal effects on many plant pathogenic fungi such as *Alternaria alternata*, *Botrytis cinerea*, *Fusarium* spp., *Macrophomina phaseolina*, *Penicillium* spp., *Phytophthora capsici*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Arslan and Karabulut, 2005). Antimicrobial effect of oregano essential oil is mainly because of its chemical constituents like thymol, carvacrol, p-cymene, terpineol, borneol, cymol and linalool (Altundağ and Aslım, 2005). Main constituents of oregano essential oil are thymol and carvacrol (Çoban and Patır, 2010) and amounts of these chemicals depend on the species. Carvacrol and thymol contents of *Origanum onites* are 72-89%, while those of *O. vulgare* are 23-79% (Başyiğit et al., 2017).

Oregano water is a by-product of oregano essential oil obtained under oil during distillation process. Active ingredients of oregano oil are also present in oregano water. Carvacrol is the main constituent of oregano water, while there is also about 10% p-mentendiol, which is rare in nature and not found in the oil (Başer, 2014). Oregano essential oil is expensive for usage in agriculture and it may have phytotoxic effects on plants. There are many studies on the effect of oregano oil against plant pathogens, but very less study on oregano water and those are mainly performed *in vitro* (Özcan and Karaca, 2016; Kaya et al., 2016).

In this study, effect of oregano water on *P. deliense* population density in soil and related severity of damping-off disease caused by the pathogen on bean plants were investigated.

Materials and Methods

Isolation and Selection of the *P. deliense* Isolate

Soil samples were taken from nurseries and greenhouses in Isparta and Antalya provinces, where seedlings showing damping-off symptoms were observed. Samples were taken to the laboratory and isolations were performed by SSDP (Surface soil dilution plate) method on VP3 selective medium. In this method, soil samples were first air dried on plastic trays for a few days and then sieved through 35 and 60 mesh screens. Twenty grams of soil retained on the 60 mesh screen was suspended in 100 ml of 0.2% sterile water agar and 1/50 dilution of the soil suspension was spread on the surface of the selective medium by using a sterile glass rod. After two days incubation at 21°C in the dark, growing mycelia were transferred to Potato carrot agar (PCA). Agar pieces taken from the growing edges of the isolates were then transferred to water culture plates made with corn and hemp seeds and sterile soil extract (Karaca et al., 2008). *P. deliense* isolates with toruloid sporangia, smooth walled oogonia, intercalary antheridia and aplerotic oospores (Figure 1), were identified according to related literature and keys (Plaats-Niterink, 1981; Yu and Ma, 1989; Dick, 1990). Isolates were grown on PCA and small agar pieces with pathogen mycelia were cut and transferred to small glass bottles with sterile distilled water. After a few days incubation, bean seedlings were dipped into the bottles after root tips were cut. After incubation for one week, bean seedlings were evaluated for damping-off symptoms and the most virulent isolate causing severe symptoms was selected for the rest of the study (Hatat, 1995).

Inoculation of Soil with *P. deliense*

Small agar pieces with virulent *P. deliense* isolate on PCA culture were cut and transferred to glass bottles with sand:water:corn meal in 9:2:1 rates and incubated at 21°C in the dark for 15 days. Soil sample used in the study was taken from an area where no chemical was used. Soil sample was autoclaved two times for 45 minutes. Some part of the soil was kept for control application. Other part was inoculated with the pathogen and checked by SSDP method if it contains at least 200 propagules per gram of soil. When inoculum concentration was lower than 200 propagules, some more inoculum was added to the soil, otherwise some sterile soil was added to dilute the concentration (Hatat, 1995).

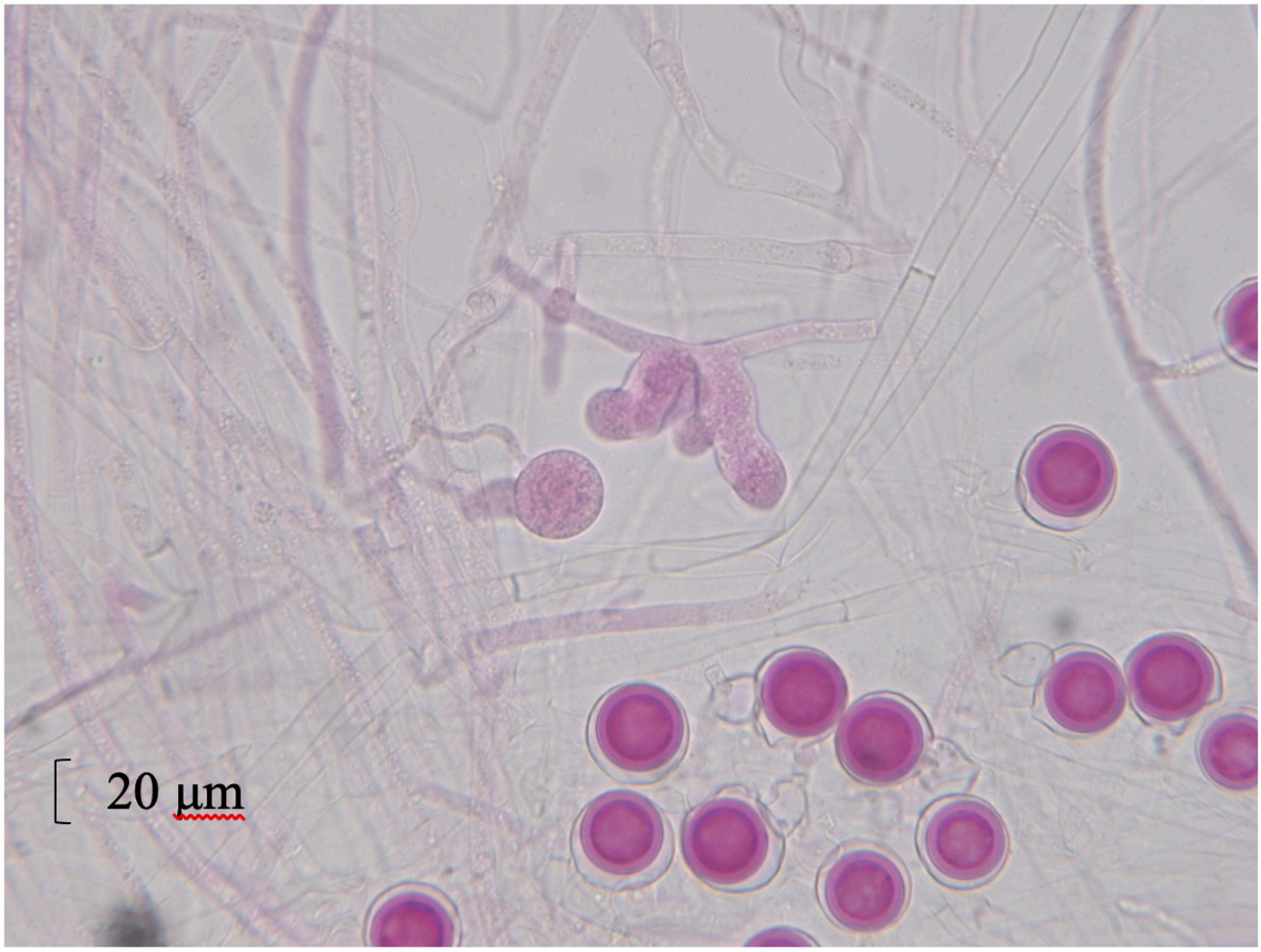

Figure 1. Distinctive features of *Pythium deliense* (toruloid sporangium, mostly intercalary antheridia and aplerotic oospores

Application of Oregano Water

Oregano water produced from *Origanum onites* by "Efecan Badem Aromatik Ürünler A.Ş." company in 2018, was used in the study. Main constituents of the oregano water determined by GC-MS analyses and their rates were given in Table 1.

Oregano water was diluted with distilled water in 5, 10, 20, 40, 60, 80 and 100% concentrations. Sterilized soil was separated into 5 kg parts and transferred into clean plastic containers. Each soil sample was sprayed with 100 ml of different oregano water dilutions. For comparison, one sample was sprayed with 1% NaOCl solution, and another one with sterile distilled water as control application. Applications were made with a hand sprayer and soil samples were mixed during spraying in order to ensure homogenized application (Özcan and Karaca, 2016).

Monitoring Pathogen Population Density in Soil

After the application of oregano water, containers with the soil samples were kept closed for three days to provide the distribution of volatiles of oregano water in the soil samples. Then necessary amounts from each soil sample were transferred to plastic pots for plant experiment, and remaining parts were kept for population monitoring. First soil samples were taken three days after the applications and sampling was continued every three days for 24 days. For each sampling, 20 g of soil were taken from the soil samples and population density of *P. deliense* was determined by SSDP method, using 5 replicate plates for each sample.

Table 1. Main chemical constituents of the oregano water used in the study and their rates

Chemicals	Rates (%)
Linalool	0.6
Terpinene-4-ol	0.6
a-Terpineol	0.5
Borneol	0.8
Thymol	1.1
Carvacrol	95.9

Determination of the Effects of Oregano Water Applications on Damping-off Disease of Bean Plants

Soil samples sprayed with different dilutions of oregano water, 1% NaOCl and sterile distilled water were transferred to plastic pots and 10 bean seeds were sown into each pot. Trial was performed with a randomized parcel design with three replications and pots were kept in a climatized room with 23±2°C temperature and 16 hours light-8 hours dark conditions. Seeds of Dermason cultivar were used and pots were irrigated with equal amounts of sterile water when necessary. One week after sowing, emergence rates were determined and four weeks after sowing, plant and root lengths, shoot and root fresh and dry weights were determined. Plants were dried in oven at 60°C for three days and weighed immediately for determination of the dry weights. In addition, disease severity rates were determined by using two scales, one for root rot and the other for hypocotyl rot (Li et al., 2014). In 0-4 root rot scale; 0=healthy roots, 1=less than 2% of roots rotted, 2=moderate rot on roots, 3=general rot on roots and less than 50% root loss, and 4=severe root rot and more than 50% root loss or dead plant. In the scale used for hypocotyl symptoms; 0=healthy hypocotyl, 1=less than 10% of hypocotyl with lesions, 2=10-25% of hypocotyl with lesions, 3=25-50% of hypocotyl with lesions, 4=50-75% of hypocotyl with lesions, and 5=more than 75% of hypocotyl with lesions or dead plant. Disease severity was calculated with the formula below, where n: number of plants with the scale value, v: scale value, N: total number of plants, and Z: highest scale value.

Disease severity rate (%)=Σ (nxv) x 100 / ZxN

After the evaluations, reisolations from the diseased plants were made in order to confirm the pathogen was *P. deliense*. All data were subjected to analyses of variance and means were compared with Tukey test by using JMP (Ver.9) program. Emergence and disease severity rates were subjected to arcsin transformation before the analyses.

Results and Discussion

Effect of Oregano Water Applications on *P. deliense* Density in Soil

It was found that the effect of soil applications of different concentrations of oregano water on *P. deliense* population density differed depending on the dilution rates. None of the applications totally destroyed pathogen propagules in soil, so the pathogen population increased again in time. *P. deliense* inoculum densities determined during the first four samplings, in the soil samples taken every three days after the applications, were given in Table 2. Pathogen inoculum reached 370 propagules per gram of soil in the samples taken from the containers sprayed with sterile distilled water representing control group, while the density of the pathogen decreased in the soil samples depending on the dilution rate of oregano water. The lowest inoculum density of the pathogen was obtained with undiluted oregano water application. Pathogen density increased in the soil sample where 5% oregano water was sprayed one week after the application and statistically arranged in the same group with control application. Samplings made 15 days after applications showed that the number of pathogen propagules per gram of soil gradually increased. However, pathogen populations were continuously lower than the others, in the soil samples taken from the containers sprayed with undiluted oregano water. Population densities obtained with the lowest concentration of oregano water were always statistically similar with the control, while all other dilutions were different in the samplings made 15 and 18 days after applications. But in the last two samplings, 10% dilution of oregano water also seemed to be ineffective to decrease the population. In a previous study, oregano water, added into culture medium in 5 and 10% concentrations, totally inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *phaseoli*, *M. phaseolina*, *B. cinerea*, *R. solani*, *Alternaria solani* and *Aspergillus parasiticus* (Özcan and Boyraz, 2000). The cause of the difference between the results of two studies maybe the difficulty of the homogenous application of the solution into the soil. Another reason may be the susceptibility differences between the pathogens.

Effects of Oregano Water Applications on Growth and Damping-off Disease Severity of Bean Plants

It was found in the experiment that oregano water applications affected emergence rates and growth of bean seedlings. Emergence rates decreased in all applications, when compared to controls without pathogen inoculation. However, emergence rates of the bean seedlings in the pots with undiluted oregano water and solutions with 80 and 60% oregano water applications arranged in the same group with control. The lowest rate of emergence was found in pots where 5% oregano water was applied and this rate was statistically similar with that of pathogen inoculated positive control group (Table 3).

In the previous studies, it was found that oregano essential oil had negative effect on seed germination. Aydın and Tursun (2010) reported that germination rates of some weed seeds decreased with the increasing doses of onion, garlic and oregano (*Origanum dubium* L.) essential oils and higher doses totally inhibited the germination. In another study, doses over

5% of clove, mentha and oregano essential oils significantly decreased or totally inhibited the germination rates of wheat seeds (Karaca et al., 2017). Similarly, 2.5% dose of oregano oil totally inhibited the growth of seed-borne fungi but also decreased germination rates of rice seeds (Olgunsoy and Karaca, 2018). It is known that oregano water contains same chemical constituents of oregano oil in lesser amounts. Main constituent was carvacrol but it has also p-mentendiol (%10), which is not found in the oil (Başer, 2014). This may be the reason of lower phytotoxic effect of oregano water on seed germination and plant growth. As expected, highest disease severity rates were found in the pathogen inoculated pots, both for root rot and hypocotyl rot (Figure 2). Negative control group yielded the highest emergence rates and plants showed healthy growth (Figure 3).

Table 2. *Pythium deliense* population densities in the soil samples taken every three days after applications (propagules/g soil)

Applications	Days After Applications							
	3 Days	6 Days	9 Days	12 Days	15 Days	18 days	21 Days	24 Days
5% OW[x]	353.33 a[y]	375.00 a	412.67 ab	437.67 ab	504.33 ab	575.33 ab	667.00 ab	742.00 ab
10% OW	315.00 b	330.00 b	366.67 bc	392.00 bc	458.33 bc	517.00 bc	608.67 abc	700.33 abc
20% OW	275.00 c	285.00 c	329.33 cd	367.00 bcd	396.00 c	479.33 c	554.33 bc	637.67 bcd
40% OW	255.00 cd	260.00 c	304.33 cd	337.67 cd	379.33 cd	446.00 cd	496.00 cd	583.67 cde
60% OW	216.67 ef	208.33 d	215.00 ef	275.00 def	308.33 de	362.67 de	413.00 de	504.33 def
80% OW	200.00 f	195.00 de	205.00 ef	245.00 ef	285.00 e	333.33 e	383.67 de	437.67 ef
100% OW	170.00 g	161.67 e	166.67 f	195.00 f	236.67 e	279.33 e	321.33 e	367.00 f
1% NaOCl	240.00 de	253.33 c	279.33 de	321.00 cde	379.33 cd	429.33 cd	492.00 cd	562.67 cde
Control	370.00 a	400.33 a	454.33 a	504.33 a	567.00 a	646.00 a	717.00 a	808.67 a

[x]OW: Oregano water

[y]Means in the columns shown by the same letter were not statistically different from each other according to Tukey test (P=0.05)

Table 3. Effect of oregano water applications on emergence rate, root and hypocotyl rot severity of bean plants

Applications	Emergence rate (%)	Root rot severity (%)	Hypocotyl rot severity (%)
5% OW[x]	6.67 d[y]	98.00 a	98.00 ab
10% OW	16.67 cd	97.33 a	95.33 abc
20% OW	36.67 bcd	95.33 ab	92.00 abcd
40% OW	43.33 bc	92.67 abc	92.67 abcd
60% OW	56.67 abc	80.67 bcd	84.00 bcd
80% OW	56.67 abc	72.67 cd	69.33 de
100% OW	76.67 ab	52.67 d	46.67 e
1% NaOCl	36.67 bc	87.33 abc	82.00 cd
Positive control	20.00 cd	98.00 a	99.33 a
Negative control	90.00 a	12.67 e	11.33 f

[x]OW: Oregano water

[y]Means in the columns shown by the same letter were not statistically different from each other according to Tukey test (P=0.05)

Figure 2. Pre-emergence damping-off symptoms on pathogen inoculated soil samples (Positive control)

Figure 3. Growth of bean plants in the soil samples without pathogen inoculation (Negative control)

Oregano water applications between 5-40% failed to decrease disease severity, while severity rates decreased with higher doses. However, none of the applications totally inhibited the disease. In addition, plants applied with lower doses of oregano water showed chlorosis on the leaves, depending on the unhealthy root development. Emergence rates increased with the increasing oregano water concentrations and plants showed better development with larger leaf areas. In the pots with NaOCl applied soil samples, emergence rates were also lower and plants had smaller leaves. Most of the plants totally dried before the end of the experiment.

Diluted oregano water applications failed to decrease the negative effects of pathogen inoculation on the shoot development of bean plants, while undiluted oregano water application significantly supported shoot development (Table 4, Figure 4). Similar results were obtained with the root development of bean plants. Only the highest two concentrations of oregano water application supported root development against pathogen and mean root lengths of the plants statistically arranged in the same group with negative control. But oregano water applications were not successful to decrease the effect of pathogen in terms of root fresh and dry weights.

Table 4. Effects of oregano water applications on shoot and root development of bean plants

Applications	Shoot development			Root development		
	Shoot length (mm)	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root length (mm)	Root fresh weight (mg)	Root dry weight (mg)
5% OW[x]	8.00 c[y]	89.27 c	8.57 d	4.17 c	5.13 c	1.53 c
10% OW	15.17 c	168.90 c	23.93 cd	6.00 c	11.73 c	3.13 c
20% OW	17.00 c	221.30 c	33.10 cd	9.67 bc	11.07 c	6.07 c
40% OW	22.33 c	316.10 bc	50.63 cd	10.33 bc	15.70 c	11.80 bc
60% OW	53.50 bc	536.10 bc	101.97 cd	22.50 bc	49.57 bc	14.63 bc
80% OW	50.83 c	893.80 b	115.93 bc	31.50 ab	58.77 bc	16.47 bc
100% OW	104.33 a	1596.90 a	209.67 ab	52.83 a	104.23 b	27.87 b
1% NaOCl	27.67 c	247.77 c	54.20 cd	15.50 bc	25.13 c	7.77 c
Positive control	8.67 c	106.83 c	21.53 cd	6.00 c	10.25 c	2.47 c
Negative control	102.17 ab	2038.67 a	259.13 a	53.67 a	216.23 a	53.27 a

[x]OW: Oregano water

[y]Means in the columns shown by the same letter were not statistically different from each other according to Tukey test (P=0.05)

Higher doses of oregano water were found to be more effective on disease severity and root and shoot development of bean plants. In the study, the use of high inoculum density of the pathogen may cause the lower efficiency of oregano water. Lower doses may be more effective in agricultural soils where pathogen density is lower because of complex soil conditions.

Conclusion

Intensive usage of pesticides in agriculture caused problems, such as development of resistance both in pests and disease agents, environmental pollution and residues in crops. Thus development of alternative method and pesticides has gained significance. Pesticides of plant origin are known to decompose easily in the nature and do not cause environmental pollution or residues on crops. Various secondary compounds of plants have commonly been used in agriculture in the last 20 years, especially with the development of sustainable agriculture concept (Aydın and Mammadov, 2017). Antimicrobial effects of plant extracts and essential oils on harmful agents of plants have known for many years (Arslan and Karabulut, 2005; Altundağ and Aslım, 2005; Çelen, 2006).

Figure 4. Effect of undiluted oregano water on bean growth

With its geographical location, diverse climate and vegetation and high agricultural potential, Turkey is one of the leading countries in the production and export of medicinal plants. Oregano is one of the medicinal plants mostly been studied for its antimicrobial effects. Its antifungal effects against different plant pathogens were generally studied *in vitro* by using oregano essential oil and found to have higher and wide spectrum effect, in comparison with other plant oils (Sokovic et al., 2002; Daferera et al., 2003). Antifungal effect of oregano oil is mainly attributed to its high carvacrol and thymol content (Kordali et al., 2008). Despite its efficiency on fungal plant pathogens, usage of oregano oil in the control is not common, because it is expensive and impractical. However, oregano water obtained during distillation process as a by-product of oregano oil has similar constituents (Boydağ et al., 2002), while it is cheaper and easier to use. In the present study, it was found that oregano water applications decreased *P. deliense* inoculum density in soil and supported emergence and growth of bean seedlings by decreasing the negative effect of the pathogen. These results showed that oregano water can be used against soil borne plant pathogens in small areas like nurseries and greenhouses. But it will be better to investigate its effect in other host-pathogen combinations.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

Funding

This study was funded by the Scientific Research Projects Coordination Unite of Isparta Applied Sciences University, Isparta, Turkey, with the project number 2019-YL1-0017.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

The authors would like to thank Efecan Bademi Aromatik Ürünler A.Ş. who supplied the oregano water used in the study.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.13 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):352-359 (2021)

Biostimulant priming for germination and seedling quality of carrot seeds under drought, salt and high temperature stress conditions

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Abstract

Abiotic stresses are serious problems that hinder crop production. Seed germination and seedling development are stages which are sensitive to abiotic stress. Seed priming improves the performance of seeds/seedlings and provides faster and synchronized emergence under stress conditions. The present study aimed to investigate the effect of priming with biostimulants, vermicompost (5%), karrikinolide (10^{-7} M) and seaweed (5%) using the solid matrix method (5 days, dark, 15 ˚C, 2:1:3, seed:vermiculite:organic solution, w:w:w) on germination and seedling quality of carrot seeds under abiotic stress conditions. Biostimulants were used alone and in double and triple combinations. Drought stress was simulated by PEG-6000 (-0.3 MPa), salinity by using NaCl at 100mM, and high temperature by 30 ˚C. Dry control and distilled water treated were used as controls. Priming treatment with biostimulants improved performance of seeds and seedlings, though not always significantly (p=0.05). Seaweed alone and its combination with karrikinolide showed the best performance for all the parameters. The germination percentage for dry control of carrot seeds were 37, 63 and 72% in salt, drought and high temperature stresses while distilled water treated seeds had values of 74, 79 and 77%, respectively. Seeds treated with seaweed+ karrikinolide and seaweed alone had 80 and 89% germination. The same treatments stimulated seedling emergence from 57% to 84-88%, 25 to 69-76%, 71 to 85-87% under drought, salt and high temperature stress, respectively. Seedling criteria, seedling height, fresh weight, dry weight and root fresh weight were also higher with these treatments in all stress conditions. Catalase activity of treated seeds was higher for seaweed (0.400 EUg^{-1}seed) and seaweed karrikinolide (0.411 EUg^{-1}seed) treated seeds than for both controls (non-primed: 0.299, distilled water: 0.239 EUg^{-1}seed). Biostimulants have potential as seed priming agents to enhance seed quality in carrots.

Keywords: Abiotic stresses, Karrikinolide, Seaweed, Vermicompost, Catalase activity

Introduction

Crops encounter environmental stresses which are both abiotic and biotic. Various analyses have suggested that abiotic stresses, mainly drought, salinity and extreme temperatures, are the major factors that obstruct crops from realizing their full yield potential (Wang et al., 2003; Fetri et al., 2014). According to Wang et al. (2003), drought and salinity are becoming the prevalent problems in many regions, and will account for serious salinization problems in more than 50% of all arable lands by the year 2050. Abiotic stress affects all stages of crop growth and development. However, seed germination, early growth of seedlings and flowering stages are the most sensitive

Cite this article as:
Muhie, S.H., Memis, N., Ozdamar, C., Gokdas, Z., Demir, I. (2021). Biostimulant priming for germination and seedling quality of carrot seeds under drought, salt and high temperature stress conditions. J. Agric. Environ. Food Sci., 5(3), 352-359
Doi: https://doi.org/10.31015/jaefs.2021.3.13
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Received: 14 September 2020 **Accepted:** 28 June 2021 **Published Online:** 18 August 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 352-359
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

stages (Yadav et al., 2011). Under abiotic stress conditions, it's difficult for a seed to be able to germinate and produce a good crop stand in the field. Hence, seed treatments are a useful tool for seeds to cope with the existing abiotic stress (Paparella et al., 2015).

Seed priming is a simple, low cost practice, which is used to overcome the problems of abiotic stress in crop production. Several methods of seed priming were developed in order to enhance the quality of seeds and minimize the risk of environmental stresses (Lutts et al., 2016). The effect of priming was attributed to metabolic repair and activation of seeds during imbibition (Basra et al., 2005). Solid matrix priming in which water uptake by seeds is controlled, was developed as a solution to overcome the problem of aeration in osmo-priming and the high cost of osmotic agents (Paparella et al., 2015). During matrix conditioning, seeds are mixed and incubated with a wet solid (water carrier) for a certain period of time so as to control the uptake of water by the seeds. Afterward, seeds are separated from the carrier, rinsed, and back-dried to the level of their original moisture content. The use of solid medium allows seeds to hydrate slowly and simulates the natural imbibition process occurring in the soil (McDonald, 2000). Different types of osmotica and chemicals are used for priming purposes. However, the issue of environmental problems is common due to intensive use of chemicals. The use of organic farming is receiving due consideration. Among such treatments, we considered seaweed (Balakrishnan et al., 2007, Muhie et al., 2020), vermicompost (Muhie et al., 2020) and smoke extracts (Demir et al., 2018). Such organic biostimulants were found to enhance seed quality in various crop seeds such as *Lupinus angustifolius* (Plazek et al., 2018), *Capsicum annuum* (Rekha et al., 2018) and onion (Muhie et al., 2020). Furthermore, there is not much research on priming combined (single, double and triple) with organic extracts (biostimulants) for carrot seeds, especially under abiotic stress conditions. We presume that they may synergistically affect seed quality under stressful conditions. Thus, the objective of this research is to investigate the effect of biostimulants (seaweed, vermicompost and smoke derived, karrikinolide) alone or in combination by using matrix priming on performance of carrot seed germination and seedling quality under drought, salinity and high temperature stress conditions.

Materials and Methods

Seed and biostimulants

Carrot (*Daucus carota* L. cv. Maestro F1 hybrid) seeds were obtained from a commercial company. The germination percentage of the lot was 85% and seed moisture content was 6.6%.

Karrikinolide (Kar) was provided by Prof. Van Staden from South Africa, University of Kwazulu Natal. 10^{-7}M karrikinolide (Mavi et al., 2010) was used in this experiment. Seaweed (Sw, green algae, *Ulva lactuca*) was collected from Marmara Sea in Turkey (northwestern part). The seaweed extract was prepared using the physical integration method. The particle size was gradually reduced and diluted with water to a ratio of 1:3. There was no heat, acid or any alkaline hydrolysis used for extraction (Demir et al., 2006). Then the diluted seaweed was filtered through a muslin cloth. The filtrate was considered 100% seaweed extract (Demir et al., 2006). The seaweed extract was stored at 4 ˚C for further applications. Balakrishnan et al. (2007) reported that best seedling performance was observed at 5% concentration. So, we used the same concentration of seaweed extract. Liquid vermicompost (V) was obtained from a compost producing company in Turkey (Aybasol Ltd. Polatlı/Ankara). Liquid vermicompost was diluted with distilled water to make 5% vermicompost extracts which displayed best seedling performance (Arancon et al., 2012) in our preliminary experiments. The extract was stored at 4 ˚C for further applications.

Priming procedure

Solid matrix priming was done at the ratio of 2:1:3 (Seed:vermiculite:organic solution (w:w:w)) for five days at 15 ˚C. Priming was done with single solutions of Sw (Seaweed), V (Vermicompost), Kar (Karrikinolide) and double combinations Sw+V, Sw+Kar, V+Kar and the triple combination of Sw+V+Kar. All combination treatments were done under dark conditions. Seeds were also treated with distilled water (Dw) and untreated dry seeds (Np) were considered as controls. So, 7 treatments and two controls were allocated. After treatments, seeds were dried on the laboratory bench until the initial seed weight was reached. Then, the seeds were kept at 5 ˚C to be used for germination and emergence tests.

Seed germination under abiotic stresses

Drought stress condition was simulated using Polyethylene Glycol-6000 (PEG) at 10%, -0.30 MPa (Muscolo et al., 2014). Primed and control carrot seeds (four replicates of 50 seeds) were placed on filter paper in 9 cm Petri dishes containing 3 cm^3 of -0.3 MPa PEG. The Petri dishes were sealed with stretch film to prevent evaporation and kept according to a completely randomized design in a growth chamber. Germination was carried out at 20 ˚C for 14 days in the dark. Seeds were considered germinated when the radicle emerged by at least 2 mm and is expressed as percentage. In the salt stress experiment, NaCl at concentration of 100mM was used to simulate salinity stress. The same germination procedure was followed.

Primed and control carrot seeds were allowed to germinate at 30 ˚C. Four replicates of 50 seeds were used for germination tests. Monitoring and recording of data were as described under earlier stress conditions.

Seedling emergence and quality tests

The seedling emergence test were carried out in three replicates of 25 seeds each under moisture, salinity and temperature stress conditions. For this purpose, plastic germination trays were used. The seeds were sown to a depth of 2 cm in peat moss and the appearance of the cotyledon leaves above the peat moss was considered as the emergence criterion. Trays were kept at 20±2 ˚C. Control and stress levels were induced as described above. Trays were watered with an appropriate solution according to the treatment design (-0.3 MPa PEG, 100mM NaCl, tap water) during the emergence period. Seedling emergence percentage (EP) was calculated based on percentages of seedlings appearing on the surface of the peat. After 21 days of sowing, five seedlings in each

replicate were destructively taken and shoot height (SH, cm/plant), shoot fresh (SFW, mg/plant) and dry weight (SDW, mg/plant), and root fresh weight (RFW, mg/plant) were calculated. Seedling dry weight was calculated after keeping seedlings at 80 °C for 24 hours. Vigor index was recorded according to Abdul-Baki and Anderson (1973).

Catalase analysis

The two treatments that gave best results for germination and emergence tests and two controls (distilled water primed and non-primed) were subjected to catalase enzyme analysis. An amount of 0.5 grams of three replicates in each treatment were used. CAT (Catalase) activity was analyzed based on the rate of hydrogen peroxide decomposition according to the method and is expressed as per g seed, and one unit represents 1 µmol of substrate undergoing reaction per g per seed (Abedi and Pakniyat, 2010).

Mineral analysis

Mineral nutrient analysis was completed only for the three biostimulants for macro and micro nutrients at the soil science laboratory of Ankara University. Analysis was done using an inductively coupled plasma spectrophotometer (Mertens, 2005).

Statistical analysis

Data was subjected to analysis of variance (ANOVA) using SPSS v.20 and Duncan's Multiple Range Test was applied to compare the differences among treatment means.

Results and Discussion

Effect of priming on seed germination

Germination percentages for carrot seeds under optimum conditions varied from 85% in Np to 92% in Sw treated. The other treatments varied between these values and significance also changed (Table 1). Drought, salt and high temperature stresses affected seed germination at various levels. The greatest impact was seen in Np seeds in which seed germination declined to as low as 37% in salt, 63% in drought and 72% in high temperature conditions, respectively. Under stress and optimum germination conditions, Sw treatment provided the highest germination percentages between 89 and 92% except for salt stress which had 80%. Biostimulant priming advanced carrot germination 10% in drought, 13% in salt and 12% in high temperature stress when the best treatment (Sw, Sw+Kar) was subtracted from Dw (distilled water) treatment. This difference was not significant except for high temperature germination ($p<0.05$).

Table 1. Germination percentages of carrot seeds treated with different biostimulants at optimum condition, drought (-0.3 MPa, PEG), salt (100 mM NaCl) and high temperature (30˚C) stresses. The values in a column with the same letter are not significantly different (P<0.05).

	Stresses				
Priming agent	Optimum (20 ˚C/ Dw)	Drought	Salt	High temperature	Mean
Np	85^{a}	63^{a}	37^{a}	72^{a}	64
V+Kar	85^{a}	70^{b}	73^{b}	76^{ab}	76
Sw+V+Kar	87^{ab}	78^{c}	74^{b}	77^{ab}	79
Dw	87^{ab}	79^{c}	74^{b}	77^{ab}	79
V	88^{ab}	82^{cd}	74^{b}	82^{bc}	81
Sw+V	87^{ab}	83^{cd}	75^{b}	84^{cd}	83
Kar	89^{ab}	85^{d}	79^{bc}	85^{cd}	85
Sw+Kar	89^{ab}	86^{ab}	87^{c}	88^{cd}	88
Sw	92^{b}	89^{c}	80^{bc}	89^{d}	88

NP:None primed (Control), Dw:Distilled water, Sw:Seaweed, Kar:Karrikinolide, V:Vermicompost

Effect of priming on seedling emergence and quality

The effect of drought stress was observed in the seedling emergence test at -0.30 MPa. Maximum seedling emergence of 89% was observed from seeds primed with seaweed extracts (Table 1). A significant difference ($P<0.05$) was observed against NP (none primed) in terms of EP (emergence percentage). Under drought stress the maximum EP was recorded from Sw (%88) and Sw+Kar (%84) treatments while the minimum was from the control (%57) (Table 2). Seedling parameters like seedling fresh weight, seedling dry weight, root fresh weight and vigor index were also statistically significantly different between the treatments. The maximum emergence of 76% was observed in SW and Sw+Kar at 100 mM salt stress (Table 3). Seedling height showed statistically significant difference among treatments The maximum seedling height was recorded in SW (4.9 cm) for low quality and for high quality carrot seeds. All other parameters like SFW, SDW and RFW also showed statistical significance differences at various levels among treatments (Table 3). Seedling emergence tests all showed more positive responses for all the parameters by using Sw and other biostimulants under high temperature stress. Np seeds had 71% emergence (EP) which went up to 83% when Dw was used and the Sw treatment increased this to 87% (Table 4). All seedling criteria were found to be higher in Sw-treated carrot seeds. But it was not significantly separated from the other treatments in all cases.

Catalase enzyme analysis was done on the best treatments according to germination and seedling emergence test results. From the nine treatments, Sw and Sw+Kar gave the best results for all the parameters studied in relation to germination and emergence. DW (Control 2) and non-primed (control 1) were also considered. These treatments were tested for the activity of enzymes via Catalase (CAT), which was maximum in Sw treatment. The activity of CAT was maximum in Sw+Kar

treatment but the difference was not significant compared to Sw (Table 5).

Mineral nutrient content of biostimulants

The results for mineral nutrient content are presented in Table 6. The amount recorded in Kar was lower than that of seaweed and vermicompost. Maximum amounts of Ca (12.30 $mgKg^{-1}$), Mg (4.22 $mgKg^{-1}$) and S (10.19 $mgKg^{-1}$) were recoded from seaweed extract. Vermicaompost also showed maximum results for minerals such as Fe (0.25 $mgKg^{-1}$), P (1.19 $mgKg^{-1}$), and K (175 $mgKg^{-1}$) (Table 6).

The present work indicates that biostimulant priming treatments increased tolerance to drought, salt and temperature stress in carrot seeds. Especially seaweed and the seaweed and karrikinolide combination provided the highest germination and seedling emergence. Seed germination, emergence, and seedling establishment are the most vulnerable growth processes and can be affected by an increase in abiotic stress conditions (Atia et al. 2006, Akbari et al., 2007; Nascimento and Pereira, 2007). Such abiotic conditions adversely affect seed germination which leads to slow and non-uniform emergence in the field or modules in transplant production (Pereira et al., 2009). Therefore, pre-sowing seed treatments are used to obtain faster and synchronized germination particularly under stress conditions. Biostimulants foster plant growth and development throughout the life cycle of crops from seed germination to plant maturity in a number of ways. One of which is priming of seeds with biostimulants because biostimulants include a variety of plant promoting substances such as hormones, humic substances, micronutrients, manure, seaweed extracts and/or smoke-derived karrikinolides (Kulkarni et al., 2011, Paparella et al., 2015, Muhie et al. 2020). Biostimulants are also valuable in organic farming systems and were proposed as a solution to environmental issues and to encourage the use of organic materials starting from seed treatment.

Table 2**.** Effect of priming with different biostimulants on seedling emergence and quality of carrot under drought stress at -0.30 MPa of PEG. The values in a column with the same letter are not significantly different (P< 0.05).

Priming agent	EP (%)	SH (cm/p)	SFW (mg/p)	SDW (mg/p)	RFW (mg/p)	Vigour Index
Np	57^{a}	4.9^{a}	25.3^{a}	2.3^{a}	3.0^{a}	316^{a}
V+Kar	61^{bc}	5.3^{ab}	27.0^{ab}	2.7^{ab}	3.3^{ab}	321^{a}
Sw+V+Kar	65^{bc}	5.7^{abc}	28.0^{ab}	3.0^{abc}	3.7^{c}	349^{a}
Dw	69^{abc}	5.9^{abc}	29.3^{ab}	3.0^{abc}	3.7^{c}	355^{a}
V	74^{a-d}	5.9^{abc}	29.7^{ab}	3.0^{abc}	3.7^{c}	357^{a}
Sw+V	76^{a-d}	6.0^{abc}	29.7^{ab}	3.0^{abc}	3.7^{c}	389^{a}
Kar	80^{bcd}	6.3^{abc}	33.7^{abc}	3.3^{abc}	4.2^{cd}	419^{ab}
Sw+Kar	84^{cd}	6.4^{bc}	35.67^{c}	3.7^{bc}	4.6^{de}	515^{b}
Sw	88^{d}	6.6^{c}	41.3^{c}	4.0^{c}	5.0^{e}	519^{b}

NP:None primed (Control), DW:Distilled water, SW:Seaweed, Kar:Karrikinolide, V:Vermicompost, EP:Emergence percentage (%), SH:Seedling height (cm/p), SFW:Seedling fresh weight (mg/p), SDW:Seedling dry weight (mg/p), RFW:Root fresh weight (mg/p).

Table 3. Effect of priming with different biostimulants on seedling emergence and quality of carrot under saline condition at 100mM NaCl. The values in a column with the same letter are not significantly different (P<0.05).

Priming agent	EP (%)	SH (cm/p)	SFW (mg/p)	SDW (mg/p)	RFW (mg/p)	Vigour Index
Np	25^{a}	3.5^{a}	14.0^{a}	1.3^{a}	2.0^{a}	91^{a}
V+Kar	26^{ab}	3.8^{a}	15.7^{ab}	1.5^{a}	2.3^{ab}	99^{a}
Sw+V+Kar	36^{abc}	4.2^{ab}	17.3^{ab}	1.7^{ab}	2.3^{ab}	149^{ab}
Dw	48^{abc}	4.4^{ab}	19.7^{abc}	2.0^{ab}	2.7^{bc}	200^{ab}
V	53^{abc}	4.5^{ab}	20.3^{bc}	2.0^{abc}	3.0^{c}	255^{ab}
Sw+V	57^{abc}	4.5^{ab}	20.7^{bc}	2.0^{abc}	3.0^{c}	269^{ab}
Kar	65^{abc}	4.7^{b}	21.5^{bc}	2.3^{bc}	3.7^{d}	271^{ab}
Sw+Kar	69^{bc}	4.8^{b}	23.7^{cd}	2.4^{bc}	3.7^{d}	311^{b}
Sw	76^{c}	4.9^{b}	26.0^{d}	2.7^{c}	3.7^{d}	323^{b}

Np:None primed (Control), Dw:Distilled water, Sw:Seaweed, Kar:Karrikinolide, V:Vermicompost, EP:Emergence percentage(%), SH:Seedling height (cm/p), SFW:Seedling fresh weight (mg/p), SDW:Seedling dry weight (mg/p), RFW:Root fresh weight (mg/p).

Table 4. Effect of priming with biostimulants on seedling emergence and quality of carrot at high temperature of 30°C. The values in a column with the same letter are not significantly different (P< 0.05).

Priming agent	EP (%)	SH (cm/p)	SFW (mg/p)	SDW (mg/p)	RFW (mg/p)	Vigour Index
Np	71^{a}	6.3^{a}	33.3^{a}	3.0^{a}	1.7^{a}	513^{a}
V+Kar	75^{b}	7.0^{b}	35.3^{ab}	3.3^{a}	1.7^{a}	549^{ab}
Sw+V+Kar	79^{bc}	7.0^{b}	37.3^{ab}	3.7^{a}	1.8^{a}	554^{ab}
Dw	83^{cd}	7.3^{bc}	38.3^{ab}	3.7^{a}	1.8^{ab}	579^{abc}
V	84^{cd}	7.3^{bc}	39.0^{ab}	3.7^{a}	2.4^{ab}	584^{abc}
Sw+V	84^{cd}	7.7^{c}	43.0^{b}	4.0^{a}	3.0^{bc}	601^{abc}
Kar	85^{cd}	8.0^{c}	44.0^{bc}	4.0^{a}	3.0^{bc}	672^{bcd}
Sw+Kar	85^{cd}	8.7^{d}	52.3^{cd}	5.0^{b}	3.7^{cd}	715^{cd}
Sw	87^{d}	9.0^{e}	59.6^{d}	5.7^{b}	4.1^{d}	792^{d}

Np:None primed (Control), Dw:Distilled water, Sw:Seaweed, Kar:Karrikinolide, V:Vermicompost, EP:Emergence percentage, SH:Seedling height (cm/p), SFW:Seedling fresh weight (mg/p), SDW:Seedling dry weight (mg/p), RFW:Root fresh weight (mg/p).

Table 5. Changes in catalase (CAT). activity of none primed (Np). distilled water (Dw). seaweed (Sw). seaweed and Kar (Sw+Kar) treated carrot seeds. The values in a column with the same letter are not significantly different (P< 0.05).

CAT	EUg^{-1}seed
Np	0.299 ^{a}
Dw	0.239^{a}
Sw	0.411^{b}
Sw+Kar	0.401^{b}

Table 6. Results of mineral analysis from the three biostimulants (mg kg^{-1})

Extracts	P	K	Ca	Mg	S	Fe	Zn	Cu	Mn	B
V	1.19	175.20	9.93	3.07	5.86	0.25	0.17	0.11	0.31	0.18
Sw	0.26	5.37	12.30	4.22	10.19	0.05	0.14	0.11	0.29	0.06
Kar	0.11	0.82	0.41	0.22	0.52	0.02	0.13	0.11	0.28	0.25

V:Vermicompost, Sw: Seaweed and Kar: Karrikinolide

Seedling characteristics such as EP, SH, SFW, SDW, RFW and vigor index were affected at 100mM (Table 3). The inhibitory effect of salinity on germination and emergence of carrot was previously studied (Elena and Lagunovschi, 2015). Slow germination, or inability to germinate, in carrot at 100mM or drought might be due to insufficient osmotic potential to hinder the uptake of threshold water. Moreover, high salinity has an inhibitory effect on cell division and enlargement. Sayar et al. (2010) mentioned that water uptake by a seed can be limited by a decrease in the osmotic potential of soil solution due to the accumulation of soluble salts in the growth medium, which in turn results in a decrease in the activity of physiological process such as germination, growth and development. Salt (NaCl) and PEG at the same osmotic potential were used to simulate salinity and moisture stress. But, the inhibitory effect of PEG was greater than salinity at same osmotic potential. In a similar study on wild mustard, both NaCl and PEG treatments decreased final germination percentage and seedling growth characteristics, but the effects of NaCl were lower on germination compared to PEG (Kayacetin et al., 2018). Demir and Mavi (2008) reported the effects of salt and osmotic stresses on the germination of pepper seeds of different maturation stages, and stated that the inhibition of germination at the same water potential of NaCl and PEG resulted from the osmotic effect rather than the salt toxicity. Seed priming with different biostimulants exerted a positive effect on carrot seeds. Sw and Sw+Kar were more effective in improving the performance of carrot seeds (Table 3) and seedlings under salinity stress conditions. Sw improved not only germination but also seedling characteristics such as EP, SH, SFW, SDW, RFW and vigor index. In previous reports, seaweed extract caused positive responses in different crops when used as priming agent (Kalaivanan and Venkatesalu, 2012; Shahbazi et al., 2015). A promotional effect on germination and seedling characteristics was also observed using 5% seaweed extract during priming of *Cyamopsis tetragonola* (Balakrishnan et al., 2007) as we used the same concentration. This is in line with the findings of the present study. The promotional effect of

priming with seaweed (SW) in carrot seeds might be attributed to the presence of enzymes, phytohormones, minerals and other growth-promoting substances (Godlewska et al., 2016; Masondo et al., 2018).

Carrot is a cool season crop and vulnerable to loss from thermal stress during early stages of development. Most commercial carrot cultivars have reduced and erratic germination at high temperatures (Nascimento and Pereira, 2007). The metabolic activation of the embryo is triggered by a favorable temperature preparatory to the start of germination. High temperatures hinder activation of enzyme compliments within the cells, changes in nucleic acids in the nuclei, mobilization of stored energy, and synthesis of new materials during the early stages of germination. All these changes prepare the radicle for elongation and the transition to normal seedling growth and development (Kozarewa et al., 2006). The indirect injuries include inactivation of enzymes, inhibition of protein synthesis, protein degradation and membrane integrity (Goraya et al., 2017). Under mild temperature stress (30˚C), matrix priming of seeds with seaweed extracts and seaweed plus karrikinolide combinations was effective in minimizing the problems related to temperature stress and improving the germination/emergence of carrot seeds and seedling characteristics such as seedling height, seedling fresh and dry weight, and vigor index. Effects of seed priming on germination stress were observed in muskmelon (Nascimento and Aragao, 2004), watermelon (Demir and Oztokat, 2003) and asparagus (Bittencourt et al., 2004). The positive effect of Sw as a biostimulant for seed priming was also observed in many crops by different researchers (Balakrishnan et al., 2007; Kalaivanan and Venkatesalu, 2012; Shahbazi et al., 2015).

One of the original aspects of the present work was to test the double and triple combinations of three different biostimulants in order to accelerate the priming effect. However, combinations appeared not to be more effective than treatments alone. This indicates that there is no integrated effect since combinations did not provide higher seed germination than single treatments. Seaweed alone and its combination with karrikinolide were most effective in improving the quality of carrots seeds both under normal and all stress conditions. Interestingly the positive effect of ranking in all treatments was the same under all stress conditions with Sw being the best and Sw+Kar being the second-best treatment (Tables 2, 3, and 4). The positive effect on germination under abiotic stress conditions was also reported in our earlier work in onion seeds (Muhie et al., 2020). Distilled water (humidifying, soaking etc.) is commonly used in priming. The promotional effect of priming with seaweed (Sw) in carrot might be attributed to the presence of enzymes, phytohormones, minerals and other growth-promoting substances (Sivasankari et al., 2006 Godlewska et al., 2016; Masondo et al., 2018, Muhie et al., 2020). In the present study, maximum amount of CAT was observed in seeds treated with seaweed and Sw+Kar which might contribute to the metabolic activity of seeds and enhanced seed germination. High sulfur in Sw co-relates to the production of enzymes, since S is the precursor of methionine and other amino acids (Svozil and Baerenfaller, 2017). Sulfur containing compounds play a critical role in the response of plants to abiotic stress factors including drought and salinity (Cao et al., 2014). Thus, rich mineral contents may contribute to the beneficial effect of seaweed. Researchers also revealed the presence of various sources of growth-promoting substance/hormones in seaweed (Masondo et al., 2018).

Conclusion

Solid matrix priming of carrot seeds with seaweed extract alone and its combination with karrikinolide showed the best results for carrot seed germination and seedling characteristics. Further research should be done to identify the best biostimulant priming for each crop because there is no single best suited method for priming of every crop.

Compliance with Ethical Standards

Conflict of interest

Authors declare no potential conflicts of interest with respect to the publication of the article.

Author contribution

Seid Hussen developed the theoretical idea of implementing the experiment. Ibrahim Demir modified the research idea and its methodologies. Seid Hussen carried out the experiment and wrote the manuscript which was proofread by Ibrahim Demir. Nurcan Memis and Cihat Ozdamar conducted the experimental applications. Zeynep Gokdas created figures and tables and searched the literature.

Ethical approval

Not required

Funding

No financial support was received for this study.

Data availability

Not applicable

Consent for publication

Not applicable

Acknowledgment

We thank Prof J. Van Staden of Kawazulu Natal University/ South Africa for generously donating karrikinolide. The free supply of vermicompost leach from Aybasol fertilizer company, Polatlı, Ankara was appreciated.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.14 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):360-364 (2021)

PCPM (*Prunus cerasifera X Prunus microcarpa*) hybrid rootstock candidate: Identification and production possibilities with hardwood cutting

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Abstract

This study was conducted in the laboratories and greenhouses of Eastern Mediterranean Transitional Zone Agricultural Research Institute between 2018 and 2020 to identify botanical characteristic of *PCPM* (*Prunus cerasifera* X *Prunus microcarpa*), which is natural hybrid of *Prunus cerasifera* (PC) *and Prunus microcarpa* (PM), and to investigate slip production opportunities of it at different IBA 1000 mg L^{-1}, 2000 mg L^{-1}, 3000 mg L^{-1}, 4000 mg L^{-1}. *PCPM* which was noticed in its natural environment, was followed in botanical terms for 3 years. It was determined that the average fruit weight, fruit width, and fruit length values of PCPM were 1.33 g, 11.93 mm and 12.50 mm, respectively.The soluble solid contents (SSC) values were measured as 15.03 brix in *PC*, 25 brix in *PM*, and 27.66 brix in *PCPM.* It was determined that the annual shoot development was 124.98, 8.41, and 26.15 cm, and the leaf lengths were 64.99, 21.18, and 41.93 mm in *PM, PC,* and *PCPM,* respectively. It was also determined that *PCPM* showed a botanic characteristic between *PM* and *PC*, which are its parents in general botanical terms. In rooting with hardwood cutting for *PCPM*, it showed similar values with Myrobolan 29C, which was the control rootstock, and had average rooting percentage as %80.11, and the average number of roots as 5.27 pcs/slip. The results had a positive effect on rooting increase in hormone application of 2000 mgL^{-1} concentration with IBA compared with other applications.

Keywords: Rootstock, Hardwood cutting, Hybrid, *Prunus*

Introduction

Prunus is a genus spreading widely in the northern temperate zone and Anatolia as one of the member of 250 different species of the Rosacea family, and some of which are still not identified in botanical terms (Demirsoy and Demirsoy, 2004). Another important characteristic of *Prunus* or its interspecies natural hybrids is that they can have important rootstock characteristics for other *Prunus* species (Bouhadida et. al., 2009). Prune is one of the most important fruit in the world, and many seedlings and clonal (vegetative) rootstocks are widely used in recent years (Cociu, et al., 1999; Gundesli, 2018). Also, in many countries, *Prunus* species are also used in breeding programs to obtain new tree fruit crops and rootstocks. All around the world like GF 677 (*P.persica* X *P.amygdalus*) important rootstocks formed by natural hybridization ways used extensively in modern orchards are also known. *Prunus microcarpa* (PM) is a wild fruit species that attracts attention with its scrub development in hard-seed *prunus* genus. *and Prunus cerasifera* (PC) has a wide range of use as fresh and dried fruit, and also has a wide range of usage area as rootstock (Ugur et al., 2019). It was reported in previous studies that natural hybrids occur in the nature between *P.cerasifera* X

Cite this article as:
Ugur, R., Gundesli, M.A., Palaz, E.B. (2021). PCPM (*Prunus cerasifera* X *Prunus microcarpa*) hybrid rootstock candidate: Identification and production possibilities with hardwood cutting. J. Agric. Environ. Food Sci., 5(3), 360-364
Doi: https://doi.org/10.31015/jaefs.2021.3.14
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Received: 10 February 2021 **Accepted:** 18 May 2021 **Published Online:** 26 August 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 360-364
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

P.armeniaca, *P. microcarpa X P.cerasifera, P. cerasifera X P.avium* (Mirabdulbaghi,et al., 2011; Atli et al., 2019).

This study was aimed to identify some botanical characteristic of PCPM (*P. cerasifera X P.microcarpa*)which is a hybrid rootstock candidate found coincidentally during rootstock breeding studies, by comparing its characteristics with its parents *PM* and *PC* and to determine the possibility of it for being used as a rootstock.

Material and Method

Plant Material

The material of the study was PCPM hybrid rootstock candidate and Myrobolan 29C rootstock (*P.cerasifera*) was used as the control rootstock in hardwood cutting trials.

Morphological and pomological determination

The PCPM hybrid rootstock candidate was examined in terms of pomological and morphological characteristics between 2018 and 2020. Each year, fruit, shoot, and leave samples, including PM and PC were taken and analyzed in the laboratory. All the fruit and leaf sample works were carried out for 3 years on 10 leaf and 30 fruit samples, and the mean were noted (Table 1). Weighing of the samples was made with precision scales (Dikomsan FGH 0.001 g), and the measurements were made with precision caliper (0.01 mm Gomax GMX1017020) on millimetric paper (Yılmaz, 2010).

Propagation with hardwood cutting

PCPM and Myrobolan 29C rootstocks have been taken to the production trial with hardwood cutting, which is the basic study for propagation. Annual shoots 20-25 cm in length, in the dormant period, were taken for rooting. Hardwood cuttings were kept in water with fungicide solution (% 80 Fosetyl-Al) for a day before rooting try. For the hardwood cutting, a total of 3 m^2 rooting pool and 3-4 mm thick medium size perlite was used. The hardwood cuttings were taken to rooting by applying IBA in four different mg.L-1 doses (1000-2000-3000-4000 mgL^{-1}) to the rooting pools in the greenhouse (Atli et. al., 2019).

Statistical analysis

Propagation trial was established in a factorial-random parcel trial pattern with 3 repetitions and 20 plants in each repetition. Variance Analysis at %5 significance level in JMP 5.0 Statistical Program, and the multiple comparisons were made with the LSD test at the same significance level (Table 2-3).

Result and Discussion:

It was found that the average fruit weight values ranged between 0.29 g *PM* and 25.36 g *PC* and the average fruit weight was 1.31 g in *PCPM*. Equatorial fruit width values of *PC, PM* and *PCPM* were 35.25 mm, 7.19 mm and 11.93 mm, respectively, and *PM* had a more homogeneous fruit width (Table 2 and Figure 1). Similar to the findings of this finding support the work of (Moreira et al., 2009Maghsudlu et al.,2013) who observed hardwood cuttings of *PC* that fruit height values to be 34.03 mm, 8.31 mm, 12.50 mm. Although the shape index was close to "round" in the fruits of all three species, *PM* fruits were slightly oval, and PCPM mostly had round fruits. It was found that *PC* had a fruit pulp color in yellow tones, *PM* had orange pulp color, and PCPM was between light brown and claret red color. However, Nas, et al. (2011) indicated that there has been also *PM* genotypes in different colors between red and purple. It was determined that the highest SSC value was in PCPM (27.66), and the lowest value was in *PC* (15.03). Fruit development values of *PC, PM* and PCPM were evaluated, *PM*'s fruit development was more homogeneous than *PC*, and PCPM created a full intermediate form in terms of these characteristics, as expected in a hybrid. It was determined that *PM* had a more homogeneous seed structure; however, the pulp/seed ratio was highest in *PC* (29.23), *PM* had the lowest pulp/seed ratio (2.62), and these values were similar to the results reported by (Demirsoy and Demirsoy, 2004; Mohammadi, et al., 2011). In terms of leaf width and leaf length values, PCPM was distributed between the two species, and the inter-nod distance on annual shoots was low in *PM* (12.45 mm), followed by PCPM and *PC* with 25.71 and 35.03 mm, respectively. These results were similar to the results reported by Rakonjac, et. al., (2010) (Table 1). Our results show that there is a significant difference between rooting percentages of IBA application according to variance analysis (Table 2, 3 and 4). It was observed in studies applying different IBA hormone doses that the highest percentage of rooting was in the doses of 2000 mg.L-1 (%91.07), and the lowest rooting rate was in 4000 mg.L-1 dose (%70.95) unlike expected. It was determined that the PCPM rootstock had similar characteristics in terms of rooting rate with Myrobolan 29C rootstock, which was the control rootstock (Table 2). Sulusoglu et al., (2010) conducted a study on the rooting possibilities of *Prunus laurocerasus*, which may be considered in the same category with PCPM and PM in our study, at different IBA doses, and reported that 2000 mg.L-1 IBA application yielded higher rooting results than the other (1000-4000-6000-8000 mg.L-1) applications. The increase of IBA concentration was accompanied by the decreased rooting percentage, suggesting high IBA concentrations were not suitable for the root formation process (Ercisli, 2004; Mohammadi et al., 2019; Ugur et al., 2018). Kurd, et al., (2010) reported that IBA doses, unlike what was expected, did not provide a considerable increase in rooting rates, and even there was a decrease after 2000 mg.L-1 dose. Tworkski and Takeda (2007) reported that increased IBA doses prevented rooting after a certain rate. It is possible to estimate that even the lowest results detected in our study (%70.95) were close to the highest results of these studies, and this would be considered PCPM as a great rootstock candidate (Table 3). Macmahon et al., (2015) reported that in their study that the average rooting rates were around %40-45, while Moreira et al., (2009) achieved rooting rates around %70-75. The 2000 application mg.L-1 that had the highest root length value (52.88 mm) was followed by 3000 mg.L-1 application with 47.86 mm. The 4000 and 1000 mg.L-1 IBA applications yielded close values. Although root length values were detected at higher values in Myrobolan 29C rootstock (47.52 mm), it was also noted that the PCPM results were not low. In general, both rootstocks yielded high results in root length values in 2000 mg.L-1 IBA application (Table 3). The root length values varied between approximately 40 and 50 mm in our study. In the studies conducted by Sulusoglu and Cavusoglu, (2010), Macmahon et al., (2015) and Tworkoski and Takeda, (2007)

they achieved average root length values between 10 and 25 mm. Compared to these values, it could be argued that the root length value of the PCPM have been good. It was found that applying IBA to Myrobolan 29C rootstock and PCPM rootstock did not yield a different result in terms of the root count, but both rootstocks had promising root counts. In addition, it was also found in the present study that the highest root count was also in 2000 mg.L-1 IBA application. Other applications did not increase the root counts at significant levels (Table 3).

Table 1. Some botanic characteristics of PCPM hybrid rootstock candidate in comparison with *P.cerasifera (PC)* and *P.microcarpa (PM)*

No	Parameters	PC	PM	PCPM
1	Fruit weight (g)	25.36 ±0.88	0.29 ±0.02	1.33 ±0.02
2	Fruit width (mm)	35.25 ±1.03	7.19 ±0.05	11.93 ±0.16
3	Fruit length (mm)	34.03 ±0.95	8.31 ±0.01	12.50 ±0.10
4	Fruit shape index	1.04 ±0.05	0.87 ±0.01	0.95 ±0.01
5	Fruit color (L)	35.38 ±0.88	36.09 ±0.87	31.59 ±0.56
6	Fruit color (a)	15.82 ±0.30	15.39 ±0.07	9.42 ±0.44
7	Fruit color (b)	49.96 ±0.77	43.91 ±0.74	51.45 ±0.58
8	Fruit flesh thickness (mm)	12.06 ±0.45	1.36 ±0.01	2.71 ±0.02
9	SSC	15.03 ±0.28	25.00 ±0.10	27.66 ±0.32
10	Core weight (g)	0.87 ±0.03	0.12 ±0.01	0.32 ±0.01
11	Core length (mm)	15.92 ±0.56	7.26 ±0.02	10.08 ±0.06
12	Core width (mm)	18.34 ±0.43	4.53 ±0.06	6.55 ±0.32
13	Meat-core ratio	29.23 ±0.83	2.62 ±0.12	4.12 ±0.09
14	Pedile length (mm)	12.75 ±0.23	9.46 ±0.02	16.50 ±0.14
15	Pedicular diameter (mm)	1.11 ±0.07	0.75 ±0.01	0.47 ±0.03
16	Leaf width (mm)	43.38 ±0.72	14.00 ±0.04	31.10 ±0.09
17	Leaf length (mm)	64.99 ±0.83	21.18 ±0.48	41.93 ±0.30
18	Petiole length (mm)	15.56 ±0.58	6.58 ±0.07	11.02 ±0.16
19	Annual shoot length (cm)	124.98 ±0.24	8.41 ±0.11	26.15 ±0.19
20	Distance between nodes (mm)	35.03 ±0.49	12.45 ±0.05	25.71 ±0.58
21	Leaf color (L)	33.46 ±0.43	37.34 ±0.43	32.51 ±0.54
22	Leaf color (a)	11.67 ± 0.91	15.23 ± 0.58	26.54 ±0.97
23	Leaf color (b)	32.59 ± 0.80	37.97 ±0.40	37.44 ±0.53
24	Flowering date	16-23 March	14-19 March	14-21 March
25	Ripening date	June 19-22	06-10 June	June 15-18

*Each value is expressed as mean ± standart deviation.

Table 2. Rooting percentage of PCPM hybrid rootstock candidate (%)

	Hormone Applications (mgL^{-1})				
Rootstocks	1000	2000	3000	4000	Rootstock Average
PCPM	84.59	90.79	75.28	69.81	80.11
Control	86.25	92.62	73.27	72.10	81.06
Application average	85.42B	91.07A	74.27C	70.95D	
$LSD_{Rootstock\ 0.05}$: NS		$LSD_{hormone\ 0.05}$: 2.91*		$LSD_{rootstock\ x\ hormone\ 0.05}$:NS	

NS: No significant

Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).

Table 3. Average root length (mm) and root number (mm) values of PCPM hybrid rootstock candidate

Rootstocks Root length	Hormone Applications (mgL^{-1})				Rootstock Average
	1000	2000	3000	4000	
PCPM	44.72b	51.72a	42.98bc	39.63c	44.76B
Control	40.12bc	54.03a	52.74a	43.18bc	47.52A
Hormone Application	42.42C	52.88A	47.86B	41.40C	
$LSD_{Rootstock\ 0.05}$: 2.44**		$LSD_{hormone\ 0.05}$: 3.46**		$LSD_{rootstock\ x\ hormone\ 0.05}$: 4.89**	
Root number					
PCPM	5,52	6,10	5,27	4,80	5.27
Control	5,12	6,31	5,08	5,05	5.53
Hormone Application	5.32B	6.20A	5.17B	4.92B	
$LSD_{Rootstock\ 0.05}$: NS		$LSD_{hormone\ 0.05}$: 0.65*		$LSD_{rootstock\ x\ hormone\ 0.05}$:NS	

NS: No significant

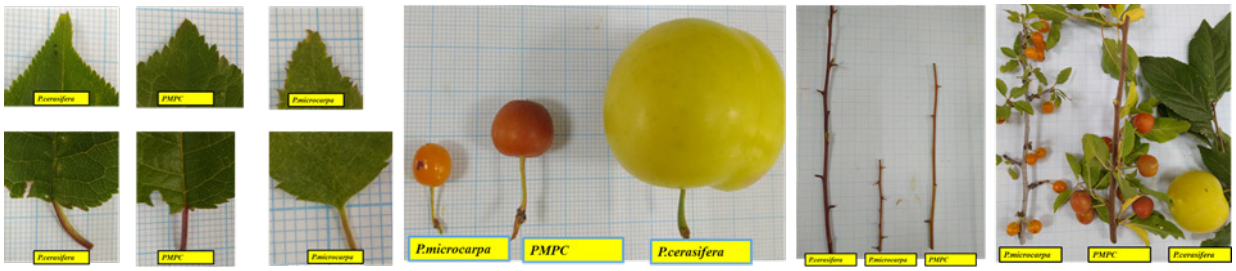

Figure 1. Shoot, Leaf and Fruit characteristics of PCPM hybrid rootstock candidate

Conclusion

As a result, it is considered that hybrids occur spontaneously in the nature among prunus species, and can be made use of for different purposes. Considering the identifications of wild plums and all the botanical characteristics of *PCPM*, it was demonstrated that it is the hybrid of *PC* and *PM* (Tables 1-2-3). In general terms, when it is considered that between %1-10 of seeds are achieved in interspecies hybridization works, the value of hybrid genotypes occur naturally and survive extrem natural conditions like this becomes clear in breeding investigations (Table 2 and 3). The importance of natural hybrids can be better understood when it is considered that the GF-677 clone rootstock, which is the hybrid of *Prunus persica* X *Prunus amygdalus*, found by chance in France, and is now widely used in modern fruit orchards. In this study, the *PCPM* rootstock candidate genotype attracted the attention as a natural hybrid between *PC* and *PM*, its botanical examinations were completed, and the possibilities of slip production were investigated. It was concluded in the study that the fruits of PCPM's *prunus* may have the positive characteristics of being a rootstock. In the future, it will be beneficial to investigate the rootstock characteristics of this rootstock candidate for different *Prunus* types.

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 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.

Funding

This work was supported by grants from the General Directorate of Agricultural Research and Policies (TAGEM BBMB-11-14).

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgments.

Authors are thankful to General Directorate of Agricultural Research and Policies Scientific Research Projects Authority about their financial supports**.** Also thanks to Prof. Dr. Sevgi Paydaş Kargı for technical studies and recommendations.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.15 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):365-373 (2021)

Evaluation of a Household Drinking Water Purification System Performance in terms of Organic – Inorganic Water Pollution Indicators and Ecological – Health Risk Assessment Indices

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Abstract

In this study, the performance of one of the most popular household drinking water purification systems (WPS) of Turkey was evaluated. Tap and purified water samples were taken from İpsala District (Thrace Region). A total of 23 significant water quality assessment parameters including essential and toxic metals (pH, TDS, EC, turbidity, Cl, NO_3, SO_4, PO_4, BOD, COD, B, Al, Cr, Mn, Ni, Cu, Zn, As, Sr, Mo, Sb, Ba, Pb) were measured in water samples and how much the WPS improves these parameters were determined. Also Water Quality Index (WQI), Heavy Metal Pollution Index (HPI), Heavy Metal Evaluation Index (HEI), Nutrient Pollution Index (NPI), Cancer Risk (CR), Hazard Quotient (HQ) and Hazard Index (HI) were applied to data in order to assess the qualities of tap and purified water in terms of multiple effects of toxicants and possible risks of human health. As a result of this research, it was determined that the investigated WPS significantly improved the drinking water quality and significantly reduced the scores of applied ecological and health risk assessment indicators.

Keywords: Household drinking water purification systems, Water qulity, İpsala District, Ecological indicators, Health rish indicators

Introduction

Water purification history is quite old and it is known that even in the ancient times, water was purified by passing through some materials such as stones or sands or by boiling. When the history of modern water treatment systems is examined, it is seen that the first water softening device was made in 1903, the first membrane for water purification devices was developed in 1980, the purification devices with UV in its structure were developed in 1995, the first closed water purification system was made in 2001, carbon filters were developed for water purification devices in order to provide the mineral support to water in 2007 and more portable water purification systems were made in 2015 (Maden et al., 2019).

With the developing technology, many different filter types have been added to the household WPSs, whose main purpose is to get the hardness of the water. Recently, the most used technology in household WPSs is the devices equipped with reverse osmosis technology, which helps to filter the ions, heavy metals, all bacteria and all the substances harmful to the human health in the water. Also, the amount of lime, which constitutes a significant problem in drinking water, and the bad odours due to various reasons can be cleaned by the reverse osmosis method (http://www.cebilon.com.tr/).

Heavy metals, which may strongly accumulate and biomagnified in organisms, have numbers of hazardous effects both on the ecological balance of environment and on the

Cite this article as:
Tokatli, C., Ustaoglu, F. (2021). Evaluation of a Household Drinking Water Purification System Performance in terms of Organic – Inorganic Water Pollution Indicators and Ecological – Health Risk Assessment Indices. J. Agric. Environ. Food Sci., 5(3), 365-373
Doi: https://doi.org/10.31015/jaefs.2021.3.15
Orcid: Cem Tokatli: 0000-0003-2080-7920 and Fikret Ustaoglu: 0000-0002-8195-8557
Received: 24 February 2021 **Accepted:** 17 July 2021 **Published Online:** 24 August 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 365-373
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

human health. They can be adsorbed by biota and transported and bio-accumulated to human through the several food chain interactions or directly by consuming drinking water containing heavy metals. Toxic metals may cause various non-carcinogenic and carcinogenic health problems and diseases (Song et al., 2018; Mutlu and Uncumusaoğlu, 2018; Köse et al., 2020; Ustaoğlu and İslam, 2020). Chronic exposure of these toxicants at lower doses for a long time may cause many types of cancer (Park et al., 2004). It has been well documented that significant quantities of toxic metals are being discharged to the environment and threatens the health of environment and human (İslam et al., 2018; Varol and Tokatlı, 2021; Tokatlı and Varol, 2021).

Toxic metal pollution in drinking water has been a significant risk factor for human health in almost all the globe and many new methods have been developed to assess the potential risks and the multiple effects of toxicants in freshwater (Varol and Davraz, 2015; Tokatlı, 2019; Ustaoğlu and Tepe, 2019; Saleem et al., 2019; Tokatlı and Ustaoğlu, 2020; Varol, 2020; Ustaoğlu and Aydın, 2020; Tokatlı et al., 2021). In the present study, the performance of a widely used household water purification system (WPS) in Turkey was evaluated by determining some significant organic and inorganic water pollution indicators and by using some significant health risk and water quality assessment indices.

Materials and Methods

Collection of water samples

İpsala District is located in the downstream of Meriç – Ergene River Basin and known as an agricultural city. Intensive agricultural activities are conducted around it and nearly 25% of Turkey›s total rice production is produced from this region. Therefore, it is known that the drinking waters of the region are quite polluted especially in terms of organically (Tokatlı, 2015; 2017; Bülbül and Elipek, 2017; Öterler, 2017). Drinking water samples were taken from the tap water of İpsala District, where is known as an agricultural city and intensive agricultural activities are conducted around it, and from the purified tap water of the district treated by a widely used household water purification system with reverse osmosis in the winter season (2019), when the precipitation was at the highest level. Water samples were taken to the 1 L pre-cleaned and acid washed polyethylene bottles. pH of water samples to be used in the elemental analyses were reduced with nitric acid in order to make them below 2 (APHA, 2005).

Water purification stages of investigated household drinking water purification system

5 different filtering systems are used in the investigated WPS. Sediment Pre-Filter (SPF) (1) collects coarse dirt. Granular Activated Carbon Filter (GACF) (2) retains chlorine and other gases and gives clarity to water. Block Carbon Filter (BCF) (3) is a second carbon filter in addition to the GACF filter. It makes the incoming water to enter the membrane. It holds even the finest particles. Membrane (M) (4) is the heart of the reverse osmosis system. It separates all negative elements in water except water molecules. The Final Carbon Filter (FCF) (5) gives flavour to the water and removes the odour that may occur in the tank (http://www.cebilon.com.tr/).

Physicochemical and macro – micro element analysis

pH, TDS and EC parameters were measured by using a multiparameter device (Hach Lange, HQ40D), turbidity parameter was measured by using a turbidimeter device (Hach Lange, 2100Q), Cl, NO_3, SO_4, PO_4 and COD parameters were measured by using a spectrophotometer device (Hach Lange, DR3900) and BOD parameter was measured by using a BOD device (Hach Lange, BOD Trak 2).

Water samples were filtered and their volumes have been set to 50 ml with ultra – pure water. Then the macro – micro element contents were measured by an Agilent branded (7700 XX) ICP-MS in the central laboratory of Thrace University (accreditation certificated laboratory). All the macro – micro element analyses were listed as the average of triple reads (TS EN / ISO IEC 17025) (EPA, 2001).

Water Quality Index

WQI is a widely used method to assess the groundwater and sufacewater quality (Wang et al., 2017; Varol, 2020; Ustaoğlu et al., 2020). The formula of WQI is given in the Equation (1) and (2).

$$WQI = \sum \left[W_I \times \left(\frac{C_i}{S_i} \right) \times 100 \right] \quad (1)$$

$$W_I = \frac{W_i}{\Sigma W_i} \quad (2)$$

W_I is relative weight. W_i coefficients are assigned as 5 (maximum) – 1 (minimum), according to the effects of toxicants on health (Meng et al., 2016). C_i is the parameter level determined in water. S_i is the standard value for drinking water specified by WHO (2011), EC (2007) and TS266 (2005). The scale of WQI is given in Table 1 (Xiao et al., 2019).

Heavy Metal Pollution Index

HPI is an assessment method the combined effects of each heavy metal on the overall water quality (Herojeet et al., 2015; Wagh et al., 2018; Tokatlı and Ustaoğlu, 2020). The formula of HPI is given in the Equation (3) and (4) (Mohan et al., 1996).

$$HPI = \frac{\sum_{i=1}^{n} W_i Q_i}{\sum_{i=1}^{n} W_i} \quad (3)$$

$$Q_i = \sum_{i=1}^{n} \frac{M_i}{S_i} \times 100 \quad (4)$$

Q_i is the subindex of the toxicant. W_i is the unit weight. M_i is the determined levels of toxicant. S_i is the standard of the toxicant. n is the total number of toxicants considered. The scale of HPI is given in Table 1 (Saleh et al., 2018).

Heavy Metal Evaluation Index

HEI helps to determine the overall assessment of water quality in terms of toxic metals (Edet and Offiong, 2002). The formula of HEI is given in the Equation (5).

$$HEI = \sum_{i=1}^{n} \frac{H_C}{H_{MAC}} \quad (5)$$

H_c is the level of toxicant determined in water sample. H_{mac} is the maximum admissible concentration (MAC) (WHO, 2011). The scale of HPI is given in Table 1 (Bodrud-Doza et

al., 2016; Saleh et al., 2018).

Nutrient Pollution Index

NPI is an important technique to evaluate the drinking water quality in terms of nutrient contamination (Isiuku and Enyoh, 2020). The formula of NPI is given in the Equation (6).

$$NPI = (CN/MAC_N) + (CP/MAC_P) \quad (6)$$

$C_{N/P}$ are the levels of NO_3 and PO_4 detected in the water samples. $MAC_{N/P}$ are the maximum permissible levels of NO_3 and PO_4 specified by WHO (2011). The scale of NPI is given in Table 1.

Health Risk Assessment

In the present investigation, one of the most effective human health risk evaluation technique developed by EPA (2004) was applied to data. Chronic daily intake (CDI), exposed from digestion ($CDI_{ingestion}$) and absorption by dermally (CDI_{dermal}) were calculated. The formulas of $CDI_{ingestion}$ and CDI_{dermal} are given in the Equations (7) and (8):

$$CDI_{ingestion} = Cwater\ x\ \frac{(IR\ x\ EF\ x\ ED)}{(BW\ x\ AT)} \quad (7)$$

$$CDI_{dermal} = Cwater\ x\ \frac{(SA\ x\ Kp\ x\ ET\ x\ EF\ x\ ED\ x\ CF)}{(BW\ x\ AT)} \quad (8)$$

$CDI_{ingestion}$ is the chronic daily intake by ingestion. CDI_{dermal} is the chronic daily intake by dermal adsorption (ppb/day). C_{water} is the concentration of the toxicant in water. IR is the ingestion rate. EF is the exposure frequency. ED is the exposure duration. BW is the average body weight. AT is the average time. SA is the exposed skin area. $ABS_{gastrointestinal}$ is the gastrointestinal absorption factor. Kp is the dermal permeability coefficient in water. ET is the exposure time during bathing. CF is the unit conversion factor (Saleem et al., 2019; Xiao et al., 2019; Varol et al., 2020; Ustaoğlu, 2020).

The probable non – carcinogenic risks of toxicants were determined by means of risk hazard quotient formula (HQ) both for adults and children. The formulas of $HQ_{ingestion}$ and HQ_{dermal} are given in the Equations (9), (10) and (11) (Chen et al., 2018).

$$HQ_{ingestion} = \frac{CDI_{ingestion}}{RfD_{ingestion}} \quad (9)$$

$$HQ_{dermal} = \frac{CDI_{dermal}}{RfD_{dermal}} \quad (10)$$

Hazard index (HI) is being calculated by summing the total amount of $HQ_{ingestion}$ and HQ_{dermal} (Equation (11)) and shows the total of potential non – carcinogenic effects formed by all the investigated toxicants (EPA, 2004; Wang et al., 2017).

$$HI = HQ_{ingestion} + HQ_{dermal} \quad (11)$$

If HQ and HI were bigger than 1 that means probable negative effects on human health. If HQ and HI were lower than 1 that means no negative effects on human health sourced from toxicants (Yang et al., 2017).

Carcinogenic Risk (CR) is being used to determine the potential risks for human by being exposed to several carcinogens for a life and it may be found by multiplying the Chronic Daily Intake (CDI) values with the Cancer Slope Factor (CSF) coefficients (Equation (12)) (Saha et al., 2017; Gao et al., 2019) The range of acceptable carcinogenic risk suggested by the EPA (2004) is 10^{-6} – 10^{-4}.

$$CR = CDI\ x\ CSF \quad (12)$$

Table 1. Water quality classes in terms of applied ecologic indices

Value	Water Quality Classes	Usage Possibilities
	WQI	
< 50	Excellent quality	Drinking, irrigation, industrial
50 – 100	Good quality	Drinking, irrigation, industrial
100 – 200	Poor quality	Irrigation, industrial
200 – 300	Very Poor quality	Irrigation
> 300	Unsuitable for drinking purpose	Treatment is required
	HPI	
< 100	Low heavy metal pollution	Suitable
> 100	High heavy metal pollution	Not suitable
	HEI	
< 10	Low pollution	Suitable
10 – 20	Medium pollution	Not suitable
> 20	High pollution	Not suitable
	NPI	
< 1	No pollution	-
1 – 3	Moderate polluted	-
3 – 6	Considerable polluted	-
> 6	Very high polluted	-

Results and Discussion

The detected physicochemical data and macro – micro element concentration levels in tap and purified water samples and the results of applied health risk and ecological risk indices are given in Table 2. Also the percent and fractional exchanges between the tap and purified water in terms of physicochemical results and the data of risk assessment indices are given in Table 1. Monomial scores of toxic metals used in HPI and HEI and also HI and CR scores are given in Figure 1.

Table 2. Standard values, detected data and the scores of applied indices

		Standard Values	Tap Water	Purified Water	Percent Exchange[1]	Fractional Exchange[2]
pH		6.5 – 9.5	7.26	8.21	13.09	1.13
TDS (ppm)		500	319.00	21.70	**-93.20**	**-14.70**
EC (µS/cm)		300	616.00	44.10	**-92.84**	**-13.97**
Turbidity (NTU)		5	0.59	0.47	-20.34	**-1.26**
Cl (ppm)		250	76.60	10.20	**-86.68**	**-7.51**
NO3 (ppm)		50	7.89	1.39	**-82.38**	**-5.68**
SO4 (ppm)		250	30.00	12.00	**-60.00**	**-2.50**
PO4 (ppm)		5	1.49	0.21	**-85.91**	**-7.10**
BOD (ppm)		3	5.40	2.10	**-61.11**	**-2.57**
COD (ppm)		5.5	16.30	4.47	**-72.58**	**-3.65**
B (ppb)		500	52.94	28.66	-45.87	-1.85
Al (ppb)		200	3.52	1.00	**-71.42**	**-3.50**
Cr (ppb)		50	6.14	0.23	**-96.18**	**-26.16**
Mn (ppb)		50	1.41	0.27	**-81.05**	**-5.28**
Ni (ppb)		70	1.89	1.04	-44.81	-1.81
Cu (ppb)		2000	2.80	0.26	**-90.85**	**-10.92**
Zn (ppb)		3000	96.16	15.05	**-84.35**	**-6.39**
As (ppb)		10	5.59	0.69	**-87.62**	**-8.08**
Sr (ppb)		1500	753.56	21.31	**-97.17**	**-35.36**
Mo (ppb)		70	1.07	0.39	**-63.57**	**-2.75**
Sb (ppb)		20	0.09	0.06	-30.89	-1.45
Ba (ppb)		700	106.95	3.96	**-96.30**	**-27.03**
Pb (ppb)		10	0.32	0.03	**-90.08**	**-10.08**
Ecological Risk Assessment	**WQI**		42.58	11.79	**-72.31**	**-3.61**
	HPI		19.41	2.44	**-87.41**	**-7.94**
	HEI		1.60	0.19	**-87.93**	**-8.29**
	NPI		0.46	0.07	**-84.69**	**-6.53**
Non - Carcinogenic Risk Assessment	**HI – Cr** (Adult)		5.88E-02	2.25E-03	**-96.18**	**-26.16**
	HI – As (Adult)		5.35E-01	6.63E-02	**-87.62**	**-8.08**
	HI – Pb (Adult)		6.57E-03	6.52E-04	**-90.08**	**-10.08**
	HI – Cr (Child)		6.62E-02	2.53E-03	**-96.18**	**-26.16**
	HI – As (Child)		6.03E-01	7.46E-02	**-87.62**	**-8.08**
	HI – Pb (Child)		7.37E-03	7.31E-04	**-90.08**	**-10.08**
Carcinogenic Risk Assessment[3 - 4]	**CR – Cr** (Adult)		**8.77E-05**	3.35E-06	**-96.18**	**-26.16**
	CR – As (Adult)		**2.40E-04**	2.97E-05	**-87.62**	**-8.08**
	CR – Pb (Adult)		7.82E-08	7.76E-09	**-90.08**	**-10.08**
	CR – Cr (Child)		**9.82E-05**	3.76E-06	**-96.18**	**-26.16**
	CR – As (Child)		**2.68E-04**	3.32E-05	**-87.62**	**-8.08**
	CR – Pb (Child)		8.76E-08	8.69E-09	**-90.08**	**-10.08**

[1]Reduces after purification more than 50% are marked in bold, [2]Reduces after purification more than 2x are marked in bold, [3]CR scores very close to the limit value are given in bold, [4]CR scores over the limit value are given in bold – underlined

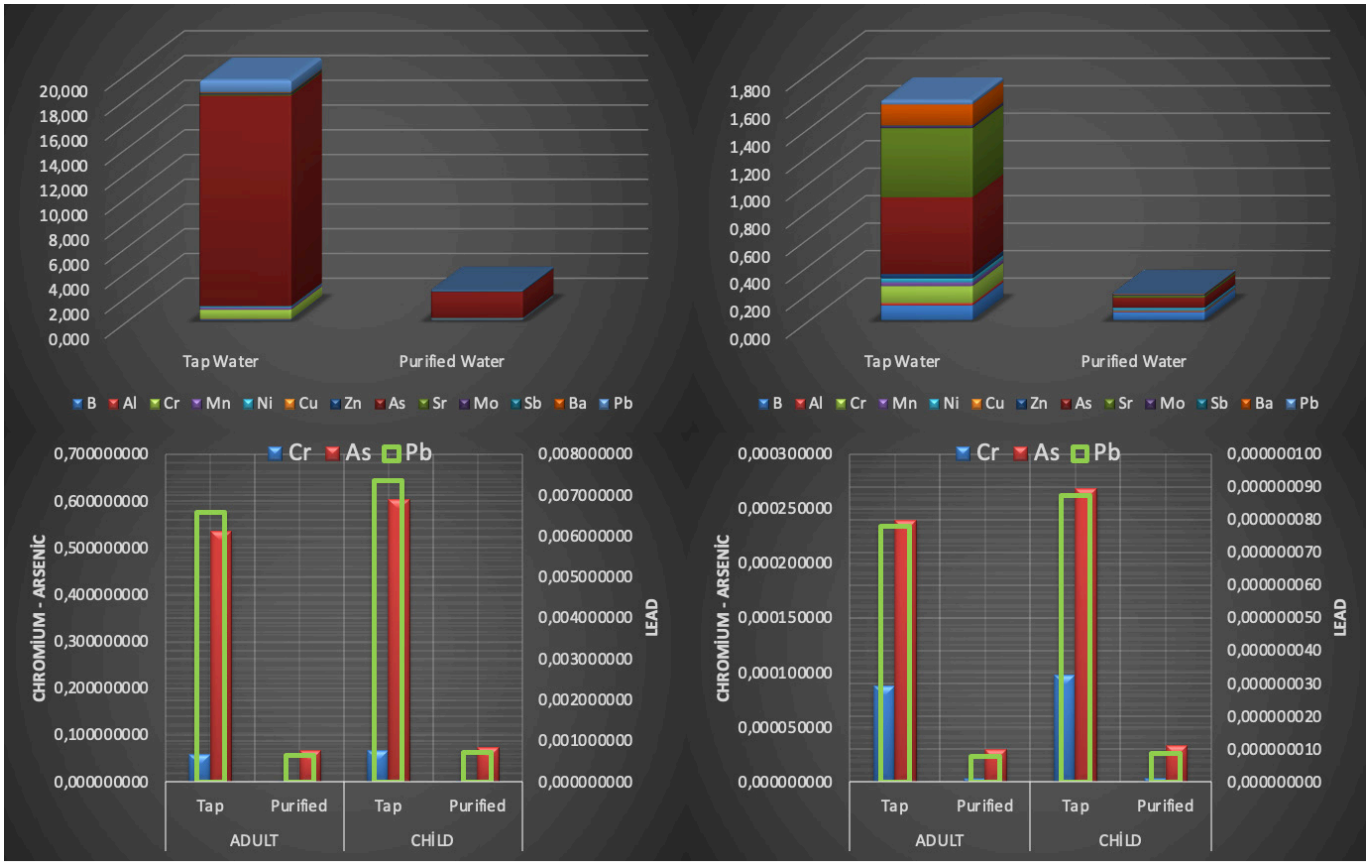

Figure 1. Monomial toxic metal scores (up) of HPI (left) and HEI (right) and results of HI (left) and CR (right)

According to the Water Pollution Control Regulation criteria in Turkey (WPCR, 2015), tap and purified water have 1st Class water quality in terms of pH, TDS, SO_4, COD and all the investigated macro – micro element levels. Tap water has 2nd Class water quality in terms of EC Cl, NO_3 and BOD and has 4th Class water quality in terms of PO_4 parameters, while the purified water has 1st Class water quality in terms of almost all these parameters.

The high or low pH value affects the drinkability of the water and according to TS266 (2005) and EC (2007) standards, the pH value of drinking water is required to be between 6.5 – 9.5. Slightly acidic pH value in drinking water indicates that the mineral content of the water is partially low and the carbon dioxide level is high, while the pH value is slightly alkaline means that the mineral content is quite higher. Also alkaline waters are known to be considered as more efficient and beneficial for human health (Li and Wu, 2019). In this study, it was determined that the investigated water purification system (WPS) increased the pH value of drinking water considerably and reaches a slightly alkaline level, which is considered to be optimum for human health.

Total dissolved solids and electrical conductivity and chlorine parameters are important for drinking water quality and also for water taste (Li and Wu, 2019). It was found that the investigated WPS reduced the levels of these parameters in drinking water of İpsala District approximately 10 times and significantly improved the taste of water.

Organic fertilizers and chemical fertilizers of inorganic origin are the most important factors that increase the amount of nitrogenous and phosphorous compounds in water. It is also known that phosphate fertilizers used in agricultural activities and phosphorus compounds in detergents are among the most important factors that increase the phosphate content of water. Nitrate in water can be caused by nitrate fertilizers used in agricultural areas, as well as the oxidation of ammonia, which occurs as a result of the decomposition of proteins contained in animal and vegetable wastes and sewage wastes (Wetzel, 2001; Manahan, 2011). It is thought that the main reasons for the quite high nitrate and phosphate detected from the drinking water of the İpsala District are agricultural activities and domestic wastes.

One of the most significant effects of nitrate on human health is methemoglobinemia, which is more common in new-borns and infants younger than six months. The stomach acid of new-born babies is not as strong as in older children and adults, and the condition causes a significant increase in the number of bacteria in the stomach that convert nitrate to nitrite. Pregnant women are susceptible to methemoglobinemia in adults with low stomach acidity and low methemoglobin-reducing enzyme activity. Nitrite is absorbed in blood cells and hemoglobin is converted into methemoglobin, which has a much lower oxygen carrying capacity (Self and Waskom, 2013; Tokatlı, 2014). The Maximum Contaminant Level (MCL) limit for nitrate reported by the EPA is 10 ppm, and the

risk of methemoglobinemia in neonates and methemoglobin – sensitive adults above this limit is extremely high (EPA, 2009). Nitrate content detected in the drinking water of the İpsala District was very close to the declared limit value.

It is known that phosphate has a carcinogenic effect and cause cancer risk, when taken in high amounts into the human body. Phosphate can be taken into the body directly by drinking water and may cause some stomach and digestive problems (Coşkunses, 2008). According to the data obtained, it has been determined that the drinking water of the İpsala District have 4th class water quality in terms of phosphate content and this parameter was found as an important risk factor for the health of the people living in this region.

In this research, it was determined that the investigated WPS decreased the nitrate and phosphate concentrations and Nutrient Pollution Index (NPI) values (being calculated by using nitrate and phosphate concentrations) in drinking water of İpsala District considerably (more than 80%) and significantly reduced the probably negative effects of these pollutants on the human health.

According to the results of applied ecological risk assessment indices, although both the investigated tap and purified water samples were found as "Excellent quality", "Low heavy metal pollution", "Low pollution" and "No pollution" in terms of WQI, HPI, HEI and NPI respectively, it was found that the Water Purification System (WPS) increased the water quality significantly and reduced the detected index data about 4 (%70), 8 (%90), 8 (%90) and 7 (%80) times for WQI, HPI, HEI and NPI respectively.

Arsenic is a carcinogenic and toxic element. Exposure of As may cause many significant health problems. Use of overly pesticides with high arsenic contents especially in the regions, where mono – cultural agricultural practices are intensive as in İpsala District, converts these toxic metals to a significant health risk factor for the local people. (ATSDR, 2012; Liu et al., 2013; Çiçek et al., 2013; Köse et al., 2015; Bhowmick et al., 2018; Tokatlı and Ustaoğlu, 2020).

Thrace Region constitutes among the most productive agricultural lands of Turkey. About 95% of the region, which means over one million hectares, is suitable for agriculture (TZOB, 2003; Anonymous, 2005). However, especially the paddy cultivation is being conducted as a mono-cultural approach in the region without any crop rotation for many years. Therefore, the soil of the reigon has weakened over the years in terms of minerals and the agricultural pests gain resistance. As a result of this mono-cultural approach, use of intensive agricultural fertilizers and pesticides have become a necessity in years for the region (Tokatlı and Ustaoğlu; Tokatlı, 2021; Varol and Tokatlı, 2021). In addition, the social studies conducted in the region show that the local people are not sensitive enough about the environmental pollution and sustainability of their soil (Tokatlı and Gürbüz, 2014; Helvacıoğlu et al., 2016).

Chromium occurs naturally in the Earth's crust and may penetrate to the water and soil a result of mainly anthropogenic applications. The main anthropogenic origin chromium in the groundwater and surfacewater are wastewater from textile manufacturing and electroplating operations (ATSDR, 2000). Besides the Thrace Region has a great agricultural potential, it has also a significant industrial capacity and there are many industrial enterprises around the region (Tokatlı and Baştatlı, 2016; Tokatlı and Ustaoğlu, 2020; Tokatlı et al., 2020).

According to the results of applied health risk assessment indices in terms of non – carcinogenic effects, calculated HI scores of Cr, As and Pb for tap and purified water samples were found as below the limit score of 1 both for adults and children. According to the results of applied health risk assessment indices in terms of carcinogenic effects, while the calculated CR scores of Pb for tap and purified water were found as below the limit score, CR scores of Cr were found at an alarming rate and CR scores of As were found as over the limit score of 1 both for adults and children for tap water. The WPS reduced the non – carcinogenic and carcinogenic risks of toxicants significantly and reduced the risky CR scores of Cr and As far below the limit value. It was also determined that HI – CR scores were decreased about 26 (%96), 8 (%88) and 10 (%90) times for Cr, As and Pb respectively after the purification process.

Conclusions

In this study, the performance of a widely used water purification system (WPS) in Turkey was evaluated in İpsala District, where is known as under effect of an intensive agricultural pressure, in terms of organic – inorganic water pollution indicators and ecological – health risk assessment indices. In conclusion, organic contents of tap water were found to be at critical levels, while the arsenic and chromium were found as the most dangerous toxicants for the drinking water of İpsala District in terms of human health. CR values of chromium in tap water of İpsala District was found to be at a very risky level and CR values of arsenic in tap water recorded as significantly higher than the limit coefficients of 0.0001. It was determined that the investigated WPS improved the drinking water quality significantly by decreasing the organic - inorganic pollutants and by increasing the pH because of increasing the mineral contents. It was also determined that WPS reduced the scores of applied ecological and health risk assessment indicators significantly and reduced the recorded coefficients of non – carcinogenic and carcinogenic effects of toxicants far below the limit values.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

Funding

The present study was funded by the Trakya University, Commission of Scientific Research Projects (Project No. 2019/279).

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

Authors are thankful to Trakya University, Commission of Scientific Research Projects for their financial supports.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.16 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):374-384 (2021)

Combined effects of acidification and high-pressure processing on microbial inactivation, bioactive compounds and antioxidant activity of liquorice root sherbet

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Abstract

High Hydrostatic Pressure Processing (HPP) has gained more attention in the fruit and vegetable industry in recent years. In this study, the optimal acidification parameters (citric acid alone and combination with ascorbic acid at the pH range 3.0 to 4.5) were determined and the effect of HPP conditions (pressures 250- 450 MPa and exposure times 1-5 min) on acidified liquorice root sherbet (ALRS) were investigated. Results showed that acidification of LRS by only citric acid had higher aroma and flavor scores. HP treatments were effective to reduce the yeast and mold (YM) count, total coliforms (TC), and inoculated pathogens (*Escherichia coli* ATCC 25922 and *Salmonella* Typhimurium ATCC 14028) in ALRS. Although acidification of LRS achieved a significant reduction in glycyrrhizic acid (GA) content, further treatment by HPP did not affect pH, the contents of total phenolic, total soluble solids, flavonoid, and GA or the antioxidant capacity of ALRS. Our results suggest that acidification and HPP treatments could be used to increase consumer acceptability and extend the shelf life of LRS.

Keywords: Liquorice root sherbet, Acidification, High pressure, Quality characteristics

Introduction

Liquorice root has been utilized in many traditional medical formulations to combat diseases in Asian and Mediterranean countries (Fung and Linn, 2017). Triterpenoid saponins and flavonoids are the main compounds in liquorice and major bioactive components are glycyrrhizin and its derivatives (Zhang and Ye, 2009). Glycyrrhizic acid (GA) in the liquorice contains one molecule of glycyrrthetinic acid and two molecules of glucuronic acid. GA is 50 times sweeter than sucrose and is generally consumed in herbal tea products and infusions (Obolentseva et al., 1999).

In the Middle East countries such as Turkey, liquorice roots are used to make a traditional drink known as ‘‘Liquorice Root Sherbet’’ which is served cold by street vendors (Aday et al., 2018; Maskan, 1999). The roots are shredded and extracted by water to prepare sherbet (Arino et al., 2007; Roden, 2008). A traditional method for producing sherbet in Turkey includes keeping the licorice roots in tap water (30-50°C) for 3 days to extract color, but the process is uncontrolled (Cınar, 2012). However, the shelf-life of sherbet is only a few days after production because of the low-acidity, high water activity and absence of pasteurization (Aday et al., 2018).

As heat treatment adversely affects the sensorial properties of LRS, there have been some attempts on utilizing non-

Cite this article as:
Aday, S., Uysal Pala, C., Ayana Cam, B., Bulut, S. (2021). Combined effects of acidification and high-pressure processing on microbial inactivation, bioactive compounds and antioxidant activity of liquorice root sherbet. J. Agric. Environ. Food Sci., 5(3), 374-384
Doi: https://doi.org/10.31015/jaefs.2021.3.16
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Received: 10 March 2021 **Accepted:** 12 July 2021 **Published Online:** 25 August 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 374-384
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

thermal techniques for cold pasteurization of LRS. Uzuner and Evrendilek (2017) applied pulsed electric field (PEF) and mild heat (40°C) on LRS and obtained a considerable reduction in microbial load and *E. coli* O157: H7 in liquorice sherbet. Additionally, PEF treatment maintained physical and sensorial properties better than those of heat treatment (90°C for 3 min). Çam et al. (2014) showed that the UV-C process did not change the physical and chemical properties of LRS. However, there was a limited decrease both total aerobic microbial count and total yeast and mold count in the same study. In our previous study, we have shown the effectiveness of HP treatment on the inactivation of microorganisms in LRS (Aday et al., 2018).

As is the case with many food preservation technologies, non-thermal technologies give the best results in extending the shelf life of foods, when they are combined with other hurdles. Based on this approach, HPP and acidification of LRS could be used to increase storage life while preserving its quality. Increasing the acidity of food can be thought of as one 'hurdle' to prolong stability and shelf life. Acidified foods are classified as foods with a pH value lower than 4.6 due to acidic food compounds or the addition of acid according to the U.S. Code of Federal Regulations (Theron and Lues, 2010). Vegetative forms of bacteria are less resistant to pressure and have low pH in foods and also acidity enhances of microorganisms during HPP (Smelt, 1998). Non-thermal techniques such as high hydrostatic pressure can be used in foods as another 'hurdle' to inactivate spoilage and pathogenic microorganisms and enzymes while preserving the colour and nutritional components of commodities (Chaikham et al., 2017). The effectiveness of the process is related to the type and count of microorganisms, duration, and level of pressure, temperature, acidity, and composition of the food. Acidity is one of the critical factors in pressure treatment to inactivate microorganisms in foods because, in acidic foods, pressure-damaged cells fail to repair themselves (Linton et al., 1999).

Our previous work has shown the effectiveness of HP treatment on liquorice sherbet and for the inactivation of microorganisms (Aday et al., 2018). In this study, the optimal acidification parameters for LRS were determined by sensory test and the impact of HPP on the inactivation of microorganisms in acidified LRS was investigated. The effects of combined acidification and HPP on nutritional and bioactive components of LRS were also determined.

Materials and Methods

Chemicals and reagents

Acetonitrile, acetic acid, ABTS, glycyrrhizic acid, ammonium salt, quercetin, trolox, and gallic acid were obtained from Sigma-Aldrich (Darmstadt, Germany). Plate count agar (PCA), Folin-Ciocalteu reagent (2 N), eosin methylene blue (EMB) agar, dichloran-rose bengal chloramphenicol agar (DRBC), xylose lysine deoxycholate (XLD) agar and violet red bile agar with mug (VRB-MUG) were supplied by Merck (Darmstadt, Germany).

Preparation of sherbet

Liquorice roots were obtained from the company located in Diyarbakır, TURKEY. Roots were gently washed with water to eliminate soil residues. Then, the length of the roots was reduced to approximately 20 cm and allowed to dry for one day at room temperature. Dried roots were ground using a mallet to obtain liquorice fibres (Aday et al., 2018). Liquorice roots were extracted according to the procedure of Cınar (2012) by using water in the ratio of 1:20 (w/w) for an hour at room temperature. The prepared solution was sealed with paraffin wax to prevent evaporation. A filter paper was used to remove impurities after the extraction process.

Optimization of the acidification process

Freshly prepared LRS was acidified by citric acid (2 M) alone or in combination with ascorbic acid (100 mg ascorbic acid/100 mL LRS) by adjusting the pH of LRS to 3.0, 3.5, 4.0 and 4.5. After the addition of acids, LRS passed through a filter paper for preventing unacceptable haze formation.

High pressure treatment

Sterile bags (4 × 10 cm) (InterscienceBagFilter®) were filled with 5ml sherbet samples and carefully sealed by a heat sealer without leaving any air bubbles inside the bags. The sealed bags were then packed for the second time in plastic pouches and sealed under vacuum using a vacuum packing machine (Model MV-20, Lipovak, Turkey). Model MSE-CIP-WB-5500 high-pressure equipment (MSE Technology, Turkey) was used for the treatment and further details of the system were previously presented by Bulut (2014) and Aday et al. (2018). The parameters used in pressure treatments were selected as 250 MPa, 355 MPa, 450 MPa for the pressure levels and exposure times of 1 and 5 min according to the earlier study that we carried out high-pressure parameters between 249 and 450 MPa and exposure times between 3 and 17 min by performing central composite design (Aday et al., 2018).

Microbiological analysis

The pour plate method was used to determine the total viable count computed from duplicate inoculated plates by using plate count agar (PCA) at 37 °C for 24-48 h. Inoculated DRBC plates were incubated for 5 days at 25 °C for the enumeration of yeasts and molds (AOAC, 2000). Violet Red Bile (VRB) plates with 4-methylumbelliferone glucuronide (*MUG*) were used to enumerate total coliforms and *Eschericihia* coli at 37 °C for 24-48 h (Halkman and Sağdaş, 2011). Salmonella spp. and Listeria spp. counts of freshly prepared ALRS analyzed according to ISO 6579-2:2012 and ISO 11290-2:2017, respectively. Inoculations of *E. coli* ATCC 25922 and *Salmonella* Typhimurium ATCC 14028 were performed by using the procedure of Bulut (2014). Eosin Methylene Blue (EMB) and Xylose Lysine Deoxycholate (XLD) agar were used to the enumeration of inoculated *E. coli* and *S.* Typhimurium according to the spread plate technique, respectively, and the plates were incubated for 24-48 h at 37 °C.

Total soluble solids and pH analyses

Total soluble solids content (TSS) and pH of the sherbet were determined using a refractometer (Atago PAL1, Japan) and pH meter (Ohaus Starter 3100, USA), respectively. Measurements were conducted at room temperature.

Colour

Determination of sherbet colour was carried out using a colourimeter (Minolta CR 400, Japan). CIELAB color space was used and readings were recorded as L^*, a^* and b^* values

(Colgecen and Aday, 2015).

Total phenolic content (TPC)

The total phenolic content was determined according to the procedure of Singleton and Rossi (1965). The absorbance of each solution (samples treated with Folin reagent) was recorded at 765 nm against reagent blank solution (Water+Folin+$NaCO_3$) and results were stated as mg gallic acid/L.

Total flavonoids content (TFC)

The procedure described by Tohma and Gulçin (2010) was used to measure total flavonoid content. The absorbance of sample solution was measured at 415 nm against ethanol (96 %) after waiting 40 min at room temperature. Results were expressed as milligrams of quercetin equivalents/L.

Trolox equivalent antioxidant capacity (TEAC)

Trolox equivalent antioxidant capacities of the samples were analyzed under Re et al. (1999). Phosphate buffer solution (pH 7.4) was used to dilute ABTS radical to 0.7 (±0.2) at 734 nm. Samples in different quantities (10, 20 and 30 µL) were added to this solution and absorbance readings were recorded after 6 min. TEAC values were expressed as µmol Trolox/mL.

Glycyrrhizic acid content (GA)

The glycyrrhizic acid content of the sherbets was determined using high-pressure liquid chromatography (Shimadzu, Japan) based on a method as described by Helmy et al. (2013). Phenomenex Gemini C18 (250 mm, 4.6 ID, 5µ) column in isocratic flow was used in the separation process. The mobile phase contains 40% acetonitrile, 60% ultrapure water and 1% acetic acid. The injection volume was 20 µL and the flow rate was selected as 1.0 ml/min. The column temperature was set at 30°C (Aday et al., 2018). Results were reported as mg glycyrrhizic acid/L.

Sensory analysis

The consumer acceptability test was performed by using a 7-point hedonic scale to choose the best acidification condition for LRS. Overall preference, appearance, aroma and flavor properties of the samples, served in 100 ml cups at room temperature, determined by participants (12 male and 3 female) who were untrained but regularly consumed this traditional beverage as part of their daily diet, using a mean liking score of 7 point scale. On the scale, 7 represent 'like extremely' and 1 represent 'dislike extremely' (Meilgaard et al., 2006).

Statistical analysis

Statistical significance of the acidification conditions and the factors (pressure levels and holding time) were determined using the ANOVA procedure. Tukey *post-hoc* test was performed to compare the means ($p<0.05$). Data analyses were carried out with SAS V8.2 software.

Results and Discussion

Selection of acidification conditions for LRS

Sensorial scores of LRS for the different acidification processes were shown in Table 1. Acidification of LRS by the combination of citric acid and ascorbic acid to different pH values did not cause any difference regarding the overall acceptability, appearance, aroma and flavor attributes. Similar trends were obtained in the LRS acidified by only citric acid, except aroma and flavor scores. Acidification with citric acid alone at pH 4.5 had significantly higher values for aroma and flavor. However, pH 4.5 is a critical point for choosing the pasteurization or sterilization operation for food products. Therefore, in order to study the effect of the HP treatments on the acidified LRS, acidification with citric acid alone at pH 4.2 was selected as the optimum treatment.

Table 1. Sensorial scores of liquorice root sherbet (LRS) for the different acidification processes (Values are expressed as the mean ± SD).

pH Values	Acidification of LRS by citric acid alone		
	Overall acceptability	Colour	Aroma and flavor
3.0	3.06^{a}±2.12	3.40^{a}±2.09	2.86^{b}±2.44
3.5	3.20^{a}±1.97	2.73^{a}±1.62	2.73^{b}±2.15
4.0	3.26^{a}±1.53	2.80^{a}±1.26	2.66^{b}±1.71
4.5	4.40^{a}±1.72	3.00^{a}±1.46	4.53^{a}±2.09
pH Values	Acidification of LRS by a combination of citric acid and ascorbic acid		
	Overall acceptability	Colour	Aroma and flavor
3.0	3.13^{a}±2.16	3.06^{a}±2.37	3.00^{a}±2.10
3.5	2.26^{a}±1.27	2.20^{a}±1.26	2.40^{a}±1.35
4.0	2.66^{a}±1.49	2.86^{a}±1.40	2.60^{a}±1.76
4.5	3.40^{a}±1.80	2.80^{a}±1.56	3.53^{a}±2.03

$^{a-c}$ Mean in the same column with different letters are significantly different ($p \leq 0.05$).

Physicochemical properties and microbiological quality of acidified LRS

The physicochemical characteristics and microbial quality of freshly made acidified LRS are reported in Table 2. With a pH of 4.21±0.09, ALRS can be classified as acidic food according to the U.S. Code of Federal Regulations (Theron and Lues, 2010). In contrast to our previous study (Aday et al., 2018) related to microbial quality, it might be explained that the acidification process resulted in higher rates of microbial inactivation. This finding corroborates the ideas of International

et al. (2009) reported that the low pH of the product causes denaturation of intracellular proteins of microorganisms. Food pathogens such as *Escherichia coli, Salmonella* spp. and *Listeria* spp. were not detected in our samples.

Table 2. Physicochemical properties and microbial loads of the acidified liquorice sherbet (Values are expressed as the mean ± SD)

Physicochemical properties and microbial loads	Acidified Liquorice Sherbet
pH	4.21±0.09
TSS (°Bx)	0.50±0.00
L*	72,30±4,42
a*	-0,76±1,66
b*	37,46±2,77
Glycyrrhyzic acid content (mg/L)	174.57±38.17
Total Phenolic Content (µg GA/mL)	308.28±42.71
Total Flavonoid (µg QE/mL)	6.56±4.29
Antioxidant Capacity (TEAC)(µmolTrolox /mL)	3,49±1,10
Total Aerobic Count (Log CFU/mL)	3,915±0.58
Yeast and Mold (Log CFU/mL)	2,038±0.47
Total Coliform (Log CFU/mL)	2,151±0.37
Escherichia coli	<10
Salmonella spp.	0/25 mL
Listeria spp.	0/25 mL

The acidification process caused an approximately seven-fold reduction in the glycyrrhizic acid content of ALRS (174.57 mg/L) compared to non-acidified LRS (1172.44 mg/L) that we previously published (Aday et al., 2018). Glycyrrhizic acid is the main compound in liquorice sherbet which gives an unpleasant, persistent aftertaste and characteristic aroma (Komes et al., 2016). Therefore, the acidification process can be a realistic option to increase the consumer acceptability of liquorice sherbets by lowering the glycyrrhizic acid content due to the breakage of bonds between glycone and aglycone in an acidic medium (Visht, 2014).

The other bioactive compounds of ALRS such as total phenolics (308.28 mg GA/L) and total flavonoids (6.56 mg QE/L) showed lower values than non-acidified LRS reported as 379.72 mg GA/L and 25.18 mg QE/L, respectively (Aday et al., 2018).

Effectiveness of high pressure on the bacterial flora of acidified LRS

Figure 1 shows the inactivation data of total aerobic microorganisms in ALRS treated by different high-pressure levels and holding times. High-pressure treatments at all levels reduced the TAC significantly by about 1.75 log CFU/mL ($p<0.05$). Obtained results are in agreement with the works of (Manas and Mackey, 2004; Tewari and Juneja, 2008) who reported that inactivation is influenced by pressure level, treatment time, and microbial types. The fact that the reduction in TAC in ALRS after a 1 m pressure treatment at 250 MPa (1.7 log CFU/mL) was slightly lower than the TAC obtained in ALRS treated at 450 MPa for 5 min (1.8 log CFU/mL), implies that the remaining microbial population (~2,5 log CFU/mL) were more likely to be aerobic spores (Aday et al., 2018). This statement could be supported by the findings of Genis et al. (2016), where they reported that the average aerobic mesophilic spore counts of LRS were 2,53 log CFU/mL and the growth of anaerobic mesophilic spores were not observed.

The initial population of yeast and mold (YM) was about 2.03 log CFU/mL. Changes in the YM counts after HPP treatments were shown in Figure 2. HPP treatments above the 355MPa were effective to achieve a complete elimination of the YM populations (min detection level 1 CFU/mL). Our results are in accordance with the results of Mukhopadhyay and Panja (2008) and Huang et al. (2013) who reported that pressures severe than 300 MPa, reduced YM counts below the limit of detection (1 log CFU/mL) in cantaloupe puree and strawberry puree, respectively.

With regard to the effects of the HPP treatments on total coliforms (TC), HPP treatments at all pressure-time combinations completely eliminated the TC in ALRS (Figure 3), which translate into a min of 2.15 log CFU/ mL reduction even after a 1 min treatment at 250 MPa. It is possible that HPP affected the morphology and disrupted the non-covalent bonds and cell membrane of the microorganisms (Casadei et al., 2002; Patterson, 2005; Tewari and Juneja, 2008). In addition, our results corroborate the results of Sreedevi et al. (2017) who showed that HPP levels above the 300 MPa resulted in a complete reduction of TC in sugarcane juice.

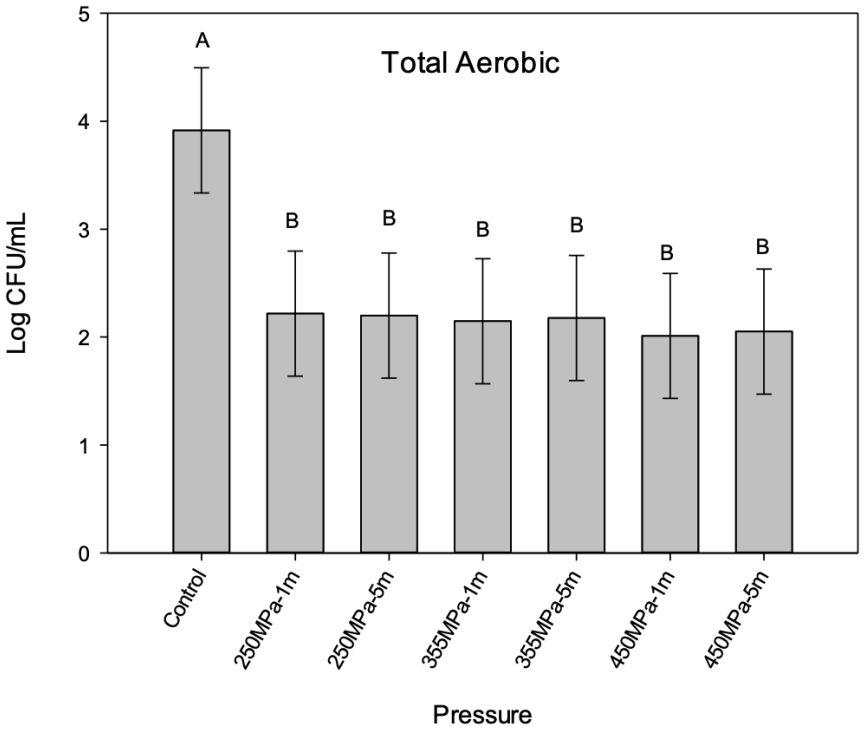

Figure 1. Changes in the counts of total aerobic microorganisms in acidified LRS after different high processing conditions. Means±SE denoted by different letters indicate significant differences.

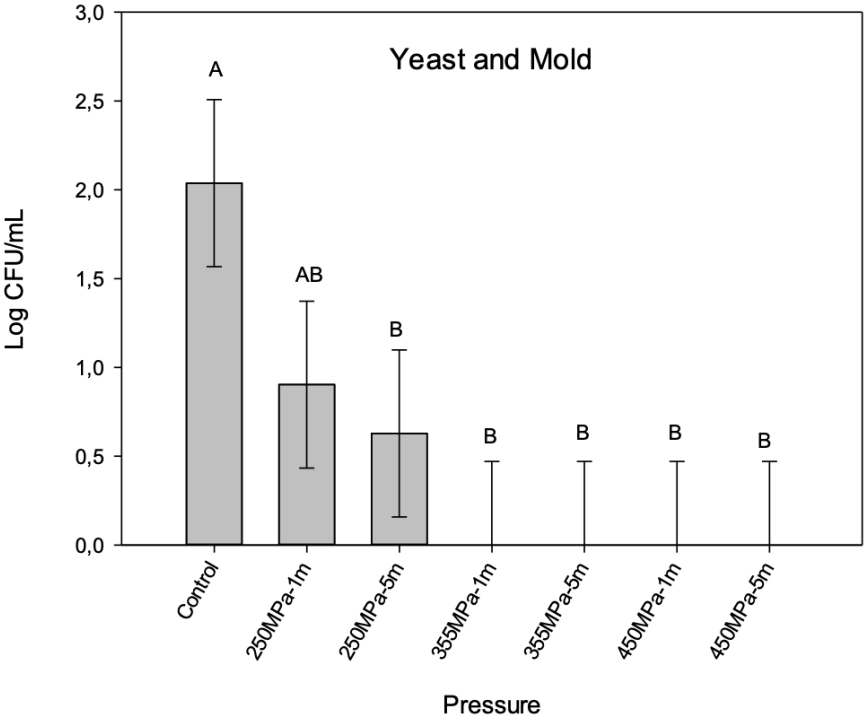

Figure 2. Changes in the counts of yeast and mold in acidified LRS after different high processing conditions. Means±SE denoted by different letters indicate significant differences.

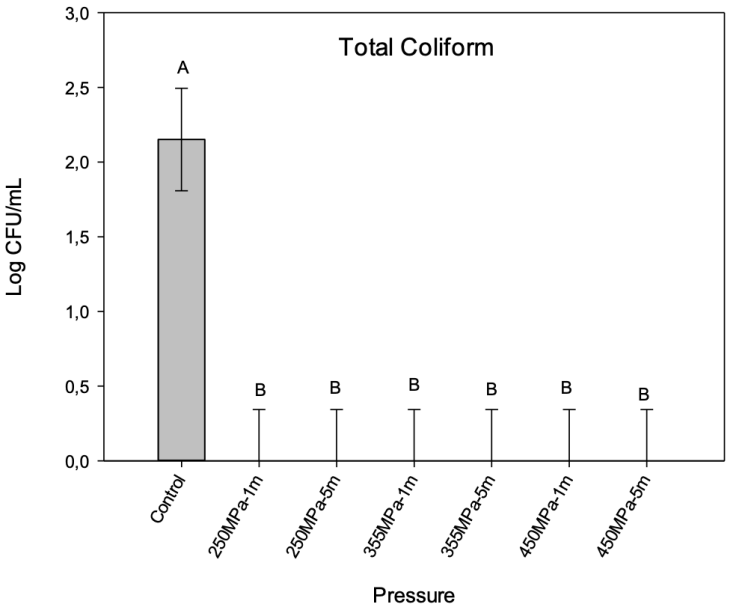

Figure 3. Changes in the counts of total coliform in acidified LRS after different high processing conditions. Means±SE denoted by different letters indicate significant differences.

Effectiveness of high-pressure treatment on pathogens

Survival counts of *E. coli* ATCC 25922 as a function of holding time and pressure are presented in Figure 4. Pressures above 355 MPa and 250 MPa-5min treatments had a more pronounced effect on the inactivation of *E.coli* whereas the effect of 250MPa-1min was limited. Pressures at 355 MPa and 450 MPa reduced the count of *E.coli* by 6 and 7 log CFU/mL cycles, respectively. Obtained results are in accordance with the report of (Bayındırlı et al., 2006) who showed that around 7 log reduction was observed in apple/apricot juices by using 350MPa-5m treatment. In our study, only 1 log reduction was observed after a 1 min treatment at 250 MPa, which is consistent with the results of Duong et al. (2015) who demonstrated about 1 log inactivation of E. coli NZRM 916 in feijoa puree after a 2 min treatment at 200 MPa.

Complete reduction of the *Salmonella* Typhimurium ATCC 1428, was achieved for the pressures severe than 355 MPa (Figure 5). These findings corroborate the results of Alpas and Bozoglu (2000) reported that HPP levels at 300 MPa for 5 min resulted in 8 log decrease in S. Typhi in orange juice. In our study, greater than 5 log reduction was noticed for 250MPa-5m treatment, while the reduction was about 3 log for the 250MPa-1m treatment.

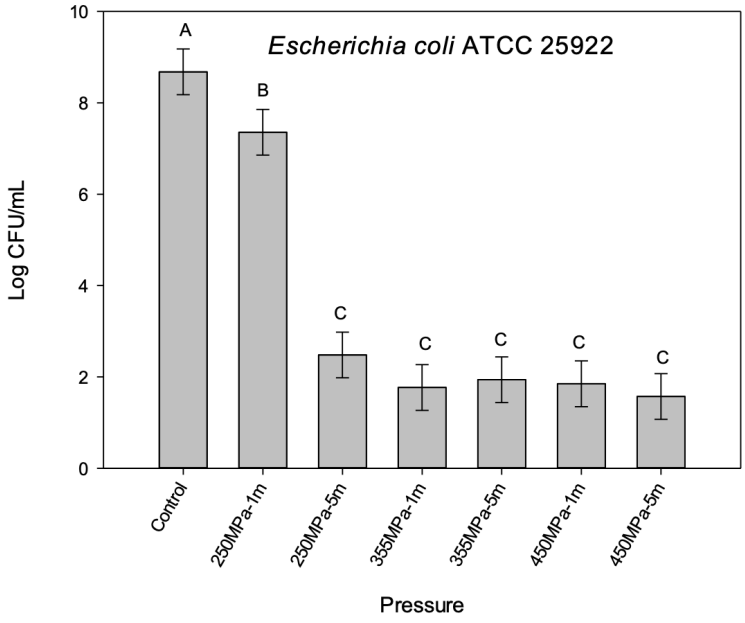

Figure 4. Changes in the counts of *E. coli* ATCC 25922in acidified LRS after different high processing conditions. Means±SE denoted by different letters indicate significant differences.

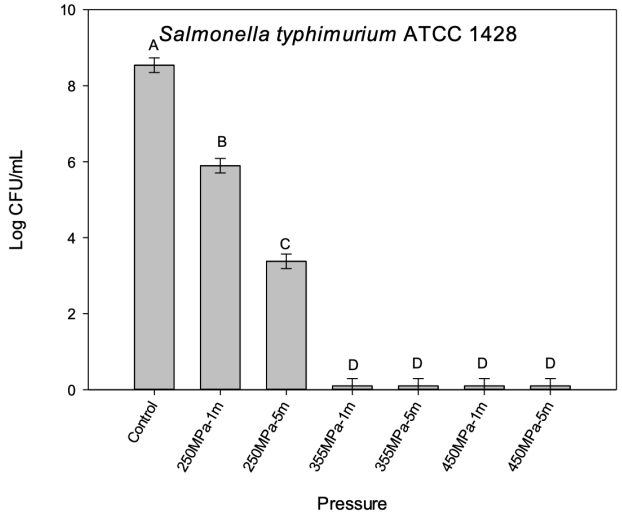

Figure 5. Changes in the counts of *S. typhimurium* ATCC 1428in acidified LRS after different high processing conditions. Means±SE denoted by different letters indicate significant differences.

Effectiveness of high-pressure treatments on physicochemical attributes

Bioactive constituents

More than 400 different chemical constituents have been identified such as triterpenoid saponins, chalcones, flavanone and their glycosides from Glycyrrhiza species (Ji et al., 2016). Roots contain significant amounts of flavonoid and phenolic contents. Major compounds of liquorice are glycyrrhizin, isoliquiritin and aglycones (Cheel et al., 2010). In our study, total phenolic and flavonoid contents of liquorice were independent of the power of the HPP ($p>0.05$) (Table 3). However, the control group had lower total flavonoid content compared to treated samples. The present findings are consistent with Plaza et al.'s (2011) findings which showed that HPP treatments (400 MPa-1m) increased the total flavanone content of the orange juices due to the higher extraction of phenolic compounds. In this work, the antioxidant capacity of the samples was not affected by different HPP treatments ($p>0.05$) (Table 3). Obtained results are in accordance with the report of Velázquez-Estrada et al. (2013) who showed that no significant difference was observed between fresh and HPP treated orange juices in the term of the anthioxidant capacity. In addition, Garcia et al. (2001a) showed that high pressure treatment did not change the antioxidant capacity of tomato puree compared to untreated samples. The study of Garcia et al. (2001b) also demonstrated that the anthioxidant potential of orange juices did not influence by high-pressure processing. However, our result differs from the studies of Del Pozo-Insfran et al. (2007) and Dede et al. (2007) who reported that antioxidant activity of muscadine grape juice and carrot juice were decreased after 400 MPa for 15m and 250MPa for 15 m HPP treatments, respectively. Inconsistency may be due to the different processing conditions or different chemical compositions. Another important finding was that the GA content of the HPP treated samples did not show any significant change compared to control samples (Table 3). This is also consistent with our earlier study (Aday et al., 2018) which showed that small molecules in liquorice such as saponins remain unaffected after HPP treatment.

Table 3. Effect of different HPP conditions on physicochemical properties of acidified LRS (Values are expressed as the mean ± SD).

Pressure (MPa)	Treatment Time (m)	pH	TSS	Total Phenolics(µg GA/mL)	Total Flavonoids (µg QE/mL)	Glycyrrhyzic acid (mg/L)	Antioxidant Capacity (TEAC)(µmolTrolox /mL)
0	1	4.21±0.09	0.50±0.00	308.28±42.71	6.56±4.29	174.57±38.17	3.49±1.10
0	5	4.21±0.09	0.50±0.00	308.28±42.71	6.56±4.29	174.57±38.17	3.49±1.10
250	1	4.21±0.09	0.53±0.06	312.83±39.28	7.13±3.76	221.80±68.18	3.47±0.88
250	5	4.23±0.11	0.53±0.06	309.39±41.88	7.28±3.29	216.21±57.49	3.45±0.97
355	1	4.24±0.13	0.50±0.00	313.17±57.78	7.36±3.51	214.55±73.92	3.44±1.15
355	5	4.23±0.12	0.53±0.06	312.28±50.97	7.00±3.06	216.00±85.56	3.62±1.31
450	1	4.29±0.06	0.55±0.07	320.67±35.12	7.21±4.18	270.12±89.32	3.55±1.88
450	5	4.22±0.11	0.53±0.06	308.28±29.35	7.74±3.00	220.91±84.18	3.38±1.31

pH and total soluble solids content

Acidity and total soluble solids (TSS) play a key role in food quality and consumer acceptability (Aday et al., 2013). From the data in Table 3, it can be seen that the pH and TSS content of the samples are not affected by HPP treatments ($p>0.05$). Our results are in agreement with the studies of Varela-Santos et al. (2012) and Barba et al. (2013) who reported that HPP treatments did not cause any significant changes in acidity and TSS values of pomegranate (350-550MPa for 30-150s) and blueberry (200-600MPa for 5-15m) juices, respectively.

Colour

Colour is an important parameter for determining food quality and affects consumers' purchase intention (Aday and Caner, 2014). Table 4 presents the L* (Lightness), a* (red-green) and b* (yellow-blue) parameters of the HPP treated and control liquorice samples. Statistical analyses revealed that interactions of two factors (pressure levels and treatment time) were significantly important regarding the L* values. Samples treated with 250 MPa for 1 m had significantly higher L* values as compared to the control group, but the differences among the HPP treated samples were not significant. Samples treated with 450MPa-5m pressure had higher L* values than control group. Obtained results are in accordance with the report of Calligaris et al. (2012) showed that an increase in L* values and a decrease in a* values of banana juices were observed as a consequence of high pressure treatment. Saldo et al. (2009) and Tribst et al. (2011) also reported that L* values of apple juice increased and * values of mango juice decreased after HPP treatments, respectively. In our study, exposure times were found significant when 450 MPa pressure was applied. However, that finding does not support the research of the Keenan et al. (2012) who reported that fruit smoothies pressurized with 450MPa-5m resulted in lower L* values than the smoothies pressurized with 450MPa-1m. This contradictory result might be due to the lower pH value (3.78) and composition (strawberry, apple, banana and orange) of the smoothie.

Statistical analysis of the redness (a*) values of the LRS showed that the interaction effect of factors was found statistically important. Samples treated with 450 MPa pressure had significantly lower a* values than control samples when the treatment time was applied as 5 minutes. A possible explanation for this might be that HPP treatments at higher pressure levels and treatment times may accelerate the degradation of anthocyanin and colour (Su et al., 2016). Flavonoid compounds are responsible for the yellow colour of sherbet. Pressure level and treatment time had a significant interaction effect on the yellowness of the LRS. However, this study showed that no differences were observed in the b* values of LRS when the holding time was chosen as 1 or 5 m. In addition, samples treated with 450MPa-5m pressure had lower b* values than control group. This is also consistent with the study of Patrigrani et al. (2019) who showed that HPP treatment caused a decrease in b* values of kiwifruit juice.

Table 4. Effects of the different high-pressure processing (HPP) conditions on colour values of acidified LRS. (Values are expressed as the mean ± SD).

	Pressure Levels (MPa) / L* values			
Treatment Time (m)	0	250	355	450
1	$72,30^{Aa}$+4,42	$78,99^{Ba}$+2,52	$77,65^{ABa}$+3,69	$72,39^{ABa}$+2,74
5	$72,30^{Aa}$+4,42	$77,76^{ABa}$+3,76	$77,96^{ABa}$+5,19	$82,04^{Bb}$+5,35
	Pressure Levels (MPa) / a* values			
Treatment Time (m)	0	250	355	450
1	$-0,76^{Aba}$+1,66	$-2,79^{Aa}$+0,99	$-2,57^{Aba}$+1,12	$-0,14^{Ba}$+1,05
5	$-0,76^{Aa}$+1,66	$-2,30^{ABa}$+1,39	$-1,99^{ABa}$+1,30	$-3,08^{Bb}$+1,16
	Pressure Levels (MPa) / b* values			
Treatment Time (m)	0	250	355	450
1	$37,46^{Aa}$+2,77	$32,26^{Aa}$+3,06	$31,70^{Aa}$+2,72	$38,93^{Aa}$+1,77
5	$37,46^{Aa}$+2,77	$33,97^{ABa}$+3,70	$28,37^{ABa}$+11,85	$27,98^{Ba}$+8,38

$^{A-B}$ Mean in the same row with different letters are significantly different ($p \leq 0.05$)
$^{a-b}$ Mean in the same column with different letters are significantly different ($p \leq 0.05$).

Conclusion

The results of this study showed that sherbets acidified by using citric acid only at pH 4.5 received higher liking scores based on aroma and flavor attributes. Pressures above 355MPa had a more pronounced effect on the inactivation of *E.coli* and *Salmonella* Typhimurium ATCC 1428 (> 6 log CFU/ml) in ALRS. Total phenolic, pH, total soluble solids (TSS), flavonoid contents, antioxidant capacity and GA contents of ALRS were not influenced by different HPP treatments. The acidification process caused an approximately seven-fold reduction in the glycyrrhizic acid content of ALRS compared to non acidified LRS. However, reduction in GA content of ALRS could be considered as an advantage, as the excess amounts of GA gives an unpleasant persistent aftertaste to LRS. Therefore, the acidification process and HPP treatments can be a realistic option to increase the consumer acceptability

and extend the shelf life of liquorice sherbets. Future research should concentrate on HPP treated non-acidified and acidified liquorice sherbet quality under different storage temperature conditions.

Compliance with Ethical Standards

Conflict of interest

The authors report no conflict of interest.

Author contribution

Serpil Aday: All analysis, Pressure treatments, Writing - review and editing.

Ciğdem Uysal Pala: Conceptualization, Supervision, Writing - review and editing.

Belgizar Ayana Cam: Sensory analysis.

Sami Bulut: Conceptualization, Pressure treatments, Review and editing.

Ethical approval

Not applicable

Funding

This research has benefited from a grant from Scientific and Technological Research Council of Turkey (TUBITAK) under grant #114O087.

Data availability

Not applicable

Consent for publication

Not applicable

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.17 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):385-392 (2021)

Closure of Göbekli Tepe: Erosion?

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Abstract

With the discovery of Göbekli Tepe, Urfa has proven to be one of the oldest settlement in history and has been named "Zero Point of History" due to this feature. What happened in the history left countless artifacts and historical mysterious in Urfa and turned it into an open-air museum. One of the most important current mystery is why the life of Göbekli Tepe disappeared. It is an important fact that civilizations disappear not only for reasons such as war, but also due to environmental problems and sometimes, on the contrary, they have survived for many years. Based on these facts; this study aimed to draw attention to the issue of "erosion", which continues to be a major environmental threat and problem for today's world, and this issue has been studied specifically for Göbekli Tepe. In the light of all this information, an answer was sought for the question of whether Göbekli Tepe was undergrounded by human or natural means.

Keywords: Göbekli Tepe, Environmental Problem, Erosion, History

Introduction

After living as nomads for many years, human beings settled down with the prevalence of favorable climatic conditions after the last glacial period. With the favorable climatic conditions, people started to settle in regions that are suitable and rich in terms of access to living resources with the development of civilization. Upper Mesopotamia, which was first named as the "Fertile Crescent" by the American archaeologist James Henry Breasted, was one of the most popular of these settlements. With its location at the intersection of Europe, Asia and Africa, it has been accepted as the witness of important historical events and the place where civilization began. It is thought that the first seeds of both agriculture and humanity were blooming in the region where the transition to settled order started and there were many non-pottery Neolithic centers (Atar,2017). Due to many more justifiable reasons, Mesopotamia has been an important center of attraction throughout history and has been a region where great civilizations were established and developed. Urfa, one of the ancient cities in the region, hosted most of these important civilizations. With Göbekli Tepe (Figure 1) discovered within the city's boundaries, it proved to be one of the oldest settlements where monumental architecture emerged and began to be known as the "Zero Point of History".

In addition to the great discovery Göbekli Tepe, which has a history of 12,000 years, Urfa, where numerous historical artifacts are located, is also described as an open-air museum. While the rich memory of the city's historical texture easily meets with humanity due to the generosity of its genes, the codes of other parts are waiting to be deciphered. An important part of the information that is aimed to be brought to light is the "environment" issues that are of vital concern to today's societies and their futures. Because it is an important fact that civilizations have survived for many years not only because of war, but also due to environmental problems or vice versa.

Cite this article as:
Yenigun, I., Oral, M., Karacizmeli, M., Uyanik, S. (2021). Closure of Göbekli Tepe: Erosion? J. Agric. Environ. Food Sci., 5(3), 385-392.
Doi: https://doi.org/10.31015/jaefs.2021.3.17
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Received: 27 March 2021 **Accepted:** 29 July 2021 **Published Online:** 24 August 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 385-392
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

Based on these facts; it is aimed to draw attention to the issue of "erosion", which continues to be a major environmental threat and problem for today's world, and this issue has been tried to be addressed in the context of Göbekli Tepe, which is accepted as a great discovery for the world. In the light of all this information, an answer was sought to the question of whether Göbekli Tepe was undergrounded by human or natural means.

Figure 1. Göbekli Tepe.

Environmental problems from past to present

According to some theories, the existence of environmental problems goes back to the first formation of our planet. As one of the data that makes this idea strong, it is shown that there is no oxygen in the process of the formation of the world, and even the highest rate of carbon dioxide. While carbon dioxide, known to be one of the leading pollutants, posed a great danger even with its presence, it initiated the first formations of the greenhouse effect, perhaps one of the biggest environmental problems of today's world. In the following formation processes of the earth, some of the carbon dioxide reacted with rocks and the other part became liquid by dissolving in the oceans and the cooling of the planet took place (Yenigün,2017). However, the possible dimensions and dangers of environmental problems with such an old past cannot be fully grasped even today.

Mankind and its environment have been in a continuous and dynamic interaction since the early ages. With the feature of being the only creature capable of thinking on the planet, human beings have moved away from this approach due to scientific developments since the seventeenth century, although they did not dominate nature before. Primarily, due to the dominant thoughts of Bacon "knowing is dominating" and Descartes' "mechanical world view", the understanding that human is positioned at the center and that the main measure is human has prevailed. This new approach has greatly changed the way human beings view both themselves and the environment they live in. This point of view, which sees man as the owner of the planet he lives in and evaluates every resource within him as his own, was the spark at the beginning of environmental problems. Environmental problems, which gained momentum especially with the industrial revolution, have reached today's dimensions that signal danger when they come together with other human-induced factors (Gül,2013).

Although countries have advanced their welfare and economic levels since the twentieth century, they have become limitless in their lives due to the endless demands of human beings. Combined with other factors such as population growth, natural resources have come to the limit of depletion, they are polluted, the balance is disrupted and the spaces have shrunk. Developments that mortgage our future have made new approaches obligatory. Therefore, orientations aiming at "sustainable" approaches, where the environment is not separated and the next generations can live healthily, have started to gain weight (Baykal et al.,2008)

Countries that have brought the level of social development to the highest levels but cannot show the same synchronization in human-environment relationship have consumed fossil fuel, which corresponds to an average of one million years each year compared to the pre-industrial revolution. This consumption has led to common problems that concern the whole world, such as the high rate of carbon dioxide released into the atmosphere and, accordingly, climate change and greenhouse effect. As can be seen in this and many other issues, the transformation of problems from a micro dimension to a macro dimension has taken the concern of the environment beyond being a temporary issue and has exposed humanity to the greatest danger of history. It has been seen that the only salvation of a person who is proud of his development will be through his education and awareness (Kayan,2018). In addition, the idea has been formed that addressing environmental problems in the filter of history

will make a great contribution to the targeted environmental awareness and education. Because the environmental problems experienced in the past will open new doors of curiosity and create a focus of attention in the perception of today's people. With the awareness provided, people will not be able to isolate themselves from the environmental problems experienced, on the contrary, they will see themselves as responsible. However, concrete steps to be taken with this perspective will prevent past mistakes and ensure that correct practices and approaches are taken as role models. Depending on the subject of this study; the issue of "erosion", which continues its current threat even today, and even increases its threat with effective factors such as global climate change and increasing deforestation, has been discussed.

One of the oldest environmental problems in the history of humanity; erosion

Erosion has been one of the oldest environmental problems in the history of the world and with its consequences. The discovery of fire, which is considered to be destructive in terms of nature, combined with its destructive aspect, has been a turning point for the fact of erosion. Plant tissue destroyed by burning formed the most important ground for erosion formation. In time, the desire of man to take everything under control has accelerated this situation. Although the results of erosion, which manifested itself especially due to forest and plant deficiencies, did not make itself felt until the industrial revolution, the situation has reversed in the last century and has deeply felt its effect (Karabıçak et al.,2004).

Plants and animals that lived in the aquatic environment until an estimated 350 million years ago have adapted themselves to living on land. In the later process of millions of years, the remains of the plant and animal species that died mixed into the land part of the planet, causing a thin layer of soil to form. Human beings, who have started to adopt the sedentary life understanding, have started to benefit from this soil in many aspects, especially the supply of food sources. Depending on the increasing demands, the amount of use and cultivation of the land also increased in parallel. However, this situation caused the soil to be exposed to many forces resulting in erosion. People have sometimes been able to control these forces that cause erosion, but most of the time they have not been able to prevent what happened and the destruction caused by erosion was only a spectator. Based on the historical findings, it has been found that many fertile regions have been destroyed as a result of erosion and, depending on, many civilizations and societies have come to an end (Çanga,2016).

Erosion due to major factors such as water and wind; It extensively and permanently affects most ecosystems around the world, such as forest areas, agricultural lands and pastures. According to the latest analysis, approximately 80% of the areas used for agricultural purposes around the world are exposed to erosion ranging from moderate to severe and over 75 billion tons of fertile soil is lost in each passing year. This loss corresponds to an average of 15 times compared to natural soil formation (Orgiazzi et al.,2016).

With erosion, which impairs soil health, the top layer of the soil, which is the richest in terms of organic matter and biology, disappears. This loss, which is extremely vital for plant life, renders the soil inefficient (Pimentel et al.,1998). The inefficient soil causes chain consequences leading to desertification and brings with it multiple economic damages (Blanco et al.,2008).

The loss of the soil, which takes hundreds of years to form, in very short periods due to negative factors, critically affects the vital balances in the world. Because the land, which is indispensable not only for the present but also for the future existence of humanity, is both a natural resource and a shelter for all other ecosystem creatures. However, especially in recent years, the increase of misapplications and mistakes has increased the rate of erosion and has paved the way for global problems such as climate change and migration (Tüfekçioğlu et al.,2016).

Considering the recent global epidemic of problems that threaten humanity, it has once again been revealed that the danger of erosion is closely related to all living beings, especially human beings. Therefore, it is vital that erosion, which constitutes one of the main sources of global risks, escapes from the agenda of humanity, and that it is always in the focus of attention and serious measures are taken. From this point of view, it is of great importance that academic circles, which are expected to make significant contributions, are involved, regardless of their area of expertise, and take a task. The research, which reveals different disciplinary approaches based on all these thoughts, aims to evaluate the situation on Göbekli Tepe, one of the great and popular discoveries of human history, and to present an irreversible problem such as erosion, which is closely related to all living things on the planet, as the focus of attention.

Possible landfill scenarios in Göbekli Tepe

The discovery of Göbekli Tepe required the reconsideration of what is known in the name of human history. While this evaluation enabled the answers of some questions to be obtained more clearly, it left the answers of some questions at the probability level. One of the questions remaining at the level of estimation in question, which is quite intriguing, was how Göbekli Tepe was undergrounded. Because, the answer to this question constitutes an important step for the interpretation of later periods. Based on this, research, analysis and interpretations have gathered Göbekli Tepe's landfill scenarios in two main views. These are discussed in detail below; it is expressed as opinions formed that it is filled with erosions or by human hands.

As can be seen in Figure 2, the cross (a) and lateral (b) sections of the soil fill layer clearly reveal that it consists of soil and stone materials with different grain diameters. When this situation is considered fictional, the process in Figure 2 and the sequential formation that will occur afterwards (Figure 3. - I, II, III and IV) suggests a similar model. Therefore, all these evaluations appear as sources that reveal and support the idea of erosion. Besides erosion, possible factors such as tectonic, volcanic movements and climatic changes bring to mind the lives of people who lived here resulting in migration. But most importantly, it emphasizes once again the unchanging destiny programming of erosion that deeply affects human life.

Figure 2. Front (a) and side (b) cross section of fillings.

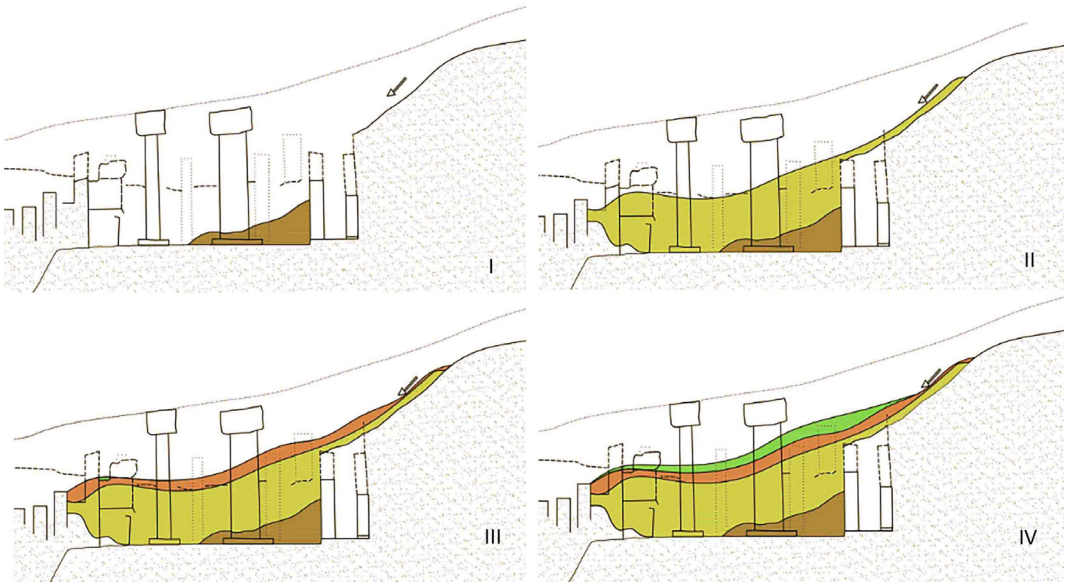

Figure 3. Göbekli Tepe›s filling with possible erosion.

While today's people can control factors such as climate and geography at a certain level, this was not possible in prehistoric times. In order to provide a partial solution to this impossibility, areas with lower altitudes than their surroundings were deemed more suitable and preferred in terms of living conditions. In addition to being convenient in terms of water resources compared to high regions, factors such as reducing the damage of people from harsh climatic conditions constituted the main reasons for preference (Erol,1971). Similar approach also revealed that the place of establishment of Göbekli Tepe was not randomly determined. Based on these considerations, it remains within the possibilities that Göbekli Tepe acted in accordance with the approach specified in the location selection. Its current location reveals that it is on the slope of the hill next to it, and provides limited support for this possibility (Figure 4).

Mentioning these preliminary reasons as well as some other supporting data strengthens the idea of erosion. The foremost of these ideas is water, which has never lost its vital importance for the continuity of vital activities throughout human history. The cisterns found during the excavations in the area and the transmission channels carved into the rock for water discharge are among the important findings regarding water (Herrmann et al.,2018). Therefore, all initiatives related to water, including the water collection holes in the rock shown in Figure 5, suggest that Göbekli Tepe was made for long-term

use. Another finding is the rock-cut pit structure, which is quite deep and large in shape, located in the northwest section (Clare et al.,2015). This structure, which is the largest ever found (Figure 6) and its recent discovery, the channels through which water is transmitted through these cisterns point to another aspect of strategic issues such as water supply and storing rainwater in the Neolithic period (Dietrich et al.,2014).

In addition, the carefully prepared special floor, whose existence is encountered in the outer surface area of the area, covered with small mosaic stone-like material known as terraso (Figure 7), suggests that the people who lived here actually used Göbekli Tepe not as a temporary place, but permanently.

Recent research has focused on the filling material in the building. The main reason for this is that it sheds light on the view that the building was filled in natural ways, contrary to the view that it was consciously filled by humans in the first years. Because the detailed research results make it necessary to reconsider the idea that it was filled by human hands. The analyzes made on the filling material show that the filling in the area occurs more than once, not once (Clare et al.,2019). Hence, according to the data obtained as a result of radiocarbon measurements and the determination that they came from the slopes can be seen as signs of small-scale erosions in different periods in the area (Clare,2020). Therefore, it is believed that the surrounding slopes into Göbekli Tepe are filled as a result of the flow (erosion) of various materials, especially soil, and is associated with natural events such as rain, wind or earth movements.

Figure 4. The picture showing the location of Göbekli Tepe on the slope.

Figure 5. Hollows made to collect water.

 DOI: 10.31015/jaefs.2021.3.17

Figure 6. Hollow structure carved into the rock (Clare et al.,2019).

Figure 7. Terraso floor.

The second important theory about Göbekli Tepe's landfill is that it was filled by human hands. Although the idea of erosion has gained weight according to the latest information obtained by experts, it also suggests that the area was filled with human hands before it was abandoned by those who used it. The first reason that suggests this view is the knowledge that such practices were applied in some neolithic settlements that can be considered contemporary, especially in the Çayönü settlement (Özdoğan and Özdoğan,1998).

Some archeology experts constitute an important part of the ideas that support that Göbekli Tepe was filled in later years due to possible reasons such as not being damaged or used by others. The first of these experts is Klaus Schmidt, who carried out the first and long-term studies in the region. In his view, the interior of the building was deliberately filled and closed in the Neolithic period. He explains this idea by the fact that the relief decorated T-shaped obelisks that characterize Göbekli Tepe were not damaged or intervened in the following processes

(Schmidt,2011).

According to another research conducted; although the geological structure of the region is composed of limestone and basalt, the first layer where the remains are composed of soft soil and the presence of stone tools in the same layer suggests that Göbekli Tepe was consciously covered with soil, not naturally (Kurt,2017).

Conclusions and Recommendations

Mankind has always looked at the past as well as its future with great curiosity and evaluated it as one of the important research topics. This search sometimes made him happy with great inventions and sometimes disappointed. However, in both cases, he used the data he obtained from the discoveries and tried to decipher his past memory. Göbekli Tepe, which is regarded as one of the most important discoveries of recent years, has caused great excitement on humanity even with the detection of the first findings of its existence. The historical structure has presented us with the information it has kept, as if fulfilling the necessity of meeting with such excitement, and opened its doors to new ones. However, the process of thousands of years has not made access to some information beyond just the level of theory. This situation did not pose a great obstacle for the enthusiast, and by trying different scientific methods and possibilities, it further stimulated the stubbornness of humanity, one of the leading virtues. Thus, the curiosity of the people living in the information age for Göbekli Tepe has increased even more, and new research topics have emerged. One of these research topics has been about Göbekli Tepe's filling scenarios.

The researches have collected the filling scenarios of Göbekli Tepe under two important theories. While the thought that it was filled with erosion has gained more weight in recent years, the findings and interpretations that it was filled with human hands have remained at a certain level, although not negligible. However, some experts who believe that the search for the future with the compass of the past will make great contributions will continue their research and perhaps reach different facts based on brand new findings. While seeking answers to these and many other questions, Göbekli Tepe, which has almost made a new beginning in human history with its discovery, continues to convey important messages to today's people and even its future as a requirement of its well-deserved reputation. One of these important messages is about the prevention of erosion, which will save us from great disasters in the future. Because researches on this subject confirm that all living things, especially human, living on the planet are on their way to great disasters. Our duty due to this situation will be to heed this cry from thousands of years ago and to take action for future generations even further.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

For their contributions to the study; endless thanks to Harran University Rector Prof. Dr. M. Sabri Çelik, to Harran University Göbekli Tepe working group, to Göbekli Tepe Science Board Member and Head of Karahantepe Excavation Prof. Dr. Necmi Karul and to MSc. Arch. Esra Tuğalan.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.18 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):393-397 (2021)

Contributions to the terrestrial Parasitengona Fauna (Acari: Trombidiformes: Prostigmata) of Diyarbakır and Mardin (Turkey)

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Abstract

Larval forms of some species belonging to the terrestrial Parasitengona (Acari: Trombidiformes: Prostigmata) group, which are common in the world, are the ectoparasites on some species causing economic damage. This study was carried out in 2020 to identify ectoparasitic larval mites and their hosts in Diyarbakır and Mardin provinces located the Southeastern Anatolia Region. As a result of the study, *Grandjeanella bella* Zhang, 1996 and *Leptus* sp. belonging to Erythraeidae family; *Trichotrombidium muscarum* (Riley, 1878) belonging to Microtrombidiidae family were determined. All two species and one genus determined in this study are the first records for Southeastern Anatolia Region acari fauna.

Keywords: Ectoparasit, Larval mite, New records, Southeastern Anatolia, Turkey

Introduction

Terrestrial Parasitengona (Acari: Trombidiformes: Prostigmata) is a widespread group in the world and has 247 genera and 19 families. Larvae of species belonging to the superfamilies Calyptostomatoidea, Erythraeoidea, and Trombidioidea in this group are the ectoparasites on some species causing crop damage (Saboori, 2016; Konikiewicz and Mąkol, 2018; Buğa and Sevsay, 2019).

Erythraeidae family (Acari: Trombidiformes: Prostigmata), belonging to the Erythraeoidea superfamily, has approximately 300 species. Erythraeidae family species have a significant role in terms of biological control due to being mostly ectoparasitic on arthropods (Gerson *et al.*, 2003). In Turkey, 23 species have been identified in this family so far (Sevsay, 2017).

Microtrombidiidae family (Acari: Trombidiformes: Prostigmata), belonging to the Trombidioidea superfamily, has about 450 identified species in the world. Larval forms of this family species are mostly specialized as ectoparasitic on arthropods similar to the Erythraeidae family (Mąkol *et al.*, 2017). In Turkey, 15 species belonging to Microtrombidiidae family have been identified by now (Sevsay, 2017).

Access to healthy food has become important due to the Covid-19 pandemic. In this period, the importance of alternative biological control methods to chemical control methods has increased even more. In addition to this development, it has become more important to know the impact of global climate change on biodiversity especially in terms of harmful and beneficial insects, mites and their relations. There have been no studies on ectoparasitic larval mites, key players in biological control, in Diyarbakır and Mardin provinces located Southeastern Anatolia Region. In this context, this study was conducted in Diyarbakır and Mardin provinces to identify ectoparasitic larval mites and their hosts.

Materials and Methods

This study was carried out in Diyarbakır and Mardin province, Turkey, in 2020. Çınar, Sur districts from Diyarbakır province and Artuklu district from Mardin province were selected for the study. Each district is divided into two sub-regions, provided that they are in different directions. Thus, at least six sampling regions were determined for each province.

Cite this article as:
Maral, H. (2021). Contributions to the terrestrial Parasitengona Fauna (Acari: Trombidiformes: Prostigmata) of Diyarbakır and Mardin (Turkey). J. Agric. Environ. Food Sci., 5(3), 393-397.
Doi: https://doi.org/10.31015/jaefs.2021.3.18
Orcid: Hasan Maral: 0000-0001-9201-8758
Received: 28 May 2021 **Accepted:** 12 July 2021 **Published Online:** 31 August 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 393-397
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

Samples were taken from the places that could best represent the working area.

In the surveys, almond, apricot, cherry, apple, pear, and walnut trees cultivated in agricultural areas as well as poplar, sycamore, willow, oak, pine, and wild fruit trees grown in non-agricultural areas, and herbaceous plants were inspected. Surveys were carried out in 15-day periods from the beginning of June to the end of September, as long as the weather conditions were appropriate. One branch from four different directions of the randomly selected trees was shaken gently, so the insects fell onto white cloth measuring 50 x 50 cm (width x length). Herbaceous plants, on the other hand, were shaken quickly several times, allowing insects to fall on the same cloth.

Insects falling on the cloth were carefully examined and mites were detached from host insects by a needle. The specimens were preserved in 75% ethanol and were sent to experts for identification. The identification of larval mites was made by Prof. Dr. İbrahim Çakmak (Department of Plant Protection, Faculty of Agriculture, Aydın Adnan Menderes University, Turkey). Measurements are given in micrometers *(µm)*.

Results and Discussion

As a result of the study, two species (one of them is at genus level) from the Erythraeidae family and one from the Microtrombidiidae family (Acari: Trombidiformes: Prostigmata), were determined.

***Grandjeanella bella* Zhang, 1996**

(Acari: Trombidiformes: Prostigmata: Erythraeidae)

Material examined: 1 larva (Figure 1), 16.08.2020, Çınar (Diyarbakır) (37°41'20"N-40°26'48"E, 667 m above sea level).

Distribution in Turkey: Balıkesir (Saboori and ***Çobanoğlu***, 2010).

General distribution: Iran (Zhang and Goldarazena, 1996).

Host insect; *Sericothrips* sp. (Thysanoptera: Thripidae) (Zhang and Goldarazena, 1996), *Parlatoria oleae* (Colvee, 1880) (Hemiptera: Diaspididae) (Saboori and Çobanoğlu, 2010), from an undetermined Diptera (this study).

Remarks; This species is rare in Turkey. It is also a new record for Diyarbakır.

In the literature, *G. bella* was found to be an ectoparasit on insect species that generally have sucking mouth structure (Zhang and Goldarazena, 1996; Saboori and Çobanoğlu, 2010). Goldarazena and Zhang (1997) stated that *G. bella* was successful in controlling pest species belonging to the order Hemiptera.

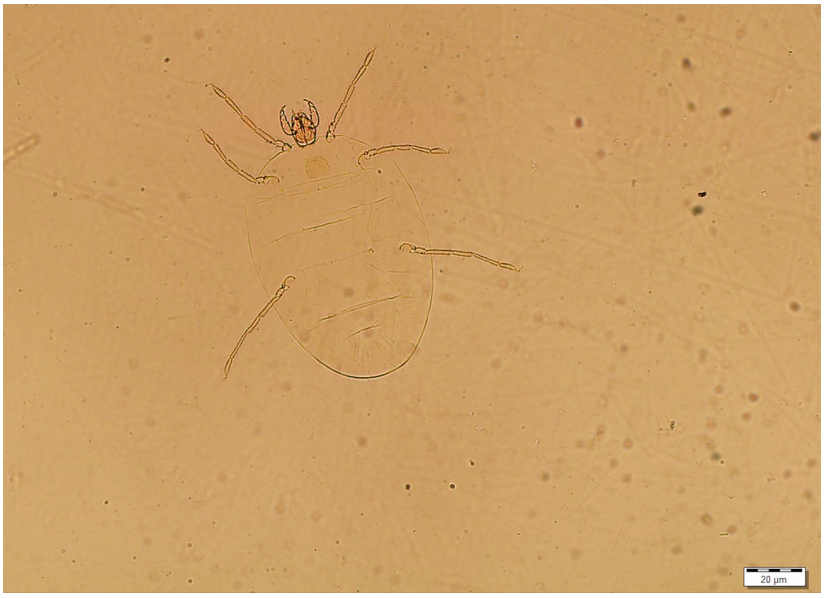

Figure 1. Larval form of *Grandjeanella bella*.

***Leptus* sp. (Acari: Trombidiformes: Prostigmata: Erythraeidae)**

Material examined: 2 larvae (Figure 2), 16.08.2020, Artuklu (Mardin) (37°26'48"N-40°38'00"E, 981 m above sea level).

Distribution in Turkey: İzmir (Haitlinger, 1999).

General distribution: Larval species of the genus *Leptus* Latreille are widely distributed throughout the World (Saboori *et al.*, 2019).

Host insect; All known species in their larval stage are associated with various arthropods, especially with insects (Baker and Selden 1997; Seeman and Palmer 2011; Saboori *et al.*, 2019), Coleoptera (Haitlinger, 1993; Mayoral and Barranco 2011). An undetermined Diptera (this study).

Remarks; This species is rare in Turkey. It is also a new record for Mardin.

In the previous studies, it was determined that the larvae of the species belonging to the genus *Leptus* are among the natural enemies of the species causing economic damage (Muñoz-Cárdenas *et al.*, 2015). In a study conducted by Tandon and Lal (1976), it was determined that *Leptus* sp., which is a host and harms mangoes in India, reduced the population density of mango mealy bug *Drosicha mangiferae* Green (Hemiptera: Margarodidae) by 15-20%. In another study, it

was also found that *Leptus* spp. negatively affected the flight behavior and fecundity of *Choristoneura fumifera* Clemens, 1865 (Lepidoptera: Tortricidae) (Houseweart *et al.*, 1980). In Turkey, few studies have been conducted about *Leptus* and were only determined one species (Haitlinger, 1999).

Figure 2. Larval form of *Leptus* sp.

***Trichotrombidium muscarum* (Riley, 1878)**
(Acari: Trombidiformes: Prostigmata: Microtrombidiidae)

Material examined: 23 larvae (Figure 3A to 3F), 31.07.2020, Sur (Diyarbakır) (38°00'20"N-40°26'16"E, 681 m above sea level).

Distribution in Turkey: Erzincan (Karakurt and Sevsay, 2013; Buğa and Sevsay, 2019).

General distribution: Australia, India, Madagascar, Iran, Hungary, Romania, Spain, USA (Hakimitabar and Saboori, 2018).

Host insect; *Musca domestica* (L.) (Diptera: Muscidae) (Karakurt and Sevsay, 2013; Buğa and Sevsay, 2019; this study), *Stomoxys calcitrans* (L.) (Diptera: Muscidae) (Kontschán and Hornok, 2019) unknown species belonging to Ulidiidae family (Diptera) (Hakimitabar and Saboori, 2018).

Remarks; This species is rare in Turkey. It is also a new record for Diyarbakır.

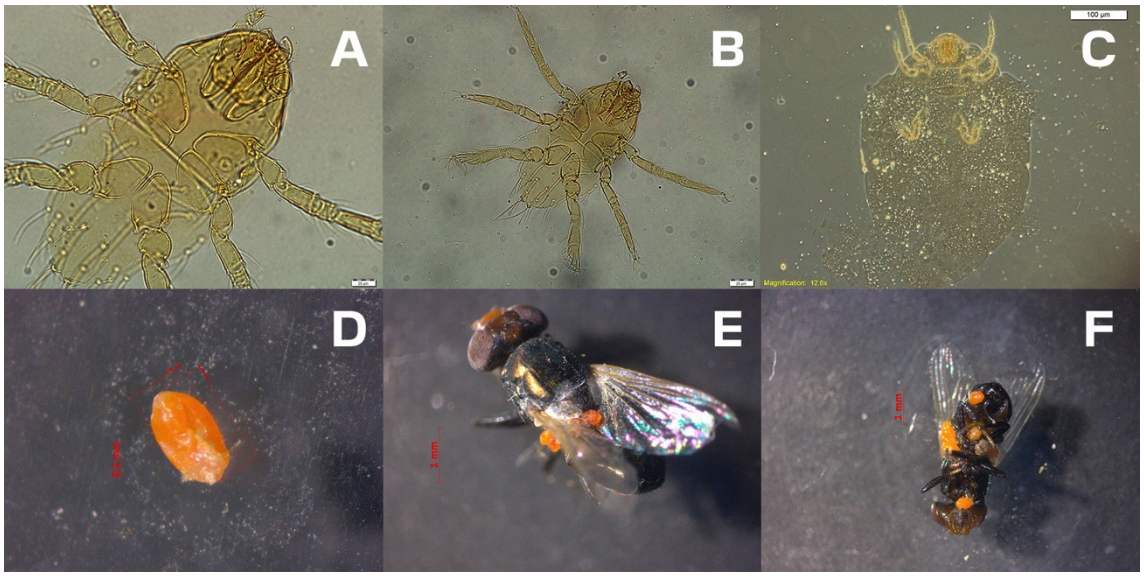

Figure 3. A, B, C, D) Larval form of *Trichotrombidium muscarum* E, F) *Trichotrombidium muscarum* on its host *Musca domestica*.

Main hosts of *T. muscarum*, one of the two species belonging to the genus *Trichotrombidium* 1951, are species belonging to the order Diptera. *T. muscarum*, to date, is only recorded from Erzincan in Turkey (Karakurt and Sevsay, 2013; Buğa and Sevsay, 2019). In the observations made in this study, it was seen that *T. muscarum* formed dense populations on its host *M. domestica*.

Conclusion

In the Southeastern Anatolia Region, where Diyarbakır and Mardin provinces are located, the Southeastern Anatolia Project (GAP) has been carried out to ensure efficient management of soil and water resources since 1989. Southeastern Anatolia Project (GAP) is one of the most important integrated irrigation and development projects in the world. The project is being carried out in stages and will be completed in 2023. Thanks to the stages that have been active in previous years within the scope of the project, significant increases have occurred in both the cultivation area and the production amount of many agricultural products. The findings obtained from this study are considered as an important opportunity in terms of suppressing pest species causing economic damage in GAP Region. For this reason, it is evaluated that the relationship between these species and especially economically damaging species should be examined well in future studies.

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 verify that the Text, Figures, and Tables are original.

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

We are thankful to Prof. Dr. İbrahim Çakmak (Department of Plant Protection, Faculty of Agriculture, Aydın Adnan Menderes University) who made the identification.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.19 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):398-404 (2021)

Statistical determination of in-vitro antimicrobial effects of extracts of marjoram (*Origanum majorana* L.) from Muğla, Turkey

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Abstract

To determine the chemical composition and antimicrobial potential of marjoram (*Origanum majorana* L.) leaves extratcs obtained from Muğla, Turkey. The extract of ethanol, methanol, hexane, chloroform, isopropanol and water of marjoram were tested for antimicrobial activity against eleven bacterial strains and one yeast by disc diffusion method. The minimum inhibitory concentrations (MICs) of isopropanolic extract of marjoram leaves were determined using broth serial dilution method. The constitituents were analyzed by gas chromatography-mass spectrometry (GC/MS). The Shapiro-Wilk of Normal Distribution Test, Kruskal-Wallis and Mann-Whitney Tests were applied for statistical analysis in the study. All of the tested extracts, isopropanolic extracts of marjoram leaves showed the best inhibition zones against *Klebsiella pneumoniae* CCM 2318 (24 mm) and *Candida albicans* ATCC 10239 (28 mm), methanolic extracts against *Staphylococcus aureus* ATCC 6538/P (26 mm). In our study, the results of the inhibition zones are varied 6 to 26 mm against tested bacteria. The results of Mann-Whitney Tests showed that isopropanolic extract is a significant different from chloroform, water and hexane extracts, statistically ($P_{isopropanolic\text{-}chloroform}$=0.003, $P_{isopropanolic\text{-}water}$=0.000, $P_{isopropanolic\text{-}hexane}$=0.000). Isopropanolic extracts of *O.marjorana* were among the most active with the MIC values ranging from 0.008-64 mg/mL. MICs of *O.marjorana* L. different extracts obtained by the broth serial dilution method. The GC/MS analyses allowed fifteen compounds to be determined; the main constituents of the of isopropanolic extract of marjoram leaves was carvacrol %86.33. Carvacrol, major constituent of the marjoram isopropanolic leaf extract in our study, had possible antimicrobial activity by testing commercial carvacrol. It has been observed that the extracts can be used in the food industry to inhibit the growth of pathogens or as natural preservatives in foods.

Keywords: *Origanum majorana* L.**,** Leaf extract, Antimicrobial activity, GC/MS analysis, Statistical analysis

Introduction

Studies in plant extracts have attracted in human beings. It can be used for both academic and industrial circles due to a growing interest in green consumerism, world-wide reduction in the composition of salt in food (health reasons), and the need of alternative techniques to assure quality and safety of perishable foods (Burt 2000; Holley and Patel 2005; Hammer et al.,1999; Dorman and Deans 2000; Lambert et al.,2001; Baydar et al.,2004).

Among the members of Lamiaceae family, the genus *Origanum majorana* L. is an aromatic, perennial, herbaceous plant. The plant has been used as a flavouring, herbal spice, perfumery, alsoused in both Ayurveda and folkloric system to cure various human diseases from time immemorial (Kirtikar and Basu., 1985; Farooqi and Sreeramu.,2004; Deans and Svoboda., 1990; Ezzeddine et al.,2001; Leeja and Thoppil.,

Cite this article as:
Ceyhan Guvensen, N., Keskin, D. (2021). Statistical determination of in-vitro antimicrobial effects of extracts of marjoram (*Origanum majorana* L.) from Muğla, Turkey. J. Agric. Environ. Food Sci., 5(3), 398-404.
Doi: https://doi.org/10.31015/jaefs.2021.3.19
Orcid: Nur Ceyhan Guvensen: 0000-0001-8753-2664 and Dilek Keskin: 0000-0003-2479-0070
Received: 29 March 2021 **Accepted:** 09 July 2021 **Published Online:** 11 September 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 398-404
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

2007; Busatta et al.,2008; Mohamed et al.,2011).

The use of natural preservatives is currently encouraged due to increasing bacterial resistance to chemical preservatives, food safety regulations, and increased awareness of the adverse effects of chemicals.Although various measures have been implemented in the past to control and reduce the prevalence of pathogenic microorganisms, reports of foodborne disease outbreaks as a result of their infection subsists (Pernin et al.,2019).

The objectives of present study were therefore to investigate the antimicrobial activities of six different solvent extracts from West Anatolian marjoramand to determine the chemical compound content to find out the relationship between antimicrobial activity and the compound content. Therefore, we have tested antimicrobial activity against bacteria and fungi, including opportunistic-pathogenic species: Gram negative bacteria; *Klebsiella pneumoniae* CCM 2318, *Salmonella typhimurium* CCM 583, *Aeromonas hydrophila* ATCC 19570, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218.Grampositive bacteria; *Bacillus cereus* CCM 99, *Staphylococcus aureus* ATCC 6538/P, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* ATCC 8043, *Bacillus subtilis* ATCC 6633. fungus; *Candida albicans* ATCC 10239.

The antimicrobial activity of marjoram extracts was measured by using disc diffusion method and minimal inhibitory concentration (MIC) and the data obtained were evaluated statistically in this study.

Materials and Methods

Plant material

Marjoram leaves samples were obtained from market place in Mugla, Turkey in 2013. The specimens were identified and authenticated by botanist Prof. Dr. Aykut Guvensen from the Department of Biology, Ege University, Turkey. The specimens were air-dried. The leaves were taken and used inthis study to determine its antimicrobial activity against the test microorganisms.

Preparation of plant extracts

Dried leaves of marjoram were mechanically graded, and 2g of plant was extracted with 20 ml of ethanol, methanol, hexane, chloroform, isopropanolor water then was gently heated after rinsed for 24 h atroom temperature (Ates and Erdogrul., 2003; Ozdemir et al., 2004).

Microorganisms and growth conditions

Test microorganisms were as follows: *Salmonella typhimurium* CCM 583, *Aeromonas hydrophila* ATCC 19570; *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* CCM 2318, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* ATCC 8043, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* CCM 99, *Staphylococcus aureus* ATCC 6538/P, and *Candida albicans* ATCC 10239.

The bacterial strains were inoculated on nutrient broth (Merck) and incubated for 24 h at 37ºC, *C. albicans* was inoculated on potato dextrose broth (Merck) and incubated for 48 h at 25 ºC.

Antimicrobial assays

Disc diffusion method

The disc diffusion method was used to screen the antimicrobial activities. The test were done according to Phadke and Kulkarni., 1989). Penicillin (10 µg/disc) (Oxoid), ampicillin (10 µg/disc) (Oxoid), erythromycin (10 µg/disc) (Oxoid), chloramphenicol (30 µg/disc) (Oxoid) and nystatin (30 µg/disc) (Oxoid) were used as positive controls. Ethanol, methanol, hexane, chloroform and isopropanol were also used as negative controls. The three sterile discs of 6 mm diameter were place ontoeach agar plates containing microorganisms with sterile forceps. Then 30 µl of extracts were absorbed onto discs under sterile conditions. Agar plates containing strains were incubated at 35 ºC at 24 h. All experiments were done inthree times (Phadke and Kulkarni., 1989).

Carvacrol, major constituent of the marjoram isopropanolic leaf extract in our study, was tested to detect the possible antimicrobial activity. For this purpose, 86.33% solution of commercial carvacrol (98%, Sigma-Aldrich) was prepared by dissolving in dimethyl sulfoxide (DMSO) (99.9%, Sigma-Aldrich) and used. DMSO was used as negative control to determine the sensitivity of the tested strains.

Minimum inhibitory concentrations (MICs) tests

MIC tests were carried out according to Kim et al., (2005), using a microplate (96 wells). The stock solutions of the extracts were diluted and transferred into the first well, and serial dilutions were performed so that concentrations in the range of 0.008-128 mg/mL were obtained. Erythromycin, chloramphenicol and nistatin (Oxoid) was used as the reference antibiotic control and pure isopropanol was also tested.

Chromatographic analysis

The steam-distilled components were analysed by Gas Chromatography-Mass Spectrometry(GC/MS) according to Adams,1995.

Statistical analysis

Marjoram leaves, solvents and standard antibiotics data were taken as variables. The Shapiro-Wilk of Normal Distribution Test is applied to variable groups. The variables not having normal distribution were tested by Kruskal-Wallis, non-parametric statistical test. Mann-Whitney Tests were performed for multiple comporisons. In this way difference between zone diameters is tested.

Results and Discussion

In our study, the results of the inhibition zones are varied 6 to 28 mm against tested bacteria (Table 1) with all extracts. Among the tested marjoram extracts, isopropanolic extract showed the best inhibition zones against all bacteria and *C. albicans* (15-28mm) in Table 1. The results of Mann-Whitney Tests showed that isopropanolic extract is a significant different from chloroform, water and hexane extracts, statistically ($P_{isopropanolic\text{-}chloroform}$=0.003, $P_{isopropanolic\text{-}water}$=0.000, $P_{isopropanolic\text{-}hexane}$=0.000). Isopropanolic extract had the highest antibacterial activity on *K. pneumoniae* (24mm) (Table 1). Besides, the inhibition zone diameters of the tested extracts against the test microorganisms were shown Table 2. In this table, all of the tested solvents showed no inhibition zones

against *S.typhimurium* and *S.epidermidis* in Table 2.

Mohamed *et al*., 2011 reported that *Origanum marjorana* L. at the concentration of 170 µl showed a strong growth inhibition effect (11, 10, 10, 9, 10 and 9 mm) against *E. coli, S. enteritidis, S. aureus, B. cereus, Aspergillus* spp. and *Penicillum* spp. respectively (Mohamed et al., 2011). However, water extract at low concentration did not show strong antimicrobial activities against all of investigated microorganism, especially *S.aureus, E. coli, Aspergillus* spp. and *Penicillum spp*. This observation is completely concurrent with the observation confirmed by Adwan et al., 2009. Leeja and Thoppil., (2007) reported that in-vitro microbicidal activity of the methanol extract of *Origanum majorana* L. was tested against seven fungi and six bacteria (Leeja and Thoppil., 2007). The methanol extract of *O. majorana* can be used as an effective herbal protectant against different pathogenic bacteria and fungi. Ramos et al., (2011) reported that it was active against *S.aureus, Enterococcus faecalis, E. coli* and *Klebsiella pneumoniae.* Previous study conducted by Ben *et al*., 2001 suggests that the essential oil of *O. majorana* posses antibacterial activity like Farooqi and Sreeramu., (2004).

Tablo 1. Antimicrobial activity of marjoram (*Origanum majorana* L.) extracts against test microorganisms by disc diffusion method.

Microorganisms	Extracts of marjoram								
	Methanol (mm)			Ethanol (mm)			Hexane (mm)		
	Methanol	Water	% 10 DMSO	Ethanol	Water	% 10 DMSO	Hexane	Water	% 10 DMSO
S. faecalis	16	13	13	20	12	17	12	9	17
S. typhimurium	17	11	18	15	9	11	13	8	13
E. coli	22	11	20	20	20	14	8	8	10
P. aeroginosa	18	9	16	15	15	8	8	9	15
A. hydrophila	13	7	14	14	8	7	8	7	14
S. epidermidis	21	9	16	16	8	6	8	11	13
S.aureus	26	23	21	22	18	15	10	7	16
K. pneumoniae	16	10	14	24	11	15	9	10	10
B. cereus	17	7	20	19	12	11	8	8	18
B.subtilis	20	11	21	23	11	20	12	11	15
C. albicans	25	13	25	24	15	14	19	11	21

-: no inhibition

Tablo 1. Antimicrobial activity of marjoram (*Origanum majorana* L.) extracts against test microorganisms by disc diffusion method (continuation).

Microorganisms	Extracts of marjoram							
	Chloroform (mm)			Isopropanol (mm)			Water (mm)	
	Chloroform	Water	% 10 DMSO	Isopropanol	Water	% 10 DMSO	Water	% 10 DMSO
S. faecalis	20	19	8	21	-	13	15	18
S. typhimurium	14	6	9	21	8	10	13	11
E. coli	14	20	8	23	9	12	10	20
P. aeroginosa	7	9	9	19	9	9	12	9
A. hydrophila	9	9	8	16	8	10	8	12
S. epidermidis	6	10	9	15	15	11	8	13
S.aureus	-	14	8	21	11	11	13	19
K. pneumoniae	16	12	19	24	14	13	8	9
B. cereus	12	7	8	22	10	8	13	9
B.subtilis	21	19	14	23	9	17	20	19
C. albicans	22	12	24	28	12	24	9	20

-: no inhibition

Table 2. Antimicrobial activity of solvents against tested bacteria by disc diffusion method.

Microorganisms	Solvents					
	Methanol(mm)	Ethanol(mm)	Hexane(mm)	Chloroform(mm)	Isopropanol(mm)	DMSO(mm)
S. faecalis	14	13	-	9	11	-
S. typhrium	-	-	-	-	-	-
E. coli	-	-	-	11	15	-
P. aeroginosa	10	10	-	-	13	9
A. hydrophyla	7	8	-	7	10	9
S. epidermidis	-	-	-	-	-	-
S.aureus	17	14	-	-	21	16
K. pneumoniae	9	10	-	8	23	10
B. cereus	-	9	8	-	12	8
B.subtilis	17	17	11	-	12	11
C. albicans	18	11	-	-	12	10

In this study, 5 standard antibiotics were used as positive control. These include, ampicillin (10µg/disc), chloramphenicol (30µg), nystatin (30µg/disc), erythromycin (15µg/disc) and penicillin G (10µg/disc). Ampicillin, penicillin and chloramphenicol very strongly inhibited the growth of *B.subtilis* whereas, erythromycin exhibited a very big zone of inhibition against *S. typhimurium* and *K.pneumoniae. E. coli*. Nystatin weakly inhibited the growth of *C. albicans* in Table 3.

The lowest MIC (2 mg/mL) was recorded for the isopropanolic extract with *E. coli* ATCC 35218, *K. pneumoniae* CCM 2318, *B.subtilis* ATCC 6633 (Table 4). Isopropanolic extracts of *O.marjorana* were among the most active with the MIC values ranging from 0.008-64 mg/mL.

The GC/MS analyses allowed fifteen compounds to be determined; the main constituents of the of marjoram leaves extract was carvacrol %86.33 in Table 5. Previous studies showed that carvacrol is well known antimicrobial compounds isolated from different plant species (Lambert et al.,2001; Ben et al.,2006; Bnyan et al.,2014).

Ramos et al., (2011) reported that the major constituents of the oil were: cissabinene hydrate (30.2%), terpinen-4-ol (28.8%). French and Italian studies reported similar results Novak et al., (2004), but the oil from Turkey (Baser et al., 2014) was reported to have a completely different composition, because *O.majorana* from Turkey contained 78% carvacrol like our study. Many researchers reported that chemical composition of essential oils of *O.majorana* from Cuba (Pino et al., 1997). Brazil (Busatta et al., 2008), Hungary (Vági et al., 2005) and Tunisia (Ben et al., 2001) and Egyptian (Selim et al., 2013). Helal et al., (2006) reported that *O.majorana* oil contain mainly 51.5% 3-cyclohexen-1-01,4-methyl-1(1-methylethyl)-(CAS). It would also be worthy to be cited here that the composition of any plant essential oil is influenced by the presence of several factors such as; local, climate, plant species, methodology and experimental conditions (Mishra et al., 1994; Prudent et al., 1995; Daferea et al., 2000). These factors may alter the biological and antimicrobial activities of the oils produced (Shu and Lawrence., 1997; Vardar-Ünlü et al., 2003).Distillation time and temperature can also significantly affect the oil constituents (Janssen et al., 1987).

Table 3. Inhibition zone diameters (mm) of the standard antibiotics against test microorganisms.

Microorganisms	Standard antibiotics				
	Ampicillin	Penicillin G	Erythromycin	Chloramphenicol	Nystatin
S. faecalis	28	30	22	25	NT
S. typhimurium	25	32	27	23	NT
E. coli	29	32	24	33	NT
P. aeruginosa	28	33	22	28	NT
A.hydropyhila	27	31	25	26	NT
S. epidermidis	23	30	23	25	NT
S. aureus	29	27	24	20	NT
K. pnemuoniae	28	29	26	25	NT
B. cereus	26	30	25	24	NT
B. subtilis	32	35	16	30	NT
C. albicans	NT	NT	NT	NT	12

NT: not tested

Table 4. MICs of isopropanolic extract of marjoram leaves, isopropanol and standard antibiotics against test microorganisms.

Microorganisms	MIC(mg/mL)				
	Isopropanolic extract	Isopropanol	Antibiotics		
			Eritromycin	Chloramphenicol	Nystatin
S. faecalis	8	128	4	2	NT
S. typhimurium	8	128	0,016	4	NT
E. coli	2	64	2	0,008	NT
P. aeruginosa	16	128	4	0,016	NT
A.hydrophila	32	128	2	0,16	NT
S. epidermidis	64	128	4	0,16	NT
S.aureus	8	64	2	8	NT
K. pneumoniae	2	128	2	2	NT
B. cereus	8	128	2	4	NT
B.subtilis	2	64	32	0,008	NT
C. albicans	0,16	32	NT	NT	16

Table 5. Volatile components of the isopropanolic extract of marjoram leaves (GC-MS analysis).

Component[a]	Area(%)	Rt[b]
n-Decane	%0.38	5.13
Methane, oxy bis (Dichloro)	%0.24	5.31
1-Propanol (CAS)	%0.23	5.46
Toluene	%0.30	5.67
2- Pentanol ,2 methyl	%0.31	6.34
Trans sabinenen hydrate	%0.37	15.87
Dimethyl sulfoxide	%0.42	19.04
Trans Caryohyllene	%1.13	20.19
Endo-Borneol	%1.09	23.08
Beta- bisabolene	%0.45	23.99
Caryophyllene oxide	%0.34	31.37
S-2-methlene- 1-cyclohexanol	%1.22	35.75
Thymol	%0.94	36.03
Carvacrol	%86.33	36.76
18-Crown 6-ether	%0.43	40.80
Undefined	%5.83	

[a] Components listed in order of elution from a HP-1capillary column

[b] Retention time (as min).

Table 6**.** Antimicrobial activity of carvacrol against tested microorganisms by disc diffusion method.

Microorganisms	*S.f*	*S.t*	*E.c*	*P.a*	*A.h*	*S.e*	*S.a*	*K.p*	*B.c*	*B.s*	*C.a*
Inhibition zones (mm)	16	15	16	16	12	15	21	20	14	15	22

S.f: Streptococcus faecalis ATCC 8043, *S.t*: *Salmonella typhimurium* CCM 583,*E.c*:*Escherichia coli* ATCC 35218, *P.a*:*Pseudomonas aeruginosa* ATCC 27853, *A.h*:*Aeromonas hydrophila* ATCC 19570, *S.e*:*Staphylococcus epidermidis* ATCC 12228, *S.a*:*Staphylococcus aureus* ATCC 6538/P, *K.p:Klebsiella pneumoniae* CCM 2318, *B.c*:*Bacillus cereus* CCM 99, *B.s: Bacillus subtilis* ATCC 6633, *C.a*:*Candida albicans*

The results of inhibition zones of carvacrol showed 12 to 22 mm against tested microorganisms like tested extracts in Table 6. It can be suggested that antimicrobial activity of marjoram leaves based on carvacrol.

Conclusion

Based on these results, it is possible to conclude that six different extracts of marjoram from Muğla, Turkey had different level antibacterial and anticandidal activity. Carvacrol which is the main compound of isopropanolic extract of marjoram exhibited high levels of antimicrobial activity against all of the tested strains. Our results support that carvacrol have antimicrobial activity against the most important opportunistic pathogenic bacteria which leads to infection of human and food contaminants. It is clear that there is a significant correlation between the chemical compositions and antimicrobial activity of marjoram extracts. The marjoram extracts studied have the ability to inhibit growth of pathogens for the food industryor as flavoring agent and natural preservative and the use of this plant in traditional medicine for the search for new drugs. These data demonstrate the effectiveness of marjoram extractstested, at the projected concentrations, against the opportunistic-pathogenic microorganisms investigated and, as such, may allow the formulation of new antimicrobial products for potential use as food additives.

As for the statistic test results, it was tested whether there is a statistically significant difference between groups by applying Kruskal-Wallis Test. According to the test results, there is a statistically significant difference between groups. To see differences between variables, pairwise comparisons were performed by applying Mann-Whitney Test. As a result, it can be said that isopropanolic marjoram leaf extract and carvacrol can be used instead of the standard antibiotics such as erythromycin. In our findings, there is no statistically a significant difference between isopropanolic extract and standard antibiotic erythromycin (P=0.176).

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

Funding

This project financially supported by Adnan Menderes University Research Fund (BAP, Project Number: BAP-CMYO 13-001).

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

Authors are thankful to Prof. Dr. Aykut Guvensen for the taxonomic identification of plant material and thanks to Serdar Demir for his valuable contribution to statistical analysis.

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International Journal of
Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2021.3.20

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 5 (3):405-412 (2021)

Determination of the optimum operating parameters of an axial fan used on the conventional air blast orchard sprayer

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Abstract

The pesticide droplets are carried to the tree canopy with the assist of the air flow which is generated by an axial fan of orchard sprayer. The designers manufacture the fan of orchard sprayer with ability of 6 different working conditions under constant PTO speed. Previous studies were related to evaluating the effectiveness of sprayers. In this study, 6 different options that presented to farmers was evaluated with view of energy consumption and air flow. A Conventional orchard sprayer having an axial fan with 36" diameter was operated at two fan revolutions (1890 and 2430 rpm) and at three blade angles (15º, 30º, 45º), and measured air capacity, the fuel consumption, variations of air velocities in air jet profile of the fan and calculated power consumption for each operating parameters potentially to be chosen by farmers. As a result, for each blade angle, if the fan speed increase, the air jet of the fan become more uniform than before. The maximum fan efficiency and system efficiency were %73,25 and %59,98 respectively which were obtained 30º blade angle and 2430 rpm fan rotation speed condition. The minimum fuel consumption and torque need was obtained under conditions of 15º blade angle and 1890 rpm fan rotation speed. However, considering maximum air capacity and the best air velocity uniformity in the profile of fan jet was established at, 45º blade angle and 2430 rpm fan rotation speed operating parameters.

Keywords: Air assisted orchard sprayers, Axial fan efficiency, Air jet velocity profile

Introduction

In recent decades there is a clear tendency to reduce the use of pesticides in agriculture (Ozluoymak et. al., 2019). For new designed field and orchard sprayers, precisely consumption of pesticide is essential for both. With this view, controlling the air volume and quantity of pesticide with technologies is milestone of the designs for air blast orchard sprayers. The axial fan of the orchard sprayer must be flexible to supply enough airflow and had variable rate technology (VRT) of pesticides to different types of trees for precisely pest application (Hołownicki et al., 2017). The efficiently usage of an axial fan can reduce tractor fuel consumption and offers more economical and environmental friendly pesticide application. To do this, the parameters that affected the air flow must be regulated. In air assisted orchard sprayers, the spray droplets produced by the nozzles are carried to the tree crown by the air flow that provided by fans. During the pest application of orchard, the airflow is needed to atomize the droplet, to carry the droplets to the target and to get better penetration (Matthews, 2000). The amount and the distribution of the pesticides on the tree crown varies depending on the characteristics of the air jet (Khot et al., 2012). The air volume flow rate of the orchard sprayer and penetration of the air flow must be set to be sufficient to different tree crowns (Panneton et al. 2001, Delele

Cite this article as:
Itmec, M., Bayat, A. (2021). Determination of the optimum operating parameters of an axial fan used on the conventional air blast orchard sprayer. J. Agric. Environ. Food Sci., 5(3), 405-412.
Doi: https://doi.org/10.31015/jaefs.2021.3.20
Orcid: Medet Itmec: 0000-0003-0714-6232 and Ali Bayat: 0000-0002-7104-9544
Received: 29 March 2021 **Accepted:** 04 July 2021 **Published Online:** 31 August 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 405-412
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

DOI: 10.31015/jaefs.2021.3.20

et al. 2005, Chen et al. 2011, Garcia et al. 2012). Beside this, the airflow need of orchard depends on the crown geometry of the tree, tractor velocity, vegetative development of the tree, foliage density, wind velocity (Chen et al. 2012). Canopy geometry, foliage density and vegetative development of a tree affects the pesticide penetration and deposition of droplets on the tree. According to Balsari et al. (2008) 5m/s air velocity was sufficient to give higher spray deposits on the leaves in vineyard.

In the real world, spraying involves a changing droplet size distribution, turbulent airflow, fluttering and wobbling objects of non-uniform size and shape, and an ever-changing canopy situation. Leaves used as targets can to some extent be regarded as ribbons or disks (Fox et al., 2008). The forced air jet transports the spray droplets throughout the target, moving and lifting the foliage to allow penetration and depositing the droplets on the plant surface including the undersides of leaves (Cross et al. 2003). There are two approaches to dealing with the problems of inefficiency and inadequate coverage associated with applying pesticide treatments: improving the dosing system and improving the application machinery. Adjusting the spray dose is useless if the application equipment is not adapted to the target canopy. Therefore, efforts have been undertaken to improve air blast sprayers (Miranda-Fuentes et al. 2017). The economically produced sufficient airflow is one of the main mission of the pest application of a orchard also for spray deposit.

The fans are used in different areas and for each blade angle of the fan, the performance and volumetric flow rate of the fan differs (Izadi and Falahat 2008, Patel and Patel 2012, Huang and Gau 2012). The airflow that produced with axial fans can be controlled by changing the fan blade angles and fan rotation speed. The blade performance may be predicted from the aero-dynamic characteristics such as *lift* and *drag coefficients* of the chosen blade section and given by the following equations:

$$C_l = \frac{L}{\frac{1}{2}\rho V^2 A_l} \qquad (1)$$

$$C_d = \frac{L}{\frac{1}{2}\rho V^2 A_d} \qquad (2)$$

where C_l is the coefficient of lift, C_d is the coefficient of drag, L is the lift force, D is the *drag force*, ρ is the density of air, V is the velocity of undisturbed airflow. The related areas of the fan blades are shown in Figure 1. The top view of the fan blade is A_l *and* the side view of the fan blade is A_d (Panigrahi and Mishra 2014). As the blade angle (α) of the fan increases, the exit velocity and the volume flow rate of the fan also increase (Logan and Roy, 2003). The fuel consumption and power need also increase depending upon the blade angle increase. Therefore, to get better performance with high working efficiency, the correct fan blade must be determined for processes.

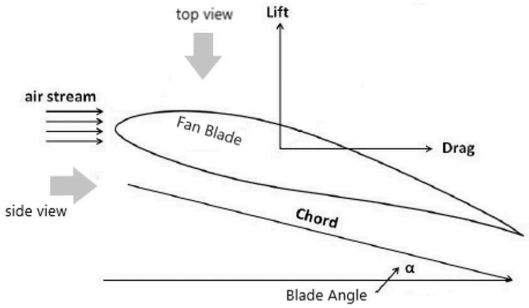

Figure 1. Lift and Drag forces acting on the blade and the blade angle change.

The branches and leaves which resist to the air flow, decreases their kinetic energy of air flow. The designers manufacture the orchard sprayer with 6 different air capacity options (1890 rpm and 2430 rpm via transmission and 15º, 30º, 45ºblade angles) under constant PTO speed. However, they did not make any advice about the correct option for related tree kind during the pesticide application for each options. The recent studies which was made according to pesticide application. In these studies, the energy consumptions was not taken into account. In this study, the axial fan which is designed for the variable airflow with the help of fan blade angle change, was used. The air velocities, the torque, fuel consumption values were measured under conditions of the two fan revolutions (1890 rpm and 2430 rpm) and three different blade angles (15º, 30º, 45º) with 540 rpm of PTO. By this way, the fan power, average air velocity, air jet velocity profile, PTO power, approximate fuel power and efficiency was calculated. Then, the axial fan of the orchard sprayer performance and efficiency was compared to determine optimum axial fan working conditions. The optimum working condition was determined according to with view of less energy consumption and high air velocity application.

Materials and Methods

Establishment of the experimental unit

The tests were carried out at the laboratory conditions with no air flow. New Holland TD 95 (Turkey) tractor which was stationary, was used to drive PTO. The maximum power of the tractor was 62,5 kW and it had diesel motor with four cylinders. To evaluate axial fan efficiency of the orchard sprayer; the pump, pressure regulator unit, and the tank were removed from the orchard sprayer. The orchard sprayer axial fan was connected to the three-point linkage system of the tractor and PTO was used to drive axial fan. Before the axial fan blades, there were flow redirecting stationary blades of 19 which were located as 55º angle according to air inlet axis. After the air passed from the fan blades, at the exit side of the fan the air flow directed to the nozzles (Figure 2a) with an air deflector. The diameter of axial fan was 900 mm (36'') and it had 9 blades which was made of polyethylene, was arranged in an aluminum hub at angle of 40º. The Exit side of the fan area calculated as 0,31 m^2. The fan blades had holes to set the angle of the blade as 15º,30º,45º via the bolts on the aluminum hub (Figure 2b). The axial fan transmission system

had the two stages as 1:3,5 and 1:4,5 transmission rates which corresponded 540 rpm to 1890 rpm and 2430 rpm respectively. Before the experiment started, each nozzle axis was turned perpendicular direction of the circumference of the axial fan to eliminate the turbulences at fan exit.

Measurement of the experimental results and data calculation

To calculate the PTO power () and fan efficiency, the torque sensor was connected between the transmission unit of the axial fan and the shaft. The torque and angular velocity was measured via torque sensor with data logger (Grant 2020 series) (Figure 3).

The real time fuel consumption values were measured by two flow-meter (AICHI OF05ZAT Aichi Tokei Denkei Co. Ltd. Japan). One of the flow meter measured the amount of fuel passing from the fuel supply line between the fuel tank and the injection pump (Figure 4a). The other was used for measuring the amount of fuel returning line of the fuel tank from the injection pump and the injectors. By the way, the difference between the two measurements represented real time fuel consumption (Figure 4b). The both flowmeters were calibrated according to diesel fuel before the experiments.

Figure 2. (a) The axial fan of the orchard sprayer and (b) aluminum fan hub blade angle change unit.

Figure 3. Connection of the torque sensor between transmission unit PTO and data logger

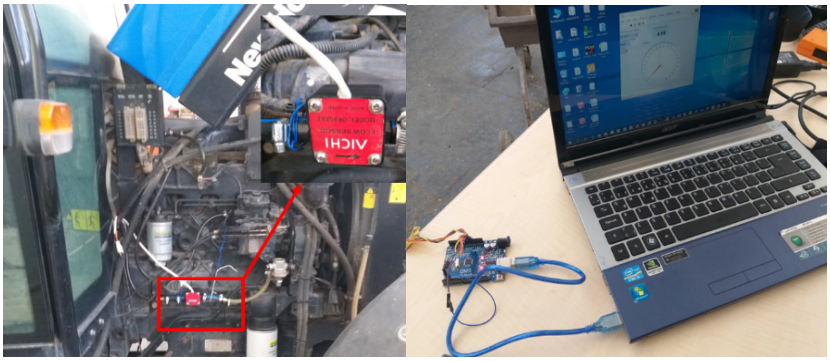

Figure 4. (a) Flowmeter of the supply line between the fuel tank and the injection pump, (b) real time gauge that show the differential difference of the two flowmeters.

DOI: 10.31015/jaefs.2021.3.20

To determine the fan power and air jet velocity of the axial fan, the measurement of the velocity was practiced with a hot wire anemometer. From the bottom side of the axial fan there was no air flow exit. The rest of the circumference was separated 12 regions and numeri zed to measure air velocity (Figure 5). The velocities that measured from the regions 1 to 6 are the left side of the axial fan and 7 to 12 are the right side of the axial. The air velocity measurement from each region was conducted with three replicates for each experiment to calculate the average exit velocity.

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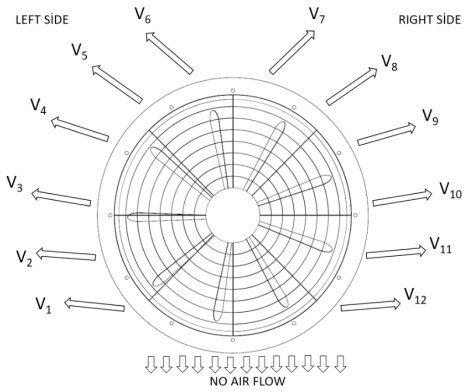

Figure 5. Air velocity measurement regions for air jet profile determination from back view of the sprayer.

The air jet velocity's uniformity of distribution was evaluated according to coefficient of variation (CV) method;

$$CV = \frac{\sigma}{\mu} \qquad (3)$$

where indicates the standard deviation and μ also means mean values of the air velocities that measured from 12 regions. The value of CV was calculated for left, right sides and totally for each experiment conditions. Reason for calculating the CV value of each experiment condition is, determining disturbance of the air jet. As the CV value increase, distribution uniformity of the jet gets worse.

The average value of each air exit velocity that measured from exit side of the axial fan was used for the Bernoulli equation to calculate fan power (N_{fan});

$$N_{fan} = \dot{m}\left(k\frac{V_2^2}{2}\right) \qquad (4)$$

Where is the mass flow rate, is kinetic energy correction factor (=1.1). P_1 is the inlet air pressure and P_2 is the exit air pressure of the air, V_1 is the inlet velocity, V_2 is the exit velocity, is air density, the g is the gravitational acceleration, z_1 and z_2 is the elevation respectively inlet and outlet side of the fan. P_1 and P_2 are equal to the atmospheric pressure P_{atm} so these two values can be neglected and there was negligible elevation difference between inlet and outlet so z_1 and z_2 were also negligible. Inlet side of the fan was large, therefore V_1 was also neglected. The mass flow rate and fan average exit velocity fan power can be calculated with these assumptions (Çengel and Cimbala 2004).

The mass flow rate of the axial flow also calculated as;

$$\dot{m} = \rho.A.V_{ave} \qquad (5)$$

In equation; is air density (kg/m^3), A is the exit area (m^2) of the axial fan and is (m/s) the average velocity of the exit side.

After measuring the torque and angular velocity data of the experiment the PTO Power (N_{PTO}) can be calculated as:

$$N_{PTO} = \frac{M_d x n}{9550} \qquad (6)$$

Where is the torque (Nm) and n is the angular velocity (rpm). Then fan efficiency of the experiment can be calculated:

$$N_{fan,eff} = \frac{N_{fan}}{N_{PTO}} \qquad (7)$$

The fuel power (can be calculated according to the fuel consumption of the each experiment data;

$$N_F = \frac{BxH}{3600} \qquad (8)$$

Where the B is the fuel consumption per hour (kg/h) and the H is the energy value of the fuel (kg/kj) which can be 42.000 kj/kg and diesel engine operates approximately %32 efficiency, hence it was used as multiplier factor (Erzurumlu, 2018). The efficiency of the whole system which meaned ratio of the fan power to the fuel power can be calculated as;

$$N_{sys,eff} = \frac{N_{fan}}{N_F} \qquad (9)$$

In recent studies, it can be clearly seen that effect of air volume, air jet capacity, air exit velocity were studied. However, energy consumption and energy components (blade angle, fan

revolution speed, torque etc.) were not evaluated (Pai et.al. 2009; Khot et. al., 2012). In the study, the energy consumption and air jet was evaluated. The axial fan of the orchard sparayer was worked at 540 rpm PTO with two different revolutions (transmission rates1:3,5 and 1:4,5 which corresponds to 1890 rpm and 2430 rpm). The fan blade angle was changed to 15º,30º,45º angles to measure fuel consumptions, torque, and local velocities (from 12 regions) with three replicates. Then the fan power, the fan efficiency, PTO power, fuel power and system efficiency were calculated. According to these values the optimum operation condition was determined according to these experimental conditions. To show operation conditions effect on air velocity The air velocity data was evaluated in SPSS 20 program, with One Way Anova Test with Duncan Post HocTest.

Results and Discussion

Air Jet Profile

To present effect of the blade angle and fan rotation speed (rpm) on the axial fan average velocity, each operation velocity data that was measured from related region (Figure 3), and evaluated statistically with One Way Anova and examined by Post Hoc Duncan Test (Figure 6). The operation conditions were statistically significant which means each operations differed. For instances, with 45º blade angle with 2430 rpm experiment condition the maximum average velocity was obtained and the 15 º blade angle with 1890 rpm was the minimum average velocity statistically. In this study, maximum average value of the velocity was 48,367 m/s. Endalew et al. (2010), used 800 mm diameter axial fan with flow regulator on deflector unit, to prevent turbulences at exit side and measured the maximum velocity at 30 m/s at exit side. The air velocity which enters the tree canopy and the exit at the other side of tree canopy must be between 5 m/s as explained before. However, it depends on vegetation, foliage density.

It was obvious that for the same blade angle, if the fan rotation speed was chosen as 2430 rpm instead of 1890 rpm, the CV (%) of the left side, right side and total CV (%) became less which means more stable velocity characteristic (Table 1). As the blade angle was increased, the velocities that measured from each region were increased. Because of transmission unit, the impeller of the fan (counter clockwise) turned reverse side of the PTO. By the way the axial fan vacuumed air firstly discharged from right side of the axial fan. Because of this reason, the turbulence occurred at the right side of the axial fan. Therefore, at V_{11} as lowest velocity of the whole air jet and V_{12} as maximum velocity of the air jet. Due to the irregularity of the right side of the axial fan, CV_{right} was larger than the CV_{left} for all experiment conditions. As the both blade angle and fan rotation speed increased, CV_{total} value became less than before. It was clear that rise of the blade angle caused the velocity increase for each region. In this study, the fan blade angle was changed to 15º, 30º, 45º. However, fan's stationary blade angles (as said before 55º) were not able to change. Because of construction, the flow redirecting blades was constant. Therefore, the flow redirecting blades must be also set according to fan blade angle. According to Liu et al. (2011), the difference between the flow regulator blade angle and fan blade angle must be 10º to get better performance.

Fuel Consumption and Power

The torque and fuel consumption values for each experimental conditions were measured as shown Table 2. It can be seen that as the rotation speed of fan increased from 1890 rpm to 2430 rpm, the torque need of the system and fuel consumption increased. If the *blade angle* increase for constant Fan Rotation Speed, both Torque and Fuel Consumption values also increased because of the resistance of the air. The maximum torque need and fuel consumption values were measured at 2430 rpm and 45º blade angle. Comparing the data that obtained from the experiments and previous studies (Işıktepe and Sümer (2010)) were similar in results of torque, power, and fuel consumption data for 540 rpm PTO. At first view, for low fuel consumption and torque, the condition of 1890 rpm and 15º blade angle was appropriate but the produced air velocity was another parameter to decide.

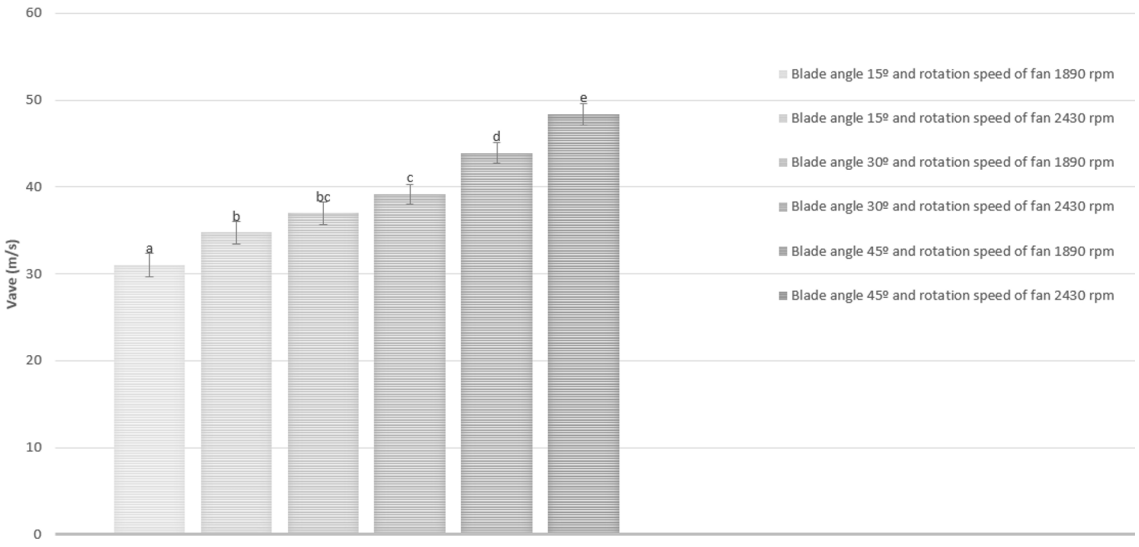

Figure 6. Statistical results of result of average fan velocity of each operation conditions.

Table 1. Measured velocity from 12 regions and distribution values under different conditions

Stage	Blade Angle (º)	V1 (m/s)	V2 (m/s)	V3 (m/s)	V4 (m/s)	V5 (m/s)	V6 (m/s)	CVleft (%)	V7 (m/s)	V8 (m/s)	V9 (m/s)	V10 (m/s)	V11 (m/s)	V12 (m/s)	CVright (%)	CVtotal (%)	Vave
3,5	15	28,96	30,8	32,6	31,03	29,9	32,23	4,45	32,23	31,2	33,8	30,5	24,8	33,87	10,80	7,89	30,99
4,5	15	35,47	36,5	37,6	39,17	36,9	37,69	3,36	38,67	39,9	37,2	35,76	29,4	39,57	10,67	7,53	36,99
3,5	30	31,46	32,2	32,8	34,8	36,4	35,2	5,73	37,2	36,8	35,2	33,88	31,8	39,2	7,36	6,92	34,74
4,5	30	41	42,9	45,4	46,93	42,5	44,2	4,87	45,93	45	45,3	42,9	38,1	46,63	7,14	5,83	43,90
3,5	45	37,97	37,9	37,4	41,5	38,4	41,43	4,74	41,63	43,3	36,5	33,93	34	45,67	12,83	9,23	39,15
4,5	45	45,5	41,3	44,7	46,73	45,1	46,7	4,44	51,15	52,6	50,1	51,57	47,9	57,13	5,95	8,89	48,37

Table 2. Comparison of the measured torque and fuel consumption values

Blade Angle (º)	Fan Rotation Speed (rpm)	Torque (Nm)	Fuel Consumption (l/h)
15	1890	207	8,56
15	2430	342	10,16
30	1890	318	10,04
30	2430	467	11,48
45	1890	426	12,03
45	2430	633	14,83

According to the results of the experiments for each angle, if the fan rotation speed was increased via shifting the stage, the volume and mass flow rate, fan power, PTO Power, Fuel Power increased. The maximum Fan Power, PTO Power, and Fuel Power were obtained at 2430 rpm and 45º blade angle (Table 4). The maximum air volume flow rate was obtained as 59040 m^3/h. According to Bayat et. al. (2020) to get better penetration, the volumetric flow rate of axial fan must be between 70000 m^3/h and 90000 m^3/h for citrus with 6 m canopy height. However, the excessive torque increase caused decline of the efficiencies. It was obvious that maximum efficiency of fan and system were obtained at 2430 rpm and 30º blade angle because of producing high average velocity, low torque need and fuel consumption.

Table 3. Calculated Fan power, PTO power Approximate Fuel Power and efficiencies of the experiment

Blade Angle(º)	Average Exit Velocity (m/s)	Volume flow rate*10^3 (m^3/h)	Mass flow rate (kg/s)	Fan Power (kW)	PTO Power (kW)	Fuel Power (kW)	Fan Efficiency (%)	System Efficiency(%)
15	30,99	34,67	11,80	6,23	11,70	24,05	53,26	25,92
15	36,99	37,62	12,81	9,64	19,34	28,54	49,83	33,76
30	34,73	42,41	14,43	9,57	17,98	28,21	53,23	33,93
30	43,91	53,60	18,24	19,34	26,41	32,25	73,25	59,98
45	39,16	47,81	16,27	13,72	24,09	33,80	56,96	40,60
45	48,36	59,04	20,09	25,84	35,79	50,09	72,20	51,59

Conclusion

To provide flexibility for spraying in orchard, the axial fan of orchard sprayer was designed with 6 different conditions (three blade angles and two different revolutions of fan). However, in user guide there was no explanation about, which condition was most efficient for general purpose and for different tree kind. According to vegetative development and leaf density, proper fan speed and blade angle must be determined for each tree kind. However, 6 different conditions were not sufficient for general purpose orchard sprayer. The air velocity must be changed instantaneously depending upon the tree canopy. Via ultrasonic sensor, LİDAR technology, stereovision technologies etc. flexibility of spraying for each tree can be provided precisely. With these technologies, energy and pesticide consumption savings also increase.

The most efficient of the average air velocity of 2430 rpm and 30º blade angle. The maximum average air velocity was calculated for 2430 rpm and 45º blade angle as 48,37 m/s. The uniformity of air jet velocity was changed depending upon the fan rotation speed. Because of fan turning side, there was turbulence at right side and the velocity of the V_{11} and V_{12} were not stable. However, the disorders of the air started to decrease with fan rotation speed increase. As the blade angle or stage increased, the rotating of the fan got resistance. Therefore, the

fuel consumption and torque need of the fan increased. The fan and system efficiencies were decreased depending upon the excessive torque rise for 2430 rpm and 45º blade angle, because the resistance of the blades were changed. The maximum fan and system efficiencies were obtained as 2430 rpm and 30º blade angle 73,25% and 59,98% respectively. However, 2430 rpm and 30º blade angle can be default value which is optimum condition for efficiency. However, the air flow need for the sufficient pest application differs according to vegetative development, tree crown geometry, foliage density. Therefore, tree kind-air volume flow rate relationship must be determined before application depending upon foliage density.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.21 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):413-424 (2021)

Covid-19 outbreak and household food waste: evidence from Turkey

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Abstract

This study aims to examine changes in food-related behaviours that occur in Turkish households due to the Covid-19 outbreak and their effect on food waste amounts. An online survey was conducted and the survey included questions about socio-demographic characteristics, food purchasing, preparation, cooking behaviour, and food waste. This study included 610 respondents. 33.9% of the participants stated that there was a decrease in the amount of food waste during the pandemic period. More than half (52.2%) of those who think that there is a decrease in the amount of food waste stated that the amount of waste decreased because the food was consumed without forgetting/spoiling due to the increase in the time spent at home. Also, a relationship was found between changes in some food-related behaviours (frequency of food shopping, preparing/cooking, the characteristic of the food purchased, the person who prepares/cooks the meal, the time spent in the kitchen, the number of meals, trying new recipes, making bread at home, ordering to home and eating out) and changes in food waste. It has been observed that the changes experienced influence the reduction of food waste. In conclusion, the necessary initiatives should be taken to make permanent the positive changes caused by Covid-19 in food-related behaviour.

Keywords: Covid-19, Food Waste, Household Habits

Introduction

World Health Organization (2020) announced the existence of the Covid-19 pandemic caused by SARS-CoV-2 on March 11, 2020. The Covid-19 pandemic is not the first pandemic in the history of humanity. Humanity has faced various pandemics in previous times (e.g., SARS, Ebola, and MERS). First detected in China in December 2019, the COVID-19 has spread to six continents in a short time after its appearance (WHO, 2020).

According to currently available evidence of the SARS CoV-2 virus that causes Covid-19, it is stated that the main route of person-to-person transmission is respiratory droplets. Hand contact with surfaces contaminated with infectious droplets and then contact with the mouth, nose, or eyes also appear to be an indirect transmission route. The SARS-CoV-2 virus is highly contagious and has very high mortality rates, especially for the elderly (CDC, 2020).

Pandemics pose a severe threat to public health and social stability. Therefore, many countries worldwide have advised their communities to stay at home and implemented extraordinary public health measures to limit the spread of the transmission since there is no specific treatment or vaccine for the disease at the start of the Covid-19 pandemic. With these measures, it was intended to reduce and delay the transmission of the pandemic in society, reduce the burden on health systems, and provide the best possible care for patients,

Cite this article as:
Songur Bozdag, A.N., Cakiroglu, F.P. (2021). Covid-19 outbreak and household food waste: Evidence from Turkey. J. Agric. Environ. Food Sci., 5(3), 413-424.
Doi: https://doi.org/10.31015/jaefs.2021.3.21
Orcid: Ayşe Nur Songür Bozdağ: 0000-0001-6412-8650 and Funda Pınar Çakıroğlu: 0000-0003-2324-6874
Received: 20 April 2021 **Accepted:** 11 June 2021 **Published Online:** 25 September 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 413-424
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

reducing case numbers to low levels by social distancing all populations (CDC 2020; IMF 2020).

According to the United Nations Food and Agriculture Organization, about a third (i.e., 1.3 billion tons) of food produced globally is wasted each year (FAO, 2019). Food loss and waste are considered one of the most important sustainability issues that cause economic, social, and environmental downsides and must be addressed globally. Food waste prevention and reduction are a critical issue for sustainable development and profitability, especially in low- and middle-income countries, and is also among the Sustainable Development 2030 targets (SDGs). Within the scope of SDG 12 (Target 12.3), it is aimed to reduce food waste at the retail and consumer level by half by 2030 (UN, 2015).

Food loss and waste occur at all stages of the food supply chain (from harvest to consumption), but it is stated that the biggest contribution comes from households (EPRS, 2014; FAO, 2011, Williams et al., 2012). Research into a better understanding of the causes of food waste generated at the household level and determining the amount of waste has recently become an important interest of the academic and social area. It is estimated that the amount of food waste generated in households constitutes more than 50.0% of the total food waste in Europe (Kummu et al., 2012) and 60.0% of the total food waste in the USA (Griffin et al., 2009). Research in the UK has shown that the amount of food and drink waste at the household level accounts for about 22.0% of all food and drink purchased (330 kg per household per year). It was stated that 65.0% of this waste (215 kg per household each year) was the edible condition before it was disposed of (WRAP, 2009). In other studies, it was determined that an average household in Denmark is approximately 105 kg per year and in Finland 63 kg (Koivupuro et al., 2012; Silvennoinen et al., 2014). Looking at the studies, it is seen that most of the studies on food waste in households were in developed countries. Information on food waste in households in developing countries is insufficient. Turkey is one of these countries. Yıldırım et al. (2016) determined that 31.3% of the participants threw away food 1-2 times a week, 27.3% created less than 250 grams of waste in a week. Ündevli et al. (2019), 34.7% of the participants found that the frequency of throwing food was less than once a week. Songür Bozdağ and Çakıroğlu (2021) stated that 32.9% of the participants generated 0-1 kg of food waste in a week and the main reasons for food waste were found to be mouldy food, food left in the refrigerator for too long, and the date expiration of food. In the COMCEC (2017) study, it has been determined that the main reason for the generation of food waste is "expired product."

During the Covid-19 pandemic, there was an increase in the time spent in homes due to measures taken by governments, which led to various changes in food production-consumption behaviour and lifestyles. Perhaps no outbreak (SARS, Ebola, MERS, etc.) in history to date has led to a rapid and radical change in society's food consumption behaviours and lifestyles as much as the Covid-19 pandemic. For this reason, this study was conducted to provide a prediction during the pandemic of Covid-19, how people make decisions about food, behavioural changes, and the impact of these changes on food waste, and shed light on future research.

Materials and Methods

Survey design and data collection

In this study, during the Covid-19 pandemic in Turkey, investigating the change in behaviour related to food in households was conducted between May and September 2020. A questionnaire was prepared by the researchers based on the studies in the literature on food waste (Aschemann-Witzel et al., 2017; Gaiani et al., 2018; Mallinson et al., 2016; Ponis et al., 2017; Stefan et al., 2013; Szabó-Bódi et al., 2018; Tucker & Farrelly, 2015).

Survey consists of 3 parts. First part socio-demographic characteristics (gender, education, marital status, income etc.), second part information on food purchasing, preparation-cooking before and during the pandemic period (food shopping person, shopping place, the person who prepared-cooking food etc.) and third part is multiple-choice and open-ended questions about their knowledge of food waste generated in their households (amount of avoidable food waste in the last week, discarded foods, change in the amount of waste etc.).

The questionnaire was applied in Turkish on Google Forms and delivered to the participants through the researcher's social media accounts. All participants read and approved the informed consent form before starting the survey. At the end of the study, between the ages of 19-65, 610 people responsible for at least half of food shopping and preparation in their household were reached.

For the study, necessary permissions were obtained from the Ministry of Health Scientific Research Platform and Ankara University Ethics Committee (2020-14/199).

Data analysis

Data were shown as the frequency and percentage for nominal variables, the median, and the interquartile range (IQR) for continuous variables. Chi-square independence tests were used for associations between changes in food purchasing, preparing, cooking, and the amount of food waste. Statistical significance was determined by $p < 0.05$ at a 95% confidence interval.

Results

According to the socio-demographic results the average age was 32.46 ± 8.75 (min: 19, max: 65) years old; it was seen that most of them were females (85.2%), have a higher education level (83.0%-undergraduate + postgraduate), working (78.4%) and more than half of them were married (53.9%). Furthermore, the median value of the monthly income of the households was 6500 TL (920 USD), the median values of the number of individuals living in the household, the number of women, and the number of children under the age of 18 were determined as 3, 1 and 0; respectively (Table 1).

Table 2 shows the changes in the food dynamics of households during the pandemic period. Before the outbreak, more than half of the participants (54.1%) did their food shopping at their homes by "themselves". In comparison, 21.1% stated that there was a change in the person responsible for food shopping in the household during the pandemic period. 37.7% of those who stated that there was a change in the person

who food shopping said that "myself/my sibling "instead of their mother/father began to do it so during the pandemic. In the pre-outbreak period, it was determined that the participants mostly (62.1%) shopped in supermarkets, that the outbreak led to changes in where 23.4% of participants shopped. Almost half of those who stated that there was a change (49.7%) now shop "online" instead of "supermarket/grocery/bazaar". When the effect of the outbreak on the frequency of shopping in households was evaluated, 50.3% of respondents stated that there was a "decrease" in the frequency of shopping. During the pandemic period, the characteristics of the food purchased in 34.3% of the households have changed. The main change was to more prefer "packaged products" (47.4%).

In the pre-pandemic period, 59.4% of the participants stated that they were responsible for preparing/cooking food at home. During the outbreak period, only 10.5% of households was a change in the person responsible. When asked if there was any change in the frequency of food preparing/cooking in households, 61.1% of respondents reported an "increased." More than half of the participants (63.9%) stated that there was an "increased" in the time spent in the kitchen to prepare meals. During the outbreak, 64.1% of households tried new recipes, 49.8% started making their bread, and 38.0% had an increase in food variety. It was also found that 65.2% of participants had a decrease in the frequency of ordering food at home, and 87.0% had a reduction in the frequency of eating out (Table 2).

Table 1. Participant profiles (n= 610)

	n	%
Gender		
Female	520	85.2
Male	90	14.8
Education		
Primary school	10	1.6
Middle school	8	1.4
High school	61	10.0
Undergraduate	379	62.1
Postgraduate	152	24.9
Occupation		
Healthcare worker	94	15.4
Private sector	88	14.4
Teacher	80	13.1
Self-employment	77	12.6
Academician	59	9.6
Student	59	9.6
Housewife	51	8.4
Civil servant	41	6.7
Engineer	40	6.6
Retired	12	2.0
Unemployed	9	1.6
Marital status		
Single	329	53.9
Married	251	41.2
Divorced/Widowed	30	4.9
	Median (IQR)	**Minimum-Maximum**
Household income	6500 TL (5000 TL) = 920 USD (710 USD)	1000 TL – 40000 TL = (145 USD – 5670 USD)
Household size	3 (2)	1-10
Number of female	1 (1)	0-5
Number of children under 18 age	0 (1)	0-5

Table 2. Changes in food purchasing, preparing, and consuming in households

	n	%
Person responsible of food shopping before the outbreak		
Myself	330	54.1
My partner	100	16.4
My father	69	11.3
My mother	61	10.0
My partner and me	23	3.8
My sibling	10	1.6
All family members	7	1.1
My mother and father	6	1.0
My child/children	2	0.3
My helpmate	2	0.3
Changing the person responsible of food shopping		
Yes	159	26.1
No	451	73.9
Changing		
My mother/My father → Myself /My sibling	60	37.7
Myself → My partner/ My home mate	58	36.5
My partner and me → My partner	12	7.5
My mother or My father → My father or My mother	8	5.0
My partner → Myself	7	4.4
Myself → My partner and me	6	3.8
All family members → Myself	3	1.9
Myself → My mother/ My father	3	1.9
Myself → My child/children	2	1.3
Food bought place before the outbreak		
Supermarket	379	62.1
Supermarket + Bazaar	123	20.2
Online	23	3.8
Supermarket + Online	23	3.8
Supermarket + Grocery	20	3.3
Supermarket + Greengrocer	17	2.7
Bazaar	15	2.5
Grocery	10	1.6
Changing food bought place		
Yes	143	23.4
No	467	76.6
Changing		
Supermarket/grocery/ bazaar à Online	71	49.7
Supermarket + bazaar à Supermarket	30	21.0
Supermarket à Grocery store, etc. (near to home)	26	18.2
Bazaar à Supermarket	5	3.5
Bazaar à Greengrocer	5	3.5
Online à Supermarket/grocery/bazaar	3	2.1
Supermarkets à Hypermarkets	3	2.1
Food shopping		
Increased	159	26.1
Not changed	144	23.6
Decreased	307	50.3
Changing characteristics of the purchased food		
Yes	209	34.3
No	401	65.7

Table 2. Changes in food purchasing, preparing, and consuming in households (continue).

Changing		
I/we get to more prefer packaged products	99	47.4
I/we get to more prefer durable foods	48	22.9
I/we get to more prefer healthy and fresh foods (vegetables, fruits, etc.)	39	18.7
I/we get to more prefer organic products	23	11.0
Person responsible of preparing/cooking food before the outbreak		
Myself	362	59.4
My mother	170	27.9
My partner	52	8.5
My partner and me	10	1.6
My helpmate	7	1.1
My mother and me	6	1.0
My sibling	3	0.5
Changing person responsible of preparing/cooking food		
Yes	64	10.5
No	546	89.5
Changing		
My mother → Myself	21	32.8
Myself/My partner → My partner/ My mother/ My child/ My sibling	18	28.1
My mother → My mother and me	12	18.8
Myself/My partner → My partner/My mother/My sibling and me	10	15.6
My helpmate → Myself/My partner	3	4.7
Food preparing/cooking		
Increased	373	61.1
Not changed	231	37.9
Decreased	6	1.0
Time spent in kitchen		
Increased	390	63.9
Not changed	215	35.2
Decreased	5	0.9
Type (number) of food prepared/cooked		
Increased	232	38.0
Not changed	360	59.0
Decreased	18	3.0
Trying new recipes		
Yes	391	64.1
No	219	35.9
Starting to make bread at home		
Yes	304	49.8
No	306	50.2
Food ordering to home		
Increased	6	1.0
Not changed	206	33.8
Decreased	398	65.2
Eating out		
Increased	-	-
Not changed	79	13.0
Decreased	531	87.0

In 63.6% of the households participating in the study, the avoidable food waste that occurred in the last week was "0-250 g" and the main raw and cooked food products were thrown into the garbage were vegetables (44.3%) and cereal/legume meals (21.3%). The main reason for throwing food into the garbage was that the food is mouldy, degraded, etc. (21.0%) (Table 3).

When evaluated the change in the amount of preventable food waste that occurred in households during the outbreak, 33.9% of respondents stated that a "decrease" in the amount of food waste, and 7.0% of them stated that an "increase."72.1% of those who thought there was an increase in the amount of food waste stated an increase in the amount of food waste because they "cook more" due to the increase in time spent at home. Also, the main reason for the decrease in the amount of food waste was food consumption without forgetting/spoiling due to the increase in the time spend at home of participants (52.2%). It was determined that 33.3% of the households made an initiative to reduce waste during the pandemic period, and the main initiative applied by the households "to make better meal planning" (Table 3).

The relationship between changes in purchasing, preparing, consuming food in households during the pandemic period and the difference in the amount of food waste was shown in Table 4.

Table 3. Information about food waste in households

	n	%
Amount of avoidable food waste (last week)		
0-250 g	388	63.6
251-500 g	124	20.3
501-750 g	42	6.9
751 -1000 g	30	4.9
1001-1500g	12	2.0
1501-2000 g	6	1.0
2001 g and above	8	1.3
Discarded raw food		
Vegetables	270	44.3
Fruits	89	14.6
Meat and meat products	36	5.9
Milk and dairy products	8	1.3
Cheese-egg	4	0.7
Oilseeds and dried nuts and fruits	4	0.7
Cereals and legume products	3	0.5
Oils	1	0.2
Discarded cooked food		
Cereal - legume meals	130	21.3
Vegetable meals	66	10.8
Soups	39	6.4
Pastry / desserts	34	5.6
Meat meals	20	3.3
Bread	18	3.0
Egg	15	2.5
Milk and dairy products	4	0.7
Reason of food waste		
Formation of mould, etc. on the food	128	21.0
Poor meal planning	111	18.2
Poor food shopping planning	87	14.3
Storage of food for an excessive period of time in the refrigerator/ store cupboard	39	6.4
Dislike of food/meal	20	3.3
Erroneous preservation (storage) method	17	2.8
Expired products	14	2.3
Fear caused by the virus	8	1.3
Amount of food waste		
Increased	43	7.0
Not changed	360	59.0
Decreased	207	33.9

Table 3. Information about food waste in households (continue).

	n	%
Reason of increase		
Preparing/cooking more food due to spending more time at home	31	72.1
Buying more food	12	27.9
Reason of decrease		
Consumption of foods in a short time without forgetting/spoilage with the increase of time spent at home	108	52.2
More conscious and careful purchasing	63	30.4
Better meal planning with increased time spent at home (the required amount of cooking etc.)	36	17.4
Extra initiatives to reduce food waste		
Yes	197	32.3
No	413	67.7
Initiatives		
Better meal planning	80	40.6
Utilizing leftover meals/foods in different ways	36	18.3
Better shopping planning	24	12.2
Ensuring that food is consumed without spoiling by paying more attention to the control of the refrigerator/food cabinet	20	10.2
Paying more attention to preservation (storage) methods	17	8.6
Eating more regularly with the increase of time spent at home	14	7.1
Better portion control	6	3.0
Effect of making bread on bread waste		
Increased	13	4.3
Not changed	172	56.6
Decreased	119	39.1

Table 4. Relationship between changes in food purchasing, preparing and consuming, and food waste in households

Changes in food purchasing, preparing and consuming	Change in the amount of food waste						p
	Increased		Not changed		Decreased		
	n	%	n	%	n	%	
Person responsible of food shopping							
Yes	8	5.0	85	53.5	66	41.5	0.05
No	35	7.7	275	61.0	141	31.3	
Food bought place							
Yes	11	7.7	75	52.4	57	39.9	0.182
No	32	6.9	285	61.0	150	32.1	
Food shopping							
Increased	24	15.1	82	51.6	53	33.3	
Not changed	8	5.6	105	72.9	31	21.5	**<0.001**
Decreased	11	3.6	173	56.4	123	40.0	
Characteristics of the purchased food							
Yes	16	7.7	95	45.5	98	46.9	**<0.001**
No	27	6.7	265	66.1	109	27.2	
Person in responsible of preparing/cooking food							
Yes	2	3.1	28	43.8	34	53.1	**0.002**
No	41	7.5	332	60.8	173	31.7	

Table 4. Relationship between changes in food purchasing, preparing and consuming, and food waste in households (continue).

Changes in food purchasing, preparing and consuming	Change in the amount of food waste						p
	Increased		Not changed		Decreased		
	n	%	n	%	n	%	
Food preparing/cooking							
Increased	36	9.7	184	49.3	153	41.0	**<0.001***
Not changed	7	3.0	17	74.0	53	22.9	
Decreased	-	-	15	83.3	1	16.7	
Time spent in kitchen							
Increased	33	8.5	197	50.5	160	41.0	
Not changed	10	4.7	160	74.4	45	20.9	**<0.001***
Decreased	-	-	3	60.0	2	40.0	
Type (number) of food prepared/cooked							
Increased	26	11.2	108	46.6	98	42.2	**<0.001**
Not changed	17	4.7	239 13	66.4	104	28.9	
Decreased	-	-		72.2	5	27.8	
Trying new recipes							
Yes	31	7.9	204	52.2	159	39.9	**<0.001**
No	12	5.5	156	71.2	51	23.3	
Starting to make bread at home							
Yes	20	6.6	165	54.3	119	39.1	**0.025**
No	23	7.5	195	63.7	88	28.8	
Food ordering to home							
Increased	-	-	6	100	-	-	
Not changed	11	5.4	148	71.8	47	22.8	**<0.001***
Decreased	32	8.0	206	51.8	160	40.2	
Eating out							
Increased	-	-	-	-	-	-	
Not changed	8	10.1	578	73.4	13	26.5	**0.002**
Decreased	35	6.6	302	56.9	194	36.5	

Food wastage decreased in 41.5% of those who had a change in the person responsible for food shopping in the household and 31.3% of those who did not.

There was no statistically significant difference between the change in the responsible person for food shopping, food shopping place and the amount of food waste in households. Food wastage decreased in 33.3% of the participants whose shopping frequency increased and 40.0% of the participants whose shopping frequency decreased. The difference between the change in shopping frequency and food waste was statistically significant ($p<0.001$).

A statistically significant difference was observed between the change in the characteristics of foods purchased and the change in the amount of food waste ($p<0.05$). There was a decrease in the amount of food waste in 46.9% of the households who stated a change in the characteristics of the food they bought, and 27.2% of those who stated that there was no change.

53.1% of those who stated that there was a change in the person preparing/cooking food at home during the outbreak, and 31.7% of those who stated that there was no change, the change in the amount of food waste generated in their household was in the direction of a decrease. It was found that the relationship between the change in the person responsible for preparing food in households and the change in the amount of food waste was statistically significant ($p=0.002$). 41.0% of those who have increased the frequency of preparing/cooking at home and 16.7% of those who have decreased stated a decrease in the amount of food waste at home. Considering the change in the time individuals spend in the kitchen to prepare meals and the change in the amount of food waste in households; 41.0% of those who stated that the time they spent in the kitchen increased, and 20.9% of those who did not change in terms of time stated that the amount of food waste in their homes decreased. The difference between the frequency of preparing/cooking at home, the change in time spent preparing/cooking food, and the change in the amount of food waste that occurs in households is statistically significant ($p<0.05$).

During the pandemic period, it was determined that 42.2% of respondents who had an increase in the variety of food preparing/cooking in their household, 39.9% of those who tried new recipes, and 39.1% of those who started making their bread at home had a decrease in the amount of food waste. It

was determined that there was a significant difference between the variety of food preparing/cooking, the changes related to trying new recipes and making own bread, and the change in the amount of food waste generated in households ($p<0.05$). It was found that 40.2% of the participants whose frequency of ordering food at home decreased, and 36.5% of those whose frequency of eating out decreased had a decrease in the amount of food waste generated at their homes. The difference between the frequency of ordering food to home and consuming food outside and the change in the amount of food waste at home are statistically significant ($p<0.05$).

Discussion

The Covid-19 pandemic has led to many changes in people's daily lives around the world. This study aimed to examine the changes in food-related behaviour in households during the Covid-19 pandemic in Turkey and the effect of these changes on the food waste.

In the household of 63.6% of the study participants, between 0-250 g of preventable food waste was generated in the last one week, and the most discarded raw and cooked food products are vegetables and cereal/legume meals. As the main causes of food waste, it has been found that, mould, deterioration, etc. and poor shopping-meal planning. When the food wastes generated in households during the outbreak are examined, 33.9% of respondents said it had "decreased, "and 7.0% said it had" increased." According to the participants, the main reason for the decrease in the amount of waste is the consumption of food without forgetting/spoiling due to the increase in time spent at home. Also, the increase in food waste was more food preparing/cooking (72.1%) and more food purchasing (27.9%). This finding suggests that the increase in food waste, even if only for a small part of the participants in our sample, is due to panic purchasing, which pushes them to buy more food than they need. 32.3% of the participants attempted to reduce food waste in their households, and their main reduction initiative (40.6%) is to do better meal planning (Table 3). Muştu et al. (2020) conducted a study in Istanbul province, it was determined that the most wasteful foods during the quarantine process are vegetables and fruits, and the most important cause of waste is food degradation. Also, in the study carried out by Güneysu (2020) in Istanbul, it was observed that food wastes were mostly in vegetables/fruits, and food wastes decreased by 26.0% compared to before the quarantine. In the study conducted in Tunisia (Jribi et al., 2020), approximately 58.0% of the participants stated that they usually discarded small amounts of food, and 29.7% did not throw away any food. It has been determined that the most wasted foods in households were bakery products and vegetables, and the main reasons why food is thrown away were overcooking, inappropriate storage, and overbuying. In the same study, the main measures applied to reduce food waste were found good food shopping management, leftovers, and attention to expiration dates. Principato et al. (2020) in Italy stated that food waste in households decreased significantly during restrictions compared to the pre-Covid-19 period and that the decrease in food waste in all food categories was found especially in bread. It has also been found that using leftovers for new recipes both in the pre-Covid-19 period and during restrictions has a strong association with reduced food waste. In the study conducted by Pappalardo et al. (2020) in Italy, 33.0% of the participants stated that food waste decreased significantly and 5.5% increased. The reasons for the decrease in waste were changes in eating and cooking habits, having more time to prepare and cook meals, and financial constraints. Also, the food waste amount has been associated increased food stock, increased in the amount of food purchased, and the amount of food cooked.In another study conducted in Italy, it was concluded that more than half of the participants (53.7%) reduced food waste in their households (Scacchi et al., 2021). In a study that included participants from the U.S. and Italy, approximately half (49.0%) of respondents reported a decrease in food waste during the Covid-19 pandemic than before the pandemic (Rodgers et al., 2021). When the studies carried out in different countries or cultures are generally evaluated, it is seen that the lockdowns experienced during the pandemic have a positive effect on the reduction of food waste generated in households. It is believed that the main reasons for this positive effect were exhibit more conscious behaviour due to the fear of not being able to access the food created by the pandemic on people and improvements in food management behaviour due to the increase in time spent in households.

Food shopping behaviour is considered one of the foremost effective factors in reducing food waste (Aschemann-Witzel et al., 2015; Farr-Wharton et al., 2014; UNEP, 2014). Giordano et al. (2019) found that increasing the frequency of shopping had a negative effect on the amount of food waste in households. In this study, it was determined that the rate of stating that the amount of food waste decreased was higher among those whose shopping frequency decreased during the pandemic compared to the other groups ($p<0.05$) (Table 4). The decrease in shopping frequency maybe since purchases are made one time and one place due to health concerns. In addition to shopping frequency, 23.4% of individuals changed their shopping places due to the pandemic. Nearly half (49.7) of those who experienced this change stated that they started online shopping (Table 2). Principato et al. (2020) also found a 3-fold increase in online shoppers during the pandemic restrictions compared to before the Covid-19 pandemic. Consumers chose to online shopping, possibly out of fear of infection, wanting to avoid densely populated areas due to long queues in shopping places such as supermarkets. Although the difference between the change in the place for shopping and the change in the amount of food waste is not statistically significant in this study that we have done, this result may change with the development of infrastructures that allow more individuals to online shopping.

The Covid-19 outbreak has caused changes in the characteristics of the food consumers to buy. Scacchi et al. (2021) has found a decrease in the purchase of fresh products that can deteriorate quickly, characterized by short shelf life. Our study determined that 34.3% of the participants had a change in the characteristics of the foods they purchased. This change was mainly in the direction of packaged products and long-lasting/durable product preferences. The effect of the change in the characteristics of the purchased food on the

amounts of food waste is statistically significant (Table 4) ($p<0.05$). Participant's preference for packaged products may be due to the possibility that they think unpacked products are more easily infected with the virus. In addition, packaged products are also products with a long shelf life. Also, participants may have intended to shop less, preferring durable products. All these reasons may have led to the purchase of durable products and decrease in discarded food products caused by deterioration.

53.1% of households with a change in the person responsible for preparing/cooking meals, 41.1% of those who increased the frequency of preparing/cooking at home, 41.0% of those who increased spent time in the kitchen preparing/cooking meals, 42.2% of those who increased the number of meals they prepared/cooked, 40.2% of those who decreased ordered food from outside to home and 36.5% of those who decreased the frequency of eating out stated decreased in food waste amounts (Table 4). Muştu et al. (2020) found that ready-made food consumption decreased during the quarantine process and that individuals usually make their meals at home. Principato et al. (2020) determined that as the number of meals consumed at home increased during the restriction period, and the amount of food waste decreased. Studies conducted before the pandemic have also shown that cooking at home is associated with fewer amounts of food waste (Marangon et al., 2014; Roodhuyzen et al., 2017). Due to the pandemic, with the increase of time spent in the homes and the restriction of eating out, individuals tended to cook and consume more at home. This situation has also increased the timely use of purchasing food products. It is believed that individual's tendency to prepare/cook their food contributes to reducing food waste positively affecting their awareness of food purchasing, preparing, and cooking.

It was observed that there was an increase in searches for recipes on Google during the pandemic period (Laguna et al., 2020). 64.1% of respondents said they had tried new recipes in the process (Table 2). 49.9% of those who tried new recipes and 23.3% of those who did not try any recipe stated a decrease in the amount of food waste (Table 4). New recipe attempts may cause food to be discarded unnecessarily if there are deficiencies in people's cooking ability (Porpino et al., 2015). In this study, the higher percentage of those who said there was a decrease in food waste among those who tried new recipes may be due to the good ability of individuals to cook. Also, food waste may have decreased, as the consumption of the meals is increased by providing variety in meals with new recipes and avoiding monotony.

Bread, one of Turkey's main food products and has a very important place in meeting daily nutritional needs, is also considered the main reason why the amount of solid waste increases over the years. 4.9 million pieces of bread are wasted daily (1.7 billion pieces per year) in Turkey (Kılınç Şahin & Bekar., 2018). Almost half (49.8%) of respondents started making their bread at home during the pandemic period (Table 2), and 39.1% of those who made their bread at home had decreased bread waste amounts (Table 3). It is highly gratifying that bread, which is important in society's nutrition and has a large amount of waste, has reduced the amount of waste. This reduction may be due to individuals baking their bread affecting their awareness of food waste.

Conclusion

Our results revealed a sudden and profound change in food-related (buying, preparing-cooking, etc.) behaviours in people in our country as well as in other countries around the world. These changes have brought along positive effects on food waste. Although food waste has always been a sensitive issue in our society, the desired level of food waste reduction has not yet been achieved. Although the Covid-19 pandemic has caused many crises in societies, it may have provided a good opportunity for sustainable food production and consumption. In order to maintain the positive behaviours that occur during the pandemic, to raise awareness about the consequences of food waste and the benefits of prevention, it is necessary to increase education and communication campaigns for the community by policymakers and practitioners.

As a result, our study has contributed to a better understanding of how such a crisis affects consumer's food-related behaviour and has raised food waste awareness during the crisis. The mechanisms underlying our findings can be fully clarified by the qualitative studies to be carried out to continue this study. At the end of the qualitative studies, various sustainable recommendations to address food waste can be developed using detailed information obtained from consumers.

Compliance with Ethical Standards

Conflict of interest

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

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

Necessary approval was obtained from the Ankara University Ethics Committee. (Decision No: 199, Protocol No: 2020/56673, Date: 24.07.2020).

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.3.22 www.jaefs.com

Research Article Int J Agric Environ Food Sci 5 (3):425-432 (2021)

Screening of Acidophilic Actinobacteria That Show Activity against Paddy Pest Fungi

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Abstract

This study aimed to isolate and identify acidophilic actinobacteria. Acidophilic actinobacteria isolates were had from a paddy field soil in Osmancık placed near Çorum province in Turkey. The dilution plate technique on seven selective media with pH 5.5 was used for isolation. 16S rRNA gene PCR amplification of acidophilic actinobacteria was performed. Three different algorithms were used in the phylogenetic analyzes made with MEGA 7.0 software. Twenty-two isolates were obtained from seven selective media, and according to 16S rRNA gene sequence analysis of 22 isolates, twenty-one *Streptomyces* isolates and one *Rhodococcus* isolate were identified. The antifungal activities of isolated acidophilic actinobacteria against *Fusarium moniliforme* and *Rhizoctonia solani*, the rice pathogenic fungi were evaluated. The isolates with antifungal activity have the potential to be used as biological control agents against rice pathogens.

Keywords: Genomic DNA isolation, 16S rRNA gene, Acidophilic actinobacteria, Paddy field

Introduction

Actinobacteria, which are in the group of gram positive bacteria, have high GC content. They are widely distributed in the soil and other different environments such as marine sediments (Veyisoglu and Sahin, 2015; Veyisoglu et al., 2016; Veyisoglu et al., 2020). It is the most economical and biologically valuable group of bacteria among prokaryotes and actinobacteria synthesize different biologically active compounds such as antitumor agents, enzymes and, antibiotics (Sanglier et al., 1996; Lazzarini et al., 2000; Procópio et al., 2012).

Acidophilic actinobacteria are divided into two main groups as neutrotolerant acidophils and strict acidophils. Typical neutrotolerant acidophils (optimum growth between pH 5.0 and 5.5) grow in environments between pH 4.5-7.5. Members of the strictly acidophilic group typically grow in environments between pH 3.5 and 6.5 and provide optimum growth at pH 4.5 (Williams et al., 1971; Xu et al., 2006; Poomthongdee et al., 2015).

Rice is a staple product that meets the needs of about half of the people living in the world. However, in rice cultivation, fungal rice diseases create significant problems (Ou, 1987). The use of chemical synthetic substances for prevention and treatment is considered an effective method, but these chemicals have harmful effects on the environment and human health (Tsukano et al., 1986; Pingali et al., 1995).

Actinobacteria are recognized as potential biocontrol agents against different phytopathogenic fungi due to the bioactive metabolites synthesis or production of enzymes that hydrolyze fungal cell walls (Basilio et al., 2003; Li et al., 2011; Patil et al., 2011; Yuan and Crawford, 1995; Gomes et al., 2000; El-Tarabily and Sivasithamparam, 2006; Xue, 2013). Numerous studies have focused on the capability of actinobacteria isolated from isolation media with neutral pH. Nevertheless, It has been reported that acidophilic actinobacteria inhibit fungi under acidic conditions more than neutrophilic actinobacteria

Cite this article as:
Veysioglu, A., Tatar, D. (2021). Screening of Acidophilic Actinobacteria That Show Activity against Paddy Pest Fungi. J. Agric. Environ. Food Sci., 5(3), 425-432.
Doi: https://doi.org/10.31015/jaefs.2021.3.22
Orcid: Aysel Veyisoglu: 0000-0002-1406-5513 and Demet Tatar: 0000-0002-9317-3263
Received: 28 June 2021 **Accepted:** 10 August 2021 **Published Online:** 25 September 2021 **Revised:** 25 September 2021
Year: 2021 **Volume:** 5 **Issue:** 3 (September) **Pages:** 425-432
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

(Zakalyukina and Zenova, 2007).

According to our screenings, there is no study on acidophilic actinobacteria isolation, molecular analysis based on 16S rRNA gene region and antifungal activity from paddy fields in Osmancık district of Çorum province in Turkey. The aim of this study is to isolate acidophilic actinobacteria from paddy fields in Osmancık district of Çorum province in Turkey, to determine the species to which the isolates belong by performing 16S rRNA sequence analysis, to make phylogenetic dendograms of the isolates according to the result of 16S rRNA sequence analysis, to identify candidate isolates to become new species and to determine antifungal activities of isolates.

Materials and Methods

Soil Sample Collection

Acidophilic actinobacteria isolates were isolated from soil samples from a paddy field (head of the field) (40°58'42.4"N 34°47'16.2"E), (middle of the field) (40°58'42.4"N 34°47'15.5"E) and (the field end) (40°58'40.1"N 34°47'14.4"E) in Osmancık located near Çorum province using a dilution plate on seven selective media with pH 5.5 (Table 1). Media used were Humic acid Vitamin Agar (Hayakawa and Nonomura., 1987), Starch-Casein Agar (Küster and Williams, 1964), Gause no. 1 Agar (Gauze et al., 1957), NZ-Amine agar-DSMZ medium 554 (Atlas, 2010), SM3 Agar (Tan et al., 2006), Nocardia Agar (Sanglier et al., 1992) and M1 Agar (Mincer et al., 2002). Soil samples were taken from a depth of 20–30 cm.

Table 1. List of selective media used.

	Name of media	Antibiotics	References
1	Humic Acid-Vitamin Agar (pH 5.5)	Nystatin (50 µg/ml) Nalidixic acid (25 µg/ml)	(Hayakawa and Nonomura, 1987)
2	Starch-Casein Agar (pH 5.5)	Nystatin (50 µg/ml) Nalidixic acid (25 µg/ml)	(Küster and Williams, 1964)
3	Gause no. 1 Agar (pH 5.5)	Nystatin (50 µg/ml) Nalidixic acid (25 µg/ml)	(Gauze et al., 1957)
4	NZ-Amine Agar- DSMZ medium 554 (pH 5.5)	Nystatin (50 µg/ml) Nalidixic acid (25 µg/ml)	(Atlas, 2010)
5	SM3 Agar	Nystatin (50 µg/ml) Rifampicin (5 µg/ml)	(Tan et al., 2006)
6	Nocardia Agar	Cycloheximide (50 µg/ml) Nystatin (50 µg/ml)	(Sanglier et al., 1992)
7	M1 Agar	Cycloheximide (50 µg/ml) Nystatin (50 µg/ml), Rifampicin (5 µg/ml)	(Mincer et al., 2002)

Acidophilic actinobacteria were isolated from four selective media with pH 5.5. The organisms were maintained on related agar slopes added cycloheximide (50 µg mL^{-1}), and stocked in glycerol (25%, v/v) at -20 °C.

Isolation of Actinobacteria

After adding 1 g of soil sample to 9 ml of Ringer's solution, it was mixed at room temperature for homogenization. Then this 10^{-1} dilution was kept for 30 min at 55 °C in a preheated water bath. Serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) were spread over the surface of related agar plates, and the plates were incubated at 28 °C for 10–14 days. Actinobacteria were subcultured onto related media and incubated for up to 4 weeks at 28 °C. Suspensions of spores and mycelia were maintained in 25% glycerol (w/v) at −20 °C.

Genomic DNA Extraction

For molecular identification and phylogenetic analysis, the genomic DNA of test organisms was isolated by using Purelink Invitrogen genomic DNA isolation kit.

Amplification and Detection of 16S rRNA Gene Sequence

PCR mixture (50 µl) included chromosomal DNA (50–300 ng), primers (20 µM), Taq polymerase (2.5 U, HotStarTaq®), Taq polymerase buffer (HotStarTaq®) and deoxynucleoside triphosphates mixture (Promega) (25 µM). The 16S rRNA genes were amplified by using specific primers 27F and 1525R. The PCR conditions were initial denaturation at 95 °C (5 min), 35 cycles at 95 °C (1 min), 55 °C (2 min), and 72 °C (3 min), and a final extension at 72 °C (10 min). Then the PCR products were separated using electrophoresis in 1% agarose gel (Merck) and were imaged with the Gene Genius Bioimaging system.

Sequencing of PCR Products

The PCR products of the 22 isolates were purified with

QIAquick purification kit (Qiagen). According to Chun and Goodfellow (1995) PCR-mediated amplification and sequencing of the 16S rRNA gene were performed as described by using an ABI PRISM 3730 XL automatic sequencer with previously mentioned oligonucleotide primers (Table 2). Chromatogram files in ABI format are turned to FASTA format using Chromas 1.7.5. An almost complete 16S rRNA gene sequences of the 48 isolates were compared to sequences of type strains in GenBank (Boratyn et al., 2013) and EzBioCloud (Yoon et al., 2017) databases.

Table 2. List of oligonucleotide primers used for 16S rRNA PCR amplification and sequencing.

Primer Code	Sequences (5'-3')	Base Length	References
27F	AGAGTTTGATC**M**TGGCTCAG	20	(Lane, 1991)
518F	CCAGCAGCCGCGGTAAT	17	(Buchholz-Cleven et al., 1997)
800R	TACCAGGGTATCTAATCC	18	(Chun and Goodfellow, 1995)
MG5F	AAACTCAAAGGAATTGACGG	20	(Chun and Goodfellow, 1995)
MG6F	GACGTCAAGTCATCATGCC	19	(Chun and Goodfellow, 1995)
1525R	AAGGAGGTG**W**TCCA**R**CC	17	(Lane, 1991)

Degeneracies according to Lane (1991) M = A:C; R = A:G; W = A:T.

Phylogenetic Analysis

The determination of phylogenetic neighbors and computation of pairwise 16S rRNA gene sequence similarity were obtained using the Ezbiocloud server (https://www.ezbiocloud.net)(Yoon et al., 2017). Multiple alignments with sequences from closely related species were applied with the program CLUSTAL W in MEGA 7.0 (Kumar et al., 2016). Phylogenetic trees were formed with the neighbor-joining (Saitou and Nei, 1987) maximum likelihood (Felsenstein, 1981) and maximum parsimony (Kluge and Farris, 1969) algorithms in MEGA 7.0 (Kumar et al., 2016). Evolutionary distances were calculated using the Kimura two-parameter (Kimura, 1980) and topologies of the resultant trees evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The 16S rRNA gene sequences obtained in this study were deposited in GenBank (Table 3).

In Vitro Antifungal Activity

Twenty-two acidophilic actinobacteria isolates were examined for their ability to inhibit the growth of two pathogens rice pests, including *Fusarium moniliforme* and *Rhizoctonia solani.*

The ability to inhibit the growth of twenty-four acidophilic actinobacteria isolates was observed using an overlay technique (Williams et al., 1983). For each isolate, 2 ml Ringer's solution was added to small bottles with lids and sterilized by autoclaving at 121 ºC for 15 minutes. The spores and substrate micelles of the isolates grown at 28 ºC in ISP 2 agar medium were transferred to small glass bottles containing Ringer's solution in aseptic conditions. Spot-inoculated colonies on modified Bennett's Agar (Jones, 1949) surface were inverted over 1-5 ml chloroform for 40 min. Killed colonies were then overlaid with 5 ml sloppy agar (0.7 %, w/v, nutrient agar) inoculated with the pathogen test organisms. Zones of inhibition were measured after 48 h at 30 ºC.

Results and Discussion

A total of 22 morphologically distinct actinobacterial isolates were obtained from a paddy field soil in Osmancık. Seven different selective isolation media were used. Seven strains were isolated on Starch-Casein agar, seven strains from Gause no. 1 agar, three strains from M1 agar, two strains from Humic Acid-vitamin (HV) agar, two strains from SM3 agar, one strain from Nocardia agar and incubated at 28°C for about 10-14 days (Fig 1 and Table 3).

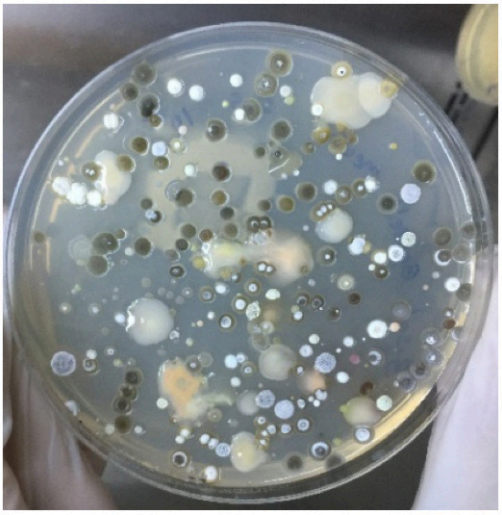

Figure 1. Isolation petri sample - Gause no. 1 agar.

16S rRNA gene sequences of all 22 isolates were amplified using universal primers (Table 2). Most of the strains belonged to the genus *Streptomyce*s (21 isolates). Other one strain belonged to the genus *Rhodococcus* (1 isolate) (Table 3).

Table 3. Nucleotide similarity of *Actinobacteria* isolates according to 16S rRNA sequence analysis.

Number	Strain	Isolation medium	Genbank Number	Highest match	Similarity (%)-Nucleotide difference
1	PT503	SM3 Agar	MZ025943	*Streptomyces bobili* NRRL B-1338^T	99.72 % - 4/1448
2	PT510	Gause no. 1 Agar	MZ026800	*Streptomyces scabiei* NRRL B-16523^T	99.86 % - 2/1448
3	PT511	Starch-Casein Agar	MZ026801	*Streptomyces bobili* NRRL B-1338^T	99.72 % - 4/1448
4	PT513	Starch-Casein Agar	MZ026802	*Streptomyces abietis* A191^T	98.34 % - 24/1444
5	PT517	Nocardia Agar	MZ026846	*Rhodococcus wratislaviensis* NBRC 100605^T	99.79 % - 3/1441
6	PT539	Humic Acid-Vitamin Agar	MZ027070	*Streptomyces bobili* NRRL B-1338^T	99.38 % - 9/1448
7	PT542	Humic Acid-Vitamin Agar	MZ027080	*Streptomyces clavifer* NRRL B-2557^T	100 % - 0/1448
8	PT557	Gause no. 1 Agar	MZ027591	*Streptomyces clavifer* NRRL B-2557^T	100 % - 0/1448
9	PT559	Gause no. 1 Agar	MZ027347	*Streptomyces fulvissimus* DSM 40593^T	99.93 % - 1/1448
10	PT564	Gause no. 1 Agar	MZ027400	*Streptomyces cyslabdanicu*s K04-0144^T	99.24 % - 11/1448
11	PT566	Gause no. 1 Agar	MZ027592	*Streptomyces fulvissimus* DSM 40593^T	99.93 % - 1/1448
12	PT572	SM3 Agar	MZ027483	*Streptomyces bobili* NRRL B-1338^T	99.72 % - 4/1448
13	PT573	M1 Agar	MZ027489	*Streptomyces bobili* NRRL B-1338^T	99.79 % - 3/1448
14	PT575	M1 Agar	MZ027494	*Streptomyces rochei* NRRL B-2410^T	100 % - 0/1448
15	PT579	M1 Agar	MZ027496	*Streptomyces caeruleatus* NRRL B-24802^T	97.51 % - 36/1448
16	PT597	Starch-Casein Agar	MZ031923	*Streptomyces aurantiogriseus* NBRC 12842^T	98.55 % - 21/1447
17	PT598	Gause no. 1 Agar	MZ031924	*Streptomyces virginiae* NRRL ISP-5094^T	100 % - 0/1446
18	PT599	Starch-Casein Agar	MZ061921	*Streptomyces fulvissimus* DSM 40593^T	99.93 % - 1/1448
19	PT600	Gause no. 1 Agar	MZ040132	*Streptomyces bobili* NRRL B-1338^T	99.72 % - 4/1448
20	PT605	Starch-Casein Agar	MZ040598	*Streptomyces bobili* NRRL B-1338^T	99.72 % - 4/1448
21	PT609	Starch-Casein Agar	MZ040488	*Streptomyces paradoxus* NBRC 14887^T	99.38 % - 9/1447
22	PT613	Starch-Casein Agar	MZ040755	*Streptomyces rochei* NRRL B-2410^T	100 % - 0/1448

In a study conducted in Thailand, acidophilic actinobacteria were isolated from the rhizosphere soil of rice plant, and the antifungal activity of these isolates was examined (Poomthongdee et al., 2015).

In another study conducted in Turkey, the effect of light intensity on the nitrogenase activities of cyanobacteria was investigated after the isolation of cyanobacteria was carried out by taking samples of irrigated soil from the regions where paddy cultivation was carried out in Osmancık district of Çorum province (Ökmen and Dönmez, 2007**)**.

Based on 16S rRNA gene sequence analysis, 21 of 22 isolates are members of the genus *Streptomyces*. Members of the genus *Streptomyces* are dominant in the paddy field located in Osmancık (Fig. 2). Based on 16S rRNA gene sequence analysis, 21 *Streptomyces* isolates were determined. Actinobacteria commonly found in acidic habitats belong to the genus *Streptomyces* (Zenova et al., 2011).

According to the neighbor-joining algorithm, the phylogenetic tree indicated that twenty-one strains were members of the genus *Streptomyces* (Fig. 2; Supp. Figs. S1 and S2). Based on the 16S rRNA gene sequence analysis, 21 *Streptomyces* isolates showed that close 16S rRNA gene sequence similarity with the type strain of *Streptomyces* which are 100% and 97.51%. Strains PT513, PT579 and PT597 may be new species belong to the genus *Streptomyces*. Strain PT513 had the closest 16S rRNA gene sequence similarity with *Streptomyces abietis* A191^T (98.34%). PT579 indicated the closest 16S rRNA gene sequence similarity with *Streptomyces caeruleatus* NRRL B-24802^T (97.51%), and Strain PT597 had the closest 16S rRNA gene sequence similarity with *Streptomyces aurantiogriseus* NBRC 12842^T (98.55%) (Table 3). Isolates with a 16S rRNA similarity rate below 98.65% have the possibility of being a new species (Stackebrandt and Ebers, 2006; Kim et al., 2014; Chun et al., 2018).

Based on the neighbor-joining algorithm, the phylogenetic tree indicated that one strain was member of the genus *Rhodococcus* (Fig. 2; Supp. Figs. S1 and S2). PT517 indicated the closest 16S rRNA gene sequence similarity with *Rhodococcus wratislaviensis* NBRC 100605^T (99.79%) (Table 3).

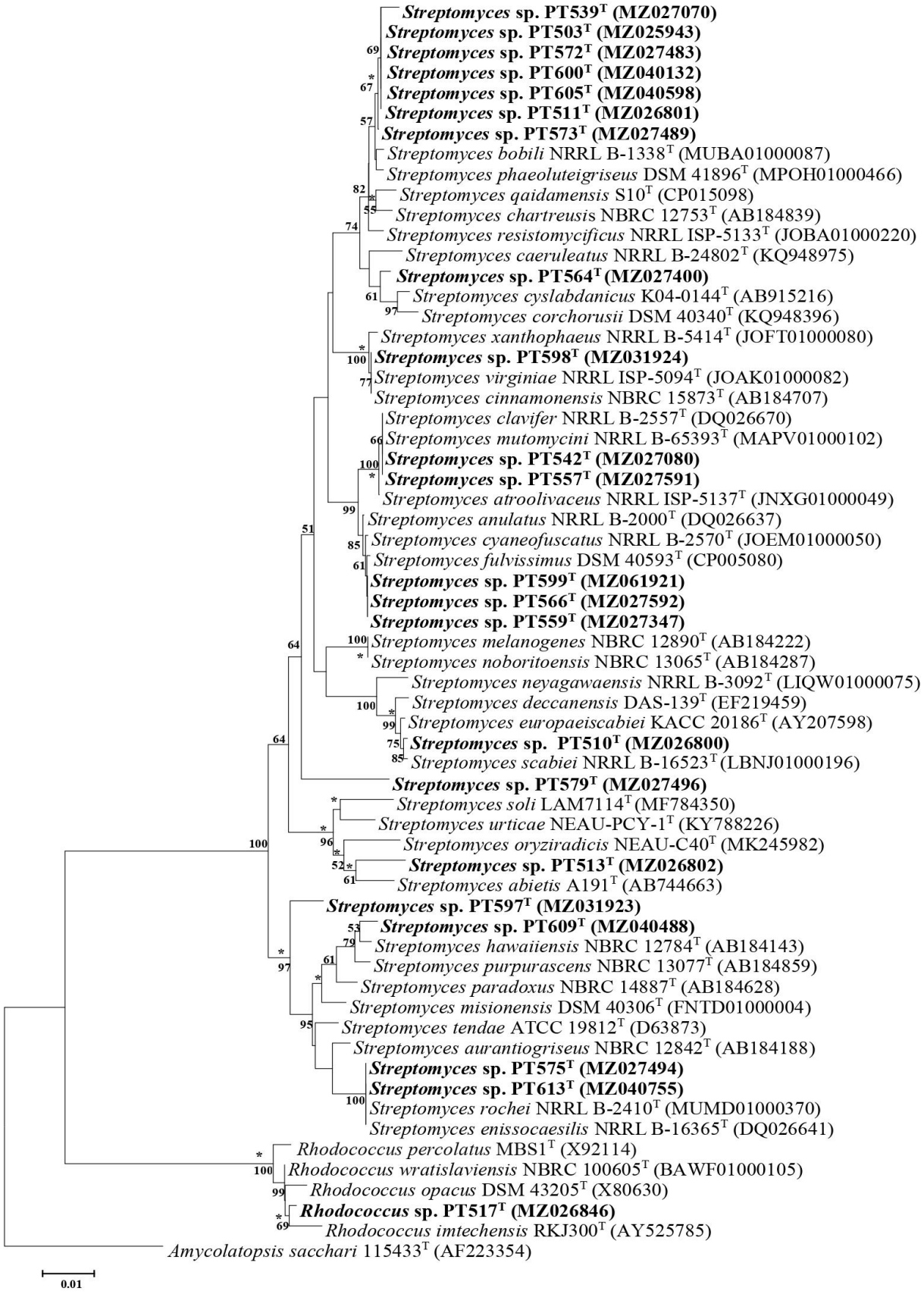

Figure 2. Neighbor-joining tree (Saitou and Nei, 1987) according to 16S rRNA gene sequences of test isolates

The antifungal activity of twenty-two acidophilic actinobacteria isolates was determined against rice pest two fungi, and their inhibition zone diameters were measured. While of the isolates 27.27 % showed antifungal activity against the rice pathogen *F. moniliforme*, 100% did not show antifungal activity against *Rhizoctonia solani*. Six acidophilic actinobacteria test isolates were found to show good antifungal activity against *F. moniliforme* fungus. PT510 coded isolate formed 38 mm, PT559 coded isolate 24 mm, PT566 coded isolate 40 mm, PT575 coded isolate 28 mm, PT599 coded isolate 14 mm and PT613 coded isolate 20 mm inhibition zone diameter. Measured zone diameters are given in Figure 3.

Poomthongdee et al. (2015) 351 acidophilic actinobacteria were isolated from 21 rhizospheric soils, and 57.8% of these actinobacteria showed antifungal effect against *Fusarium moliniforme*, 32.5% *Helminthosporium oryzae* and 50% *Rhizoctonia solani*. While 25.9% of the isolates showed activity against all pathogenic fungi tested, more than 68.1% showed activity against at least one pathogenic fungus.

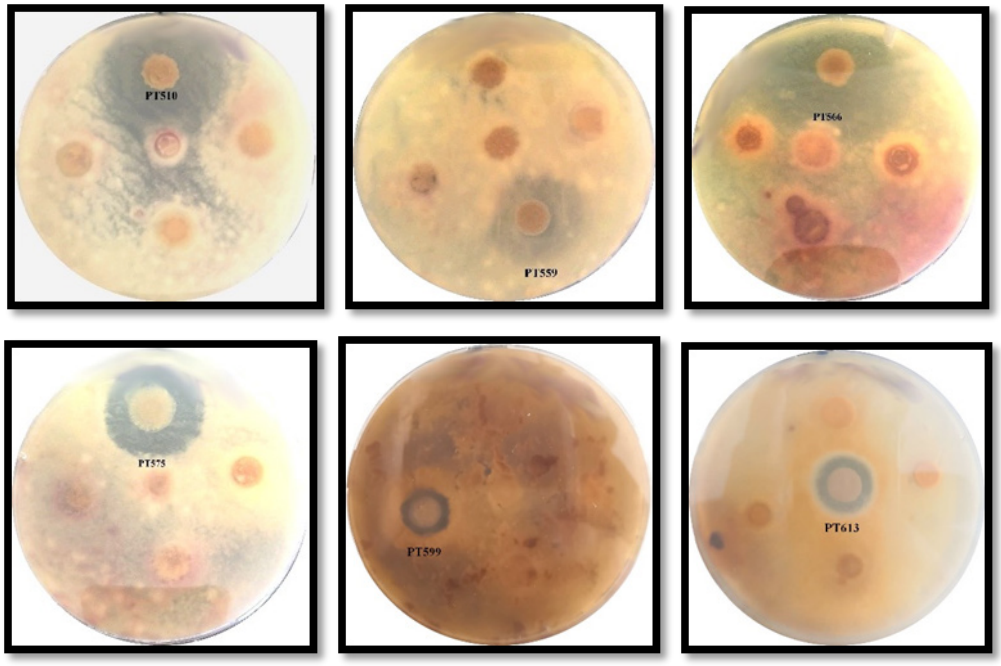

Figure 3. Zones of acidophilic actinobacteria isolates formed by against the rice pathogen *Fusarium moniliforme*

Conclusion

Consequently, isolation and phylogenetic analysis of acidophilic actinobacteria living in a paddy field soil in Osmancık were performed.

In future studies, it is possible to introduce *Streptomyces* sp. PT513, *Streptomyces* sp. PT579 and *Streptomyces* sp. PT597 isolates obtained in this study as a new species in the literature by making necessary analyzes.

Fungi cause important problems in rice cultivation. Although the use of chemical substances against fungi seems to be effective, it can be harmful to human and environmental health. Actinobacteria have the potential to be used as biocontrol agents against a variety of phytopathogenic fungi. In this study, six acidophilic actinobacteria test isolates were found to show good antifungal activity against *F. moniliforme* fungus. Isolates showing activity in this study can be used in biological control. It is more beneficial for the environment and people than chemicals.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

The contribution of the authors to the present study is equal.

Ethical approval

Not applicable.

Funding

Hitit university financed this study (ODMYO19001.20.001).

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

I would like to thank Prof. Dr Nevzat Şahin for supporting the study. The authors are grateful to Prof. Dr. Berna Tunalı for her assistance in the acquisition of fungal pathogens.

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