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Research Article

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Antioxidant and antibacterial activities of essential oils and aromatic waters of some plants grown in the highlands

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Abstract

The essential oils from twenty-two plants grown in the highlands were isolated by hydrodistillation. The plants with an essential oil yield above one percent, including *Achillea millefolium, Asparagus plumosus*, *Matricaria chamomilla*, *Mentha piperita*, *Mentha pulegium*, and *Thymus vulgaris,* were tested for the antibacterial activity and total antioxidant capacity. Their antibacterial activities against the three most common foodborne pathogens and an opportunistic pathogen were evaluated. Results indicated that *T. vulgaris* essential oil had the highest total antioxidant capacity with 11.78 ± 0.01 mmol/L TEAC. The essential oils of plants inhibited the growth of pathogen bacteria tested, while their aromatic waters showed no inhibition. *T. vulgaris* oil was the most powerful antibacterial essential oil with the inhibition zones of 49.27±7.26 mm against *S. aureus,* 44.13±4.16 mm against *L. monocytogenes,* 39.55 ± 0.52 mm against *E. coli,* and 38.09 ± 4.15 mm against *M. luteus*. Furthermore, the volatile compounds of *T. vulgaris* essential oil were detected using GC-MS. Thymol, carvacrol, caryophyllene, 1,8-cineole, 2 acetyl-4,5-dimethylphenol, and γ-terpinene were determined as major compounds in *T. vulgaris* essential oil. The obtained results suggest that the essential oils of tested plants with high antimicrobial activity and antioxidant capacity might be used as natural antioxidants and antimicrobial agents.

Keywords: Antibacterial activity, Antioxidant capacity, Foodborne pathogen, *Thymus vulgaris,* Essential oil

Introduction

Antibiotic resistance, one of the largest hazards to global health and food security, is accelerated by the misuse and overuse of antibiotics. Development of new antibiotics and use of alternatives to antibiotics are the measures taken to control and prevent the spread of antibiotic resistance (WHO, 2018). The use of essential oils as antibiotics is safer for the people and the environment because of their natural origin and they play an important role in the fight against antibiotic resistance (Daferera et al., 2003). Essential oils containing natural compounds are used in folk medicine for their beneficial effects on the health. They have not only antimicrobial effects against several microbial pathogens, but also antioxidant activity. For example, the bactericidal (against *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Enterococcus feacalis, Vibrio alginolyticus*), fungicidal (against *Verticillium fungicola* and *Trichoderma harzianum*), and antioxidant effects of thyme (*Thymus vulgaris)* essential oil have been reported (Miladi et al., 2013; Oussalah et al., 2007; Soković et al., 2010). Roby et al. (2013) evaluated the antimicrobial effect of chamomile (*Matricaria chamomilla* L.) essential oil on some pathogens (*E. coli, S. tyhpimurium, B. cereus, S. aureus, Candida albicans* and *Aspergillus flavus)* and found that chamomile oil inhibits the growth of tested pathogens. Essential oils of yarrow (*Achillea millefolium),* a plant belonging to the family *Asteraceae,* was tested for its antibacterial effect

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and it was found that its essential oil inhibited the growth of some pathogenic bacteria, including *B. cereus, Bacillus subtilus*, *S. aureus, S. typhimurium,* and *Salmonella agona* (El-Kalamouni, et al., 2017). Similarly, it has been shown that the peppermint oils (*Mentha* spp.; *M. piperita* and *M. pulegium)* exhibited antimicrobial effect against *E. coli*, *S. aureus*, *P. aeruginosa*, *Proteus* v*ulgaris*, *Enterobacter aerogenes*, *S. typhimurium*, *C. albicans, Klebsiella pneumoniae, Yersinia enterocolitica*, *L. monocytogenes*, *B. cereus*, *S. epidermidis*, *Xanthomonas campestris* pv. *phaseoli, Pseudomonas syringae* pv. *phaseolicola*, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas tolaasii* var. *tolaasii*, *Xanthomonas campestris* pv. *campestris, Verticillium fungicola var. fungicola,* and *T. harzianum* (Soković and van Griensven, 2006; İşçan et al. 2002). Moreover, Madikizela et al. (2014) reported the antibacterial effect of two *Asparagus* spp. (*A. africanus* and *A. falcatus*) on *Mycobacterium tuberculosis* and *Salmonella typhimurium.*

In the present study, the essential oils from twenty-two plants, which are grown in the highlands in Turkey and traditionally consumed due to their health benefits, were obtained. Among these, the plants containing over one percent essential oil (*Achillea millefolium, Asparagus plumosus*, *Matricaria chamomilla*, *Mentha piperita*, *Mentha pulegium*, and *Thymus vulgaris)* were tested for total antioxidant capacity and antibacterial effect on three widespread foodborne virulent pathogens, *S. aureus, E. coli*, and *L. monocytogenes,* and a low-virulence pathogen *M. luteus*.

Materials and methods

Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), Plate Count Agar and Mueller Hinton Agar were purchased from Sigma-Aldrich (St. Louis, USA). Tryptic Soy Broth, n-butanol, and methanol were obtained from Merck (Darmstadt, Germany).

Plant material and isolation of essential oils

Cynara *syriaca, Sambucus ebulus, Psoralea bituminosa* L., *Sedum pallidum* M. Bieb., *Alchemilla mollis, Trifolium repens, Verbascum blattaria* L.*, Phedimus stoloniferus, Echium vulgare, Hypericum perforatum, Prunella vulgaris* L., *Polygonum affine, Urtica dioica, Inula helenium, Rhododendron luteum, Vaccinium corymbosum* L.*, Achillea millefolium, Asparagus plumosus*, *Mentha piperita*, *Mentha pulegium, Matricaria chamomilla*, and *Thymus vulgaris* were collected from highlands in Ordu and Giresun provinces, Turkey. The plant species were identified in the departments of biology and plant protection at Ordu University. The essential oils of plants were extracted by the hydrodistillation method according to Soković et al. (2010). After drying at room temperature, the plant samples were coarsely ground for extraction. 50 g of dried plants were taken into a 1 L flask and hydrodistilled using the Clevenger apparatus to extract the essential oils. The obtained essential oil and aromatic water, the residue after isolation of essential oil, were then stored at -18 °C until analyses.

Determination of total antioxidant activity

To determine the antioxidant activity of essential oils and aromatic waters, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed with a slightly modified method described by Shahidi and Liyana-Pathirana (2007). 40 µL of the samples were added to 1500 µL of DPPH solution (0.6 mM DPPH in n-butanol for essential oil samples and 0.1 mM DPPH in methanol for plant aromatic waters) in a microcuvette in which the reaction takes place. The mixture was allowed to stand at room temperature for 30 min. After the incubation, the absorbance of the mixture was measured at 515 nm. Trolox was chosen as the standard. Total antioxidant activity was calculated using standard curves. Results were expressed as millimoles per liter trolox equivalents.

Microbial strains and culture

Staphylococcus aureus NCTC 8530, *Escherichia coli* BL21, *Listeria monocytogenes* ATCC 7644, and *Micrococcus luteus* NCIMB 8166 were gifts of Dr. Ö. F. Çelik (Ordu University, Faculty of Agriculture) and were used as the indicator bacteria to determine antibacterial activity of the essential oils. All bacteria were inoculated from frozen stocks into the Tryptic Soy Broth and incubated at 37°C for cultivation purposes. Preceding the assay, the cultures were passaged in the same medium overnight.

Determination of antibacterial activity

The antibacterial activity of essential oils against *E. coli, L. monocytogenes, M. luteus,* and *S. aureus* was determined using a disc diffusion method according to Soković et al. (2010) with some modifications. Agars of Plate Count (PCA) and Mueller Hinton (MHA) were sterilized by autoclaving and used for the growth of *E. coli* and the remaining bacteria, respectively. Then, the agars were cooled to 50 ºC and 20 mL of each agar were poured into sterile petri dishes. After inoculation with 0.1 mL of the bacterial suspension adjusted to the turbidity of a 0.5 McFarland standard, the petri dishes were allowed to dry for 30 minutes. The blank sterile disc was placed on an inoculated agar plate and 15 µL of essential oil were added to the discs. After incubation at 37 ºC for 24 hours, the diameters of the inhibition zones were measured with a digital caliper and recorded in mm. The antibiotic discs including penicillin (10 µg/disc), streptomycin (300 µg/disc), ampicillin (10 µg/disc) and gentamycin (10 µg/disc) were used as positive controls.

Determination of volatile compounds

The volatile compounds of the essential oil of *Thymus vulgaris* were analyzed according to literature (Sahin et al., 2017) with some modifications by GC/MS (Thermo Fischer Scientific, USA) equipped with a TraceGOLD TG-5MS capillary column (30 m x 0.25 mm; film thickness 0.25 µm). Helium was used as the carrier gas and the flow rate was adjusted as 1.2 mL per minute. The oven temperature was programmed at 80°C for 2 min, then 2°C/min to 270°C. The MS conditions were as follow: ionization voltage, 70 eV; ion source temperature, 250°C; electron ionization mass spectra were acquired over the mass range of 50 to 550 m/z. The identification of the compounds in *Thymus vulgaris* essential oil was based on a comparison of their mass spectra with those obtained from the NIST, WinMain, mainlip, replib, and Wiley

libraries spectra.

Statistical analysis

The results of the analysis were expressed as mean ± standard deviation (SD). Data were statistically analysed using repeated measures two-way ANOVA. The Tukey multiple comparison test was used as follow-up to ANOVA with a significance level of $p < 0.05$. Statistical analysis was performed using Minitab 18.1 (Minitab Software, Coventry, UK).

Results and Discussion

Yield of essential oils

The essential oil yields of *Cynara syriaca, Sambucus ebulus, Psoralea bituminosa* L., *Sedum pallidum* M. Bieb., *Alchemilla mollis, Trifolium repens, Verbascum blattaria* L., *Phedimus stoloniferus, Echium vulgare, Hypericum perforatum, Prunella vulgaris* L., *Polygonum affine, Urtica dioica, Inula helenium, Rhododendron luteum,* and *Vaccinium corymbosum* L. were less than one percent, while *Achillea millefolium, Asparagus plumosus*, *Matricaria chamomilla*, *Mentha piperita*, *Mentha pulegium*, and *Thymus vulgaris* were rich in essential oils. For further analysis, these six essential-oil rich plants were tested.

Antioxidant activity

The antioxidant capacities of plant essential oils and aromatic waters were detected using a DPPH radical scavenging assay and presented as mmol/L Trolox Equivalent Antioxidant Capacity (TEAC) values in Figure 1. The essential oil of *Thymus vulgaris* showed the highest antioxidant capacity, which was 3- to 11-fold higher than the other plant essential oils tested. The minimum TEAC value was found in *Asparagus plumosus* essential oil (1.07 ± 0.03 mmol/L TEAC). Many researchers reported that *Thymus vulgaris* essential oil exhibited high antioxidant capacity and there is a positive correlation between its antioxidant activity and amounts of its phenolic components like thymol and carvacrol (Chizzola et al., 2008; Kulisic et al., 2005; Miladi et al., 2013). El-Kalamouni et al. (2017) evaluated the antioxidant activity of *Achillea millefolium* essential oil using sunflower oxidation test and observed that it increased the stability of sunflower oil. The antioxidant activity of *Matricaria chamomilla* essential oil assessed by DPPH assay has been also reported (Abdoul-Latif et al., 2011; Stanojevic et al., 2016).

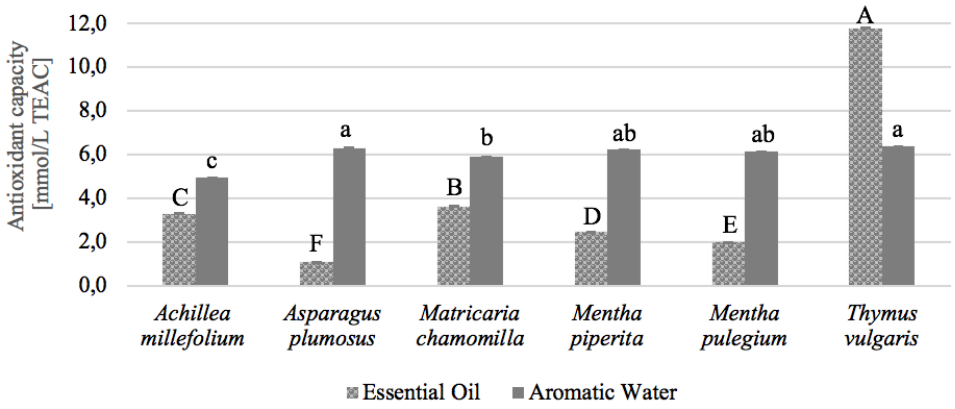

Figure 1. Total antioxidant capacity of plant essential oils and plant aromatic waters expressed as mmol/L TEAC values. The data are reported as mean ± standard deviation; n=6. The values indicated by different letters (a-c or A-F) on the columns in each group are different from each other at $p < 0.05$ level.

When the aromatic waters of plants were tested for total antioxidant capacity, it was seen that *Thymus vulgaris* and *Asparagus plumosus* aromatic waters had higher antioxidant capacity, followed by *Mentha pulegium* and *Mentha piperita*, however, no difference between the means was found among these plant aromatic waters ($p<0.05$). The *Achillea millefolium* aromatic water had the least antioxidant capacity. The results also indicated that the aromatic waters of plants-except for *Thymus vulgaris*- showed higher antioxidant capacity when compared with their essential oils. This can be explained that the aromatic water is generally rich in water soluble compounds which may have higher antioxidant activity than the water-insoluble substances found in the essential oil of *Achillea millefolium, Asparagus plumosus*, *Mentha piperita*, *Mentha pulegium,* and *Matricaria chamomilla*. On the other hand, Candan et al. (2003) observed that the methanolic extracts (water-soluble) of *Achillea millefolium* had lower antioxidant capacity compared to *Achillea millefolium* essential oil. This suggests that methanol may not be a good choice to extract the strong, water soluble antioxidants.

Antibacterial activity

The plant essential oils and aromatic waters were tested for antibacterial effects against the three most common foodborne pathogens including *S. aureus, E. coli,* and *L. monocytogenes* (Bintsis, 2017) and an opportunistic pathogen *M. luteus* (Kao et al., 2014). *S. aureus,* an aerobic Gram-positive bacteria, can cause several infections in the skin such as cellulitis, and postoperative wound infections. Some serious infections, including bacteremia, pneumonia, osteomyelitis, cerebritis, etc., have been attributed to *S. aureus* infection (Archer, 1998; Bintsis, 2017). Pathogenic *E. coli*, a Gram-negative facultative anaerobe, is a fecal indicator bacteria and an important public health problem because of its low infectious concentration and its transmission via omnipresent media such as food and

water (Bintsis, 2017). *L.monocytogenes,* a Gram-positive facultative anaerobic bacteria, is known as highly hazardous food-borne pathogen and causes some inflammation like meningitis, and gastroenteritis (Bintsis, 2017; Buchanan et al., 2017). *M. luteus,* Gram-positive aerobe, is an opportunistic pathogen in patients having a weakened immune system and found in soil, water, air, and the skin of humans and animals. *Micrococcus* species are associated with bacteremia, infection of the endocardium, inflammation of the ventricles in the brain, pneumonia, endophthalmitis, inflammation of the peritoneum, and infectious (Kao et al., 2014).

No inhibition zones were observed for the plant aromatic waters. The antibacterial activity (mean diameters of the inhibition zones of plant essential oils) on *S. aureus, E. coli, L. monocytogenes,* and *M. luteus* were given in Table 1. The highest antibacterial activity against the four pathogenic bacteria was observed in the essential oil of *Thymus vulgaris*. The essential oil of *Achillea millefolium* led to the least inhibitory effect on the growth of *S.aureus;* however, the difference between inhibition zones of *Mentha pulegium*, *Mentha piperita*, *Asparagus plumosus*, *Matricaria chamomilla*, and *Achillea millefolium* was not significant ($p > 0.05$). The essential oils of *Asparagus plumosus* and *Achillea millefolium* were the least effective against *E. coli*. The inhibition zones of *Mentha pulegium* and *Mentha piperita* on *E. coli* were similar. *Asparagus plumosus* essential oil had a high inhibition zone (17.14 ± 0.6 mm) on *L. monocytogenes*, whereas no significant difference in the mean diameters of inhibition zones of *Mentha pulegium*, *Mentha piperita, Matricaria chamomilla*, and *Achillea millefolium* was found. The essential oils of *Mentha pulegium* and *Mentha piperita* showed similar antibacterial effects against *M. luteus*. The lowest inhibition zones on *M. luteus* were determined with the essential oils of *Achillea millefolium*, *Asparagus plumosus*, and *Matricaria chamomilla*.

Table 1. Mean inhibition zones of plant essential oils [mm]. Data represent means ± SD of three replicates. Different superscript letters (a-d) in the same columns are significantly different from each other ($p < 0.05$).

Plant essential oils	Pathogenic bacteria			
	S. aureus	*E. coli*	*L. monocytogenes*	*M. luteus*
Achillea millefolium	9.4 ± 0.01^{b}	9.84 ± 0.49^{d}	11.96 ± 1.51^{c}	8.11 ± 0.10^{d}
Asparagus plumosus	10.72 ± 0.76^{b}	8.73 ± 1.68^{d}	17.14 ± 0.6^{b}	8.19 ± 0.08^{d}
Matricaria chamomilla	10.63 ± 1.01^{b}	14.63 ± 1.37^{cd}	11.04 ± 0.91^{c}	9.51 ± 2.1^{d}
Mentha piperita	15.93 ± 1.29^{b}	21.55 ± 7.36^{bc}	11.15 ± 0.42^{c}	11.69 ± 2.3^{bc}
Mentha pulegium	16.23 ± 0.91^{b}	24.3 ± 0.00^{b}	13.16 ± 2.31^{bc}	10.45 ± 0.58^{b}
Thymus vulgaris	49.27 ± 7.26^{a}	39.55 ± 0.52^{a}	44.13 ± 4.16^{a}	38.09 ± 4.15^{a}

El-Kalamouni et al. (2017) tested also the essential oil of *Achillea millefolium* grown in France for its antimicrobial activity against some pathogenic bacteria species (*B. subtilis*, *B. cereus, S. aureus, S. epidermidis, S. typhimurium, S. enteritidis, S. agona, E. coli)* and observed that the essential oil of *Achillea millefolium* had antibacterial activity against *S. aureus* but no activity against *E. coli*. Similarly, Candan et al. (2003) reported that the essential oil of *Achillea millefolium* subsp. *millefolium* grown in Sivas, in the Central Anatolian Region of Turkey, showed antimicrobial activity against *S. aureus,* while *E. coli* was resistant against *Achillea millefolium* essential oil. In our study, the essential oil of *Achillea millefolium,* which is grown in Ordu in the Black Sea Region of Turkey, exhibited antibacterial activity against both *S. aureus* and *E. coli*. Compared to other studies (El-Kalamouni et al., 2017; Candan et al., 2003), we used the PCA instead of the MHA for the growth of *E. coli* in this study, because the growth of *E. coli* on PCA was better monitored. It was also shown that the essential oils of some *Achillea* species (*A. clavennae* and *A. holosericea*) had antibacterial activity against *E. coli (*Bezić et al., 2003; Stojanović et al., 2005).

Some *Asparagus* spp. (*A. africanus* and *A. falcatus)* are grown in South Africa and known as South African medicinal plants. Madikizela et al., (2014) reported the antibacterial effect of two *Asparagus* spp. (*A. africanus* and *A. falcatus)* on *M. tuberculosis* and *S. typhimurium.* We tested here the essential oil of *Asparagus plumosus* for antibacterial activity and demonstrated that *Asparagus plumosus* essential oil inhibited the growth of *S.aureus, E. coli, L. monocytogenes,* and *M. luteus.* To our knowledge, the antibacterial effect of *Asparagus plumosus* essential oil has not been described before.

The essential oil of *Matricaria chamomilla* L. showed antimicrobial activity against all pathogenic bacteria tested (Table 1). Previously, the antibacterial effect of *Matricaria chamomilla* L. essential oil against *S. aureus* and *E. coli* was reported (Abdoul-Latif et al., 2011; Roby et al., 2013; Soković et al. 2010; Stanojevic et al., 2016). It was also shown that the essential oil of *Matricaria chamomilla* L. exhibited antibacterial effect on *L. monocytogenes* (Soković et al. 2010; Stanojevic et al., 2016).

As shown in Table 1, the essential oils obtained from *Mentha piperita* and *Mentha pulegium* inhibited the growth of pathogenic bacteria tested. Similarly, Soković et al. (2010) and İşçan et al. (2002) observed the antibacterial activity of *Mentha piperita* essential oil against *S. aureus, E. coli* and *L.*

monocytogenes. The antimicrobial effect of *Mentha piperita* essential oil on *M. luteus* has been also reported by Mattazi et al. (2015). Mahboubi and Haghi (2008) reported that the essential oil of *Mentha pulegium* showed antibacterial activity against *S. aureus* and *E. coli.*

Among the tested essential oils, *Thymus vulgaris* was the most active in terms of inhibition of bacterial growth and *S. aureus* was the most sensitive to the essential oil of *Thymus vulgaris.* Previous studies have also shown that *Thymus vulgaris* essential oils exhibited antimicrobial activity against *S. aureus, E. coli*, and *L. monocytogenes;* and *S. aureus* had the highest sensitivity (Soković et al., 2010; Oussalah et al., 2007). On the other hand, Miladi et al. (2013) reported that *S. aureus* was more resistant to the essential oil of *Thymus vulgaris* than *L. monocytogenes*, and *M. luteus* was the most sensitive among these four pathogenic bacteria.

Since *Thymus vulgaris* was the most potent antibacterial essential oil tested, its antibacterial effect was compared with the antibacterial effects of commonly used antibiotics (Table 2). Among tested antibacterials, penicillin and ampicillin were most effective against *S. aureus*, *L. monocytogenes,* and *M. luteus* and their effects were similar to the antibacterial effect of *Thymus vulgaris*. Furthermore, *Thymus vulgaris* had a higher antibacterial effect against *E. coli* than the tested antibiotics.

Table 2. Mean diameters of the inhibition zones [mm] of antibiotics. Data represent means ± SD of three replicates. Means with different superscript small letters in the same column are significantly different from each other ($p < 0.05$).

Antibiotics	**Pathogenic bacteria**			
	S. aureus	*E. coli*	*L. monocytogenes*	*M. luteus*
Ampicillin	36.40 ± 0.85^{ab}	23.85 ± 0.50^{b}	38.85 ± 0.50^{ab}	34.45 ± 0.07^{a}
Gentamycin	18.90 ± 0.00^{c}	14.10 ± 0.42^{c}	22.05 ± 0.64^{c}	24.10 ± 0.14^{b}
Penicilin	40.75 ± 1.91^{a}	14.25 ± 0.21^{c}	41.70 ± 2.69^{a}	38.35 ± 1.34^{a}
Streptomycin	24.85 ± 0.07^{bc}	$26.00 \pm 0.1b^{b}$	$31.45 \pm 0.64b^{bc}$	$26.10 \pm 0.85b^{b}$
Thymus vulgaris	49.27 ± 7.26^{a}	39.55 ± 0.52^{a}	44.13 ± 4.16^{a}	38.09 ± 4.15^{a}

Chemical composition of essential oil of Thymus vulgaris

Single volatile compounds of the essential oil of *Thymus vulgaris,* which showed the highest antioxidant and antibacterial effect, were determined by GC-MS. *Thymus vulgaris* essential oil was rich in thymol, carvacrol, 1,8-cineole, 2 acetyl-4,5-dimethylphenol, γ-terpinene, and caryophyllene (Figure 2). Soković et al. (2010) reported that the thymol, carvacrol, and 1,8-cineole isolated from *Thymus vulgaris* essential oil had antibacterial activity against *S. aureus*, *L. monocytogenes,* and *E. coli.* They found also a correlation between the levels of antibacterial activity and the percentage of the major components of essential oil (Soković et al., 2010). Thymol and carvacrol belong to the phenol-type oxygenated monoterpenes which show higher antimicrobial activity (Couladis et al., 2004; García-García et al.,2011; Soković and van Griensven, 2006; Soković et al., 2010; de Oliveira et al., 2015). Thymol and carvacrol also exhibit synergistic antibactericidal effects when used in combination (García-García et al., 2011). The synergic interaction between carvacrol and 1,8-cineole has been also reported by de Oliveira et al. (2015). Additionally, Ruberto and Baratta (2000) measured the antioxidative capacity of approximately 100 pure compounds of essential oils and observed that phenols like thymol and carvacrol have the highest antioxidant activity.

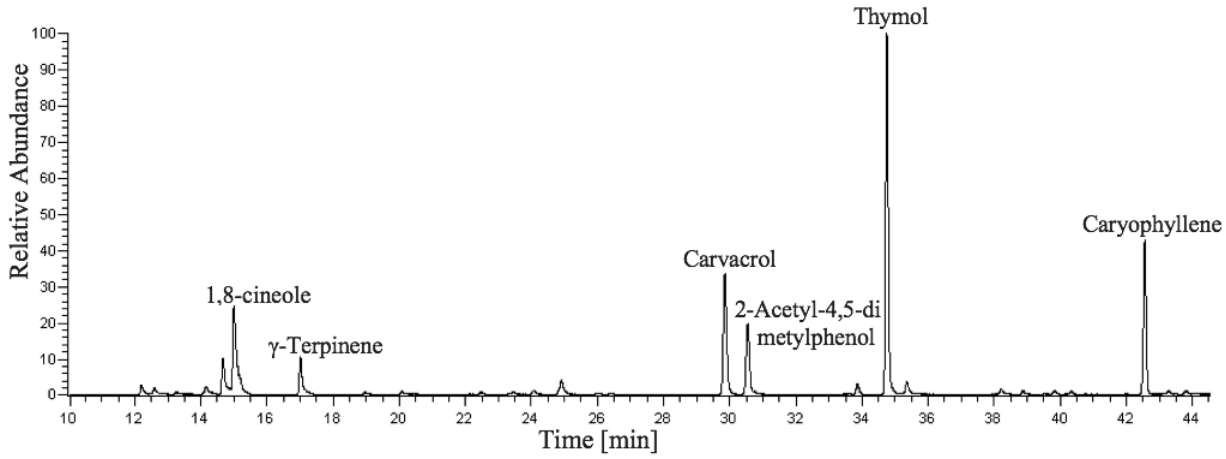

Figure 2 GC-MS chromatogram of a *Thymus vulgaris* essential oil

Conclusion

The use of natural products such as essential oils might be useful in the struggle against antibiotic resistance which is a serious problem for food security and public health. In this study, the essential oils and aromatic waters were isolated from *Achillea millefolium, Asparagus plumosus*, *Matricaria chamomilla*, *Mentha piperita*, *Mentha pulegium*, and *Thymus vulgaris*. Their antibacterial effects were detected against three foodborne pathogenic bacteria and an opportunistic pathogen. All essential oils tested indicated a high antioxidant capacity and antibacterial effect. *Thymus vulgaris* essential oil containing thymol, carvacrol, caryophyllene, 1,8-cineole, 2 acetyl-4,5-dimethylphenol, and γ-terpinene was the most effective against all pathogens tested. Further studies are now required to determine the antibacterial mechanism of the active compounds found in essential 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.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Research Article

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Feed-value of post-harvest quinoa plant sectıons growed by different cultural applications

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Abstract

The aim of the study, the grains of the quinoa plant grown in the Kahramanmaraş region, Turkey, which was sown at different times (March 26, April 2, 13 and 26, and May 11) in various row spacing applications (20, 40 and 60 cm) was separated, and the feed-worthiness of the remaining plant sections were analyzed. The study findings demonstrated that crude protein content was 9.70-19.30%, dry matter ratio was 86.18-88.20%, acid detergent fiber content was 42.95-55.95%, neutral detergent fiber content was 51.23-64.27%, acid detergent insoluble protein content was 0.88-1.37%, digestible dry matter content was 45.3-55.4%, dry matter intake rate was 1.87-2.34%, relative feed value was 66.88-96.49%, and quality standard value varied between III and V. Mineral content was determined as follows: Ca: 0.96-1.96%, K: 1.47-2.08%, Mg: 0.17-0.74%, P: 0.18-0.37%, Tetany: 1.51-1.99, milk fever: 3.69-9.49. It was determined that the sowing time with the highest feed values for quinoa straw was May 11, while the ideal row spacing was 40 cm. Thus, a feed with higher protein and mineral content but low indigestible nutrient content could be obtained. However, it was concluded that it would be more adequate to employ the feed in composite form with other feed plants for feed quality.

Keywords: ADF, ADP, Crude protein content, Mineral content, NDF

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a plant commonly exploited for its grains, and the leaves are consumed as vegetables. Primarily the grains of the quinoa plant have been investigated; however, it is also grown for animal forage production (Kakabouki et al., 2014). Previous studies reported that quinoa could be a valuable forage crop in dairy production and dairy farms (Podkowka et al., 2018). Generally, Gramineae and leguminous forage crops are used in animal feed. However, the available forage crops are not sufficient due to inadequate production and grazing activities. Thus, the increase in the forage demand and the need to fill the gap between forage supply and consumption require further research to determine alternative forage resources. The employment of the residue plant sections as animal forage after the quinoa seeds are separated, similar to the wheat straw, has been discussed as an alternative feed resource.

Sowing time and row spacing are the factors that affect yield in agricultural cultivation. Thus, the present study aimed to investigate the forage values of the remaining quinoa plant parts after deseeding in various sowing times and row spacing applications. Herewith, the study aimed at the employment of the residual parts of the quinoa plant, cultivated for the grains, to determine the availability of these parts for use as animal feed based on sowing time and row spacing nutrient and mineral content and feed quality. Furthermore, forage could play an essential role in improving the farmers' financial stability and contribute to the national economy.

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Material and Methods

Material

The trial was conducted in Kahramanmaraş province, Turkey, in 2018. In the study, quinoa (*Chenopodium quinoa* Willd.) "Q-52", which is resilient in Mediterranean climate conditions, was used as the plant material.

The analysis of soil samples collected at different depths in the trial field revealed that the soil content included moderate organic matter levels, clayey, non-saline, low phosphorus, high potassium levels, and neutral pH (Anonymous, 2018a).

According to specific climate data for 2018, total values minimum and maximum temperatures in the trial area in Kahramanmaraş province were 124.7 °C and 213.6 °C, respectively, and mean minimum and maximum temperatures were 17.8 °C and 30.5 °C, respectively. While the total precipitation in the season was 140.0 mm, the mean precipitation was 23.3 mm. During the cultivation season, the mean total temperature was 164.6°C, and the season average was 23.5°C. The average relative moisture was 336.2% in the season, and the mean moisture was 48.0% (Anonymous, 2018b).

Method

The trial was set up in 3 replicates based on the random blocks experimental design. The trials were conducted in 5 sowing times (March 26, April 2, 13, and 26, and May 11) and 3-row spacing (20 cm, 40 cm, and 60 cm) applications (4 rows per parcel). Sowing times are planned at 15-day intervals; however, due to climate conditions, 15-day application intervals varied. The seed amount sown in the trial parcels was adjusted based on the method proposed by Risi and Galwey (1991).

Based on the soil analysis findings, 5 kg^{-1} N, 6 kg^{-1} P2O5, and 6 kg^{-1} K2O were applied as essential fertilizers before sowing. Approximately 35 days after sowing, 3 kg da^{-1} N was applied as top fertilizer. Irrigation was conducted depending on the climate conditions and the water requirements of the quinoa plant. The plant samples were collected after the plant grains matured and the plant sections became yellow-brown and deseeded in the study. The plant samples were dried at 70°C for 48 hours, ground, and filtered with a 1 mm sieve.

Quality analysis was conducted on dry grass samples with a NIRS analyzer. In the analysis, crude protein content (CP), dry matter content (DMC), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent insoluble protein content (ADP), calcium content (Ca), potassium content (K), magnesium content (Mg) and phosphorus content (P) were determined. Digestible dry matter content (DDM), dry matter intake (DMI), and relative feed value (RFV) were also calculated with the ADF and NDF obtained in the analyzes (Morrison, 2003). Nevertheless, also protein content was analyzed with the micro Kjeldahl method in pulverized samples. Tetany (K: (Ca + Mg)) and milk fever (Ca: P) incidences were calculated based on milliequivalents (meq) (Aydın and Uzun, 2008).

The study data were analyzed with one-way ANOVA in SAS® 9.0 (2004) software, while Duncan multiple comparison tests were employed to determine the differences between mean scores.

Results

The analysis of variance findings and resulting groups based on DMC, CP, ADF, NDF, ADP, DDM, DMI, RFV, Ca, K, Mg, P, tetany, and milk fever parameters are presented in Table 1 and Table 2.

Crude protein content (%)

It was determined that the differences between the CP content based on ST, RS, and STxRS interactions were statistically significant in the study ($p < 0.01$). It was observed that the CP content varied between 9.70 % (II; 20 cm) and 19.30 (V; 60 cm), and the mean CP content was 13.86 %. The analysis of these figures based on the sowing times demonstrated that CP content varied between 11.96 % (II) and 17.60 % (V) and 12.69 % (20 cm) and 14.51 % (60 cm) based on RS applications.

Dry matter content (%)

Based on the ST and STxRS interaction factors, it was determined that the differences in DMC data were statistically insignificant, while they were significant based on RS ($p < 0.05$). It was observed that DMC varied between 87.07 and 87.64 % based on the RS application, and the increase in spacing led to a decrease in DMC in plants.

Acid detergent insoluble fiber (%)

It was determined that the differences in quinoa plant ADF content were statistically significant ($p < 0.01$) based on ST and STxRS interaction but insignificant based on RS ($p<0.05$). It was observed that ADF content varied between 42.95 % (V; 20 cm) and 55.95 % (III; 60 cm), and the mean ADF content was 47.80 %. The highest ADF content was obtained in the second sowing, while the lowest ADF content was obtained in the fifth sowing.

Neutral detergent insoluble fiber (%)

Based on the NDF content, the observed differences between ST, RS, and STxST interactions were statistically significant ($p < 0.01$). The quinoa plant NDF content obtained with different applications varied between 51.23 % and 64.27%, and the mean NDF content was 57.58 %. NDF content varied between 56.09 % (I) and 60.72 % (II) based on ST and between 56.28 % (40 cm) and 58.38 % (60 cm) based on RS application. Furthermore, it was determined that there was so statistically significant difference between 20cm and 60 cm RS applications (58.1 %).

Acidic detergent insoluble protein (%)

Based on ADP, the differences between the ST applications were significant at $p < 0.01$ level, and the differences between the RS applications were significant at $p < 0.05$ level, while they were insignificant between the STxRS interactions ($p < 0.05$). ADP content varied between 1.00 % (II.) and 1.25 % (V.) based on ST. Also, the ADP content in the 1st, 2nd, and 4th sowing times was statistically in the same group with the 2nd sowing. Based on RS, it was observed that ADP content varied between 1.00 % (20cm) and 1.13 % (60 cm), and 40 cm RS was the transition group.

Digestible dry matter (%)

The study data analysis demonstrated that DDM content differed significantly based on the ST and STxRS interactions ($p < 0.01$), while the impact of the RS was insignificant. DDM

content varied between 45.31 % (III; 60 cm) and 55.44 % (V; 20 cm), and the mean DDM was 51.66 %. Based on the ST application, DDM rates varied between 49.33 % (II) and 54.41 % (V).

Dry matter intake (%)

The analyses revealed statistically significant differences between DMI ($p < 0.01$) based on ST, RS, and STxRS interaction. As seen in Table 1, the DMI varied between 1.98 % and 2.15% based on the STs, and the lowest value was obtained in the 2nd ST, and the highest DMI was obtained in the 1st ST. It was determined that DMI varied between 2.06 % (60 cm) and 2.14 % (40 cm) based on RS application, and it varied between 1.87 % (II; 20 cm) and 2.34 % (IV; 40 cm) based on STxRS interaction.

Relative feed values (%)

The review of the study data on RFVs, which is an essential parameter in animal nutrition, demonstrated that the differences based on ST and STxRS interactions were significant ($p < 0.01$), and the differences based on RS applications were significant ($p < 0.05$).

While RFV varied between 75.94 % (II) and 90.46 % (V) based on ST, it varied between 82.06 % (60 cm) and 86.86 (40 cm) based on RS applications. Based on STxRS interaction, RFV varied between 66.88 % (III; 60 cm) and 96.49 % (I; 20 cm).

Calcium (%)

In the study, the analysis of the impact of applications on Ca content revealed that the differences based on RS and STxRS interactions were significant at $p < 0.01$ significance level, while STs led to significant differences at $p < 0.05$ level. Ca content varied between 1.30 % (III) and 1.64 % (V) based on ST. On the other hand, Ca content varied between 1.33 % (60 cm) and 1.60 % (20 cm) based on RS applications. It was determined that Ca content varied between 0.96 % (III; 60 cm) and 1.96 % (I; 20 cm) based on STxRS interaction.

Potassium (%)

The differences between the K content of the samples were significant at $p < 0.01$ confidence level based on ST and

Tablo 1. The analysis of variance analysis findings and resulting groups for some parameters of feed quality values

		DMC	CP	ADF	NDF	ADP	DDM	DMI
Sowing Time		NS	**	**	**	**	**	**
26 March (I)		87.59	12.73 ^{c}	46.50 ^{bc}	55.97 ^{d}	1.01 ^{b}	52.68 ^{ab}	2.15 ^{a}
2 April (II)		87.40	11.96 ^{d}	50.80 ^{a}	60.72 ^{a}	1.00 ^{b}	49.33 ^{c}	1.98 ^{d}
13 April (III)		87.09	12.19 ^{d}	49.34 ^{ab}	58.05 ^{b}	1.04 ^{b}	50.46 ^{bc}	2.07 ^{c}
26 April (IV)		87.34	14.80 ^{b}	48.08 ^{ab}	57.10 ^{c}	1.07 ^{b}	51.44 ^{bc}	2.12 ^{b}
11 May (V)		87.50	17.60 ^{a}	44.28 ^{c}	56.09 ^{d}	1.25 ^{a}	54.41 ^{a}	2.14 ^{a}
Row space (cm)		*	**	NS	**	*	NS	**
20 cm		87.64 ^{a}	12.69 ^{b}	48.04	58.09 ^{a}	1.00 ^{b}	51.47	2.08 ^{b}
40 cm		87.45 ^{ab}	14.36 ^{a}	47.01	56.28 ^{b}	1.10 ^{ab}	52.28	2.14 ^{a}
60 cm		87.07 ^{b}	14.51 ^{a}	48.35	58.38 ^{a}	1.13 ^{a}	51.23	2.06 ^{c}
Interaction		NS	**	**	**	NS	**	**
	20 cm	88.20	13.34	43.50	53.05	1.12	55.02	2.26
I	40 cm	87.26	13.43	45.81	55.55	1.03	53.21	2.16
	60 cm	87.30	11.41	50.19	59.30	0.88	49.80	2.03
	20 cm	87.27	9.70	54.21	64.27	0.92	46.67	1.87
II	40 cm	87.69	11.65	52.06	60.61	0.99	48.34	1.98
	60 cm	87.24	14.52	46.12	57.27	1.10	52.97	2.10
	20 cm	87.47	12.74	46.37	56.59	0.99	52.78	2.12
III	40 cm	87.61	13.40	45.71	54.53	1.03	53.29	2.20
	60 cm	86.18	10.44	55.95	63.04	1.09	45.31	1.90
	20 cm	87.58	10.69	53.19	62.68	0.94	47.46	1.91
IV	40 cm	87.50	16.83	46.14	51.23	1.08	52.96	2.34
	60 cm	86.95	16.89	44.91	57.39	1.20	53.91	2.09
	20 cm	87.67	16.98	42.95	53.89	1.03	55.44	2.23
V	40 cm	87.18	16.51	45.31	59.49	1.37	53.60	2.02
	60 cm	87.66	19.30	44.58	54.89	1.36	54.17	2.18

DMC: Dry matter contents, CP: Crude protein contents, ADF: Acid detergent fibre, NDF: Neutral detergent fibre, ADP: Insoluble protein content in acid detergent, DDM: Digestible dry matter content, DMI: Dry matter intake, RFV: Relative feed value, QS: Quality Standard; Ca: Calcium, K: Potassium , Mg: Magnesium, P: Phosphorus, NS: Not statistically significant, **: Important compared to $p \leq 0.01$, *: Important compared to $p \leq 0.05$.

RS, and significant at p <0.05 confidence level based on the STxRS interaction. It was determined that the K content varied between 1.62 % (V) and1.95 % (III) based on ST, between 1.69 % (20 cm) and 1.86 % (60 cm) based on RS application, and between 1.47 % (V; 20 cm) and 2.08 % (III; 20 cm) based on STxRS interaction.

Magnesium (%)

The study determined that the differences between Mg content, which is known to have a calming effect on animals, were significant (p <0.01) based on ST and STxRS interaction factors, while the effect of RS applications was insignificant on Mg content. It was determined that Mg content varied between 0.38 % (III) and 0.66 % (V) based on ST, while it varied between 0.17 % (III; 60 cm) and 0.74 % (I; 20 cm) based on STxRS interaction.

Phosphorus (%)

The impact of ST and RS applications on the P content of quinoa was statistically significant at p <0.01, while the effect of STxRS interaction was significant at p <0.05. P content varied between 0.21 % (II) and 0.33 % (V) based on ST and between 0.22 % (20 cm) and 0.28 % (60 cm) based on RS application. It was determined that P content varied between 0.18 % (II; 40 cm and IV; 20 cm) and 0.37 % (V; 40 cm) based on STxRS interaction.

Tetany

The effects of ST and RS on tetany, which is inducted by mineral imbalance and leads to paralysis in animals, were significant (p <0.01), and the impact of the STxRS interaction was significant (p <0.05). Tetany risk varied between 1.68 (V) and 1.92 (III) based on ST, 1.66 (20 cm) and 1.91 (60 cm) based on RS application, and 1.51 (V; 20 cm) and 1.99 (I; 40 cm and III; 60 cm) based on STxRS interaction.

Tablo 2. The analysis of variance analysis findings and resulting groups for some parameters of feed quality values

		RFV	QS	Ca	K	Mg	P	Tetany	Milk fever
Sowing Time		**	-	*	**	**	**	**	**
26 March (I)		87.94 ^{ab}	III	1.57 ^{a}	1.89 ^{a}	0.55 ^{ab}	0.24 ^{bc}	1.84 ^{ab}	6.94 ^{a}
2 April (II)		75.94 ^{d}	IV	1.46 ^{ab}	1.83 ^{ab}	0.51 ^{b}	0.21 ^{c}	1.80 ^{bc}	7.31 ^{a}
13 April (III)		81.52 ^{c}	IV	1.30 ^{b}	1.95 ^{a}	0.38 ^{c}	0.25 ^{b}	1.92 ^{a}	5.26 ^{b}
26 April (IV)		84.67 ^{bc}	IV	1.50 ^{a}	1.72 ^{bc}	0.54 ^{b}	0.26 ^{b}	1.71 ^{c}	6.18 ^{ab}
11 May (V)		90.46 ^{a}	III	1.64 ^{a}	1.62 ^{c}	0.66 ^{a}	0.33 ^{a}	1.68 ^{c}	5.27 ^{b}
Row space (cm)		*	-	**	**	NS	**	**	**
20 cm		83.40 ^{b}	IV	1.60 ^{a}	1.69 ^{b}	0.55 ^{a}	0.22 ^{b}	1.66 c	7.40 a
40 cm		86.86 ^{a}	III	1.56 ^{a}	1.85 ^{a}	0.57 ^{a}	0.27 ^{a}	1.80 b	6.09 b
60 cm		82.06 ^{b}	IV	1.33 ^{b}	1.86 ^{a}	0.46 ^{b}	0.28 ^{a}	1.91 a	5.08 c
Interaction		**	-	**	*	**	*	*	**
	20 cm	96.49	III	1.96	1.72	0.74	0.23	1.63	8.46
I	40 cm	89.18	III	1.35	2.00	0.44	0.27	1.99	4.97
	60 cm	78.15	IV	1.41	1.96	0.46	0.21	1.90	7.38
	20 cm	67.56	V	1.27	1.69	0.37	0.19	1.70	6.75
II	40 cm	74.21	V	1.67	1.77	0.61	0.18	1.72	9.49
	60 cm	86.05	IV	1.42	2.02	0.55	0.25	1.97	5.70
	20 cm	86.77	IV	1.47	2.08	0.49	0.24	1.93	6.43
III	40 cm	90.92	III	1.47	2.01	0.48	0.26	1.85	5.65
	60 cm	66.88	V	0.96	1.75	0.17	0.26	1.99	3.69
	20 cm	70.44	V	1.48	1.51	0.48	0.18	1.52	8.45
IV	40 cm	96.17	III	1.72	1.77	0.65	0.29	1.68	6.05
	60 cm	87.39	III	1.29	1.88	0.48	0.32	1.95	4.05
	20 cm	95.72	III	1.80	1.47	0.69	0.26	1.51	6.93
V	40 cm	83.83	IV	1.57	1.73	0.65	0.37	1.78	4.29
	60 cm	91.84	III	1.56	1.67	0.65	0.35	1.75	4.58

DMC: Dry matter contents, CP: Crude protein contents, ADF: Acid detergent fibre, NDF: Neutral detergent fibre, ADP: Insoluble protein content in acid detergent, DDM: Digestible dry matter content, DMI: Dry matter intake, RFV: Relative feed value, QS: Quality Standard; Ca: Calcium, K: Potassium , Mg: Magnesium, P: Phosphorus, NS: Not statistically significant, **: Important compared to p≤0.01, *: Important compared to p≤0.05.

Milk fever

Based on milk fever risk values, the effects of ST, RS, and STxRS interaction were statistically significant (p <0.01). While the milk fever risk varied between 5.26 (III) and 7.31 (II) based on ST, it varied between 5.08 (60cm) and 7.40 (20cm) based on RS application. It was determined that it varied between 3.69 (III; 60cm) and 9.49 (II; 40cm) based on STxRS interaction.

Discussion

Crude protein content (%) and Dry matter content (%)

The previous studies conducted on various quinoa varieties reported crude protein content between 13.5 and 17.7 % during the flowering period (Temel and Keskin, 2019), between 16.3 and 17.8 % in physiologically mature plants (Tan and Temel, 2017), between 11.3 and 13.6 % (Kaya and Aydemir, 2020). Although the present study findings were mainly consistent with other studies conducted with quinoa, specific differences were observed due to the effect of the harvest period.

Sayar et al. (2018) reported that DMC varied between 88.9 and 91.7% in certain poaceous forage crops, Khan et al. (2017) reported that DMC varied between 89.2 and 95.1% in 6 weeds, Gürsoy and Macit (2016) determined that DMC varied between 92.6 and 95.6% in certain poaceous forage crops. The present study findings were lower when compared to the data reported in other research. This difference in result could be due to the diversity between the studied plant species.

Acid detergent insoluble fiber (%) and Neutral detergent insoluble fiber (%)

It was determined ADF ratio varied between 22.8 and 26.9% (Temel and Keskin, 2019), between 17.9 and 30.5% (Kaya and Aydemir, 2020) in quinoa varieties, between 22.9 and 43.2% in certain gramineae forage plants (Sayar et al., 2018). It could be observed that previous study findings on quinoa were lower when compared to our findings. The difference in findings could be associated with the forage crop harvest dates since fodder was used as the present study material. During the maturation period, the changes in plant cell wall components lead to an increase in ADF content (Kaplan et al., 2017). On the other hand, the differences between the previous and current study findings were due to genotypical, ecological, cultural, and analytical factors (Başbağ et al., 2018).

In previous studies, NDF was determined as 42.3-45.2% for 6 quinoa varieties (Kaya and Aydemir, 2020), as 38.0-43.5% in quinoa (Temel and Keskin, 2019). Although the present study findings were similar to previous reports, it could be observed that the NDF content varied within a wide range. This variation was caused by several factors, especially plant species, varieties, development period of the plant, and ecological conditions.

Acidic detergent insoluble protein (%) and Digestible dry matter (%)

The previous studies on ADP reported that it varied between 0.08 and 0.63% in certain poaceous species (Başbağ et al., 2018). The present study findings were higher than those reported in previous studies. It was suggested that the differences were due to plant species, the harvest periods, and employed plant parts. The present study suggested that the high ADP content was since the harvested plants had reached physiological maturity and the stem volume was higher than the leaf volume, especially after harvest.

In some works of literature, DDM content varied between 65.1 and 67.2 % in various quinoa species (Kaya and Aydemir, 2020) and 68.0 and 71.1 % (Temel and Keskin, 2019) based on row and inter-row spacing applications.

Dry matter intake (%) and Relative feed values (%)

The studies conducted on quinoa reported that DMI varied between 2.8 and 3.2 % (Temel and Keskin, 2019) and between 2.7 and 2.8 % (Kaya and Aydemir, 2020). It was suggested that the compatibility of these figures with the present study was because all were conducted with the same plant species and the differences were due to harvesting in the flowering period.

Previous studies reported that RFV varied between 134.4 and 147.6 (Kaya and Aydemir, 2020), between146.3 and 173.2 (Temel and Keskin, 2019), between 68.9 and 143.1 (Başbağ et al., 2018), between 86.8 and 197.0 (Gürsoy and Macit, 2016). These figures were inconsistent. This inconsistency could be due to the employment of different species and varieties as roughage. It was determined that cultural processes, climate, ecology, and even the harvest in the phenological period also impacted RFV even when the same species and varieties are used.

Calcium (%) and Potassium (%)

Certain studies reported that Ca content varied between 1.0 and 3.3 % in 9 quinoa varieties (Tan, 2020) and between 0.83 and 1.27 % in 6 quinoa varieties (Kaya and Aydemir, 2020). Although the previous study findings were consistent with our findings, it was observed that Ca content was lower in various plant species. Debski et al. (2013) reported that Ca content varied based on variety, and the quinoa plant was quite rich in Ca.

The previous study findings that K content varied between 2.9 and 3.3% (Kaya and Aydemir, 2020) and 1.5 and 2.3% (Tan, 2020). It was observed that the present study findings were consistent with previous studies and reported ideal K levels.

Magnesium (%) and Phosphorus (%)

Mg content reported in studies investigating the feed-worthiness of various quinoa varieties was 1.0-2.3% (Tan, 2020) and 2.7-4.3 (Kaya and Aydemir, 2020). While these figures were higher than those reported in the present study, Nurfeta et al. (2008) reported that Mg content varied significantly among different varieties of the same plant species.

Furthermore, since the Mg content is higher (Chen et al., 2018) at growth extremities and young leaves compared to other sections, it is higher in plants harvested during the flowering period compared to those harvested during physiological maturity. On the other hand, it was found that quinoa had higher Mg content than certain other forage plants. It was suggested that our findings were high due to the variety and different harvest periods.

Phosphorus content reported in previous studies varied between 0.27 and 0.42 % (Kaya and Aydemir, 2020), 0.7, and 1.8 % (Tan, 2020) in different quinoa varieties. In the present study, the P content was low since we harvested the crops after physiological maturity and analyzed the residue plant sections after deseeding. However, it was determined that the P content in barley, oat, triticale straw, and their intercrop cultivation with peas were lower when compared to the quinoa straw; thus, quinoa is an excellent alternative crop.

Tetany and Milk fever

Tan (2020) analyzed the use of quinoa plants as feed and reported that the tetany risk varied between 1.5 and 3.0 and MF

value varied between 0.5 and 3.8% and also, he was reported that intensive use of certain varieties might lead to milk fever risk. It was suggested that the present study findings were higher than the ideal range, and quinoa should not be used alone and at high rates.

Conclusions

The application of the quinoa plant, global recognition, and production of which has been increasing since the last decade is not limited to the use of its grain, but the straw could be used as feed. In studies conducted on the cultivation of quinoa as a forage crop, the feedworthiness of the plant has been investigated. In sowing conducted in May with a row spacing of 40 cm, it was determined that high protein and mineral content and low hard-to-digest agents were produced. On the other hand, its use as intercrop feed with other forage crops was suggested due to non-ideal ADF, NDF, ADP, and MF content. However, the fact that the feed quality is affected by all conditions requires further studies to acquire more detailed information on quinoa cultivation. Thus, the present study concluded that the potential of quinoa as feed was high, and it would help eliminate the existing feed shortage.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Research Article

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Evaluation of University Students' Knowledge of and Practices for Sustainable Nutrition

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Abstract

The world is faced with many significant environmental challenges, such as climate change, on a global scale. Sustainable nutrition has an important role in solving these problems. In this study, we aimed to determine the sustainable nutrition knowledge and attitudes of university students. The study was completed with 889 students. It was found that 71.2% of male students think that foods have no effect on the environment. All students had a mean sustainable nutrition knowledge score of 16.0 ± 5.3. As income increased, sustainable nutrition practices scores decreased ($p<0.05$). Sustainable nutrition knowledge scores of overweight and obese individuals were found to be lower than those of participants with normal BMIs ($p<0.05$). Red meat is eaten 1-2 days a week by 73.7% of the students. Also, 85.7% of students do not separate their wastes. This study is the first to provide data on the knowledge and attitudes of Turkish university students on food sustainability from a national and holistic perspective. The results show that there is a significant lack of knowledge regarding the characteristics of sustainable nutrition. It is extremely important to help people understand how food affects the environment. In this context, various research and training approaches are needed.

Keywords: Sustainable nutrition, Environmental issues, Climate change, University student

Introduction

Sustainability is the fulfillment of the needs of current generations by preserving the existence and quality of resources that the next generations will need (Akay & Demir, 2020). The Food and Agriculture Organization of the United Nations (FAO) has stated that in 2050, it will be necessary to increase food production by at least 60% in order to fulfill the demands of world population growth and the rising demand for animal nutrition (Alexandratos & Bruinsma, 2012). The 2018 global warming report of the Intergovernmental Panel on Climate Change (IPCC) (Masson-Delmotte et al., 2018) and the sustainable nutrition recommendation report from the EAT-Lancet Commission on Food, Planet, Health (EAT2019) (Willett et al., 2019) have increased interest in this subject.

At present, more than 2 billion people worldwide are known to suffer from malnutrition (Bailey et al., 2015) and approximately 860 million people from hunger (McGuire, 2013), while about 2 billion people are overweight or obese (WHO, 2016). Taking into consideration that the world population is expected to rise by an additional 2.2 billion by 2050, both worsening hunger and obesity (Nations, 2015) and environmental pressure with scarcity of resources and climate change stand as inevitable facts that seriously threaten our future. In the last 50 years, alongside the constantly increasing need for food, there has been a rapid jump in production in the food industry and livestock sector. Increasing animal food production, in particular, is shown as one of the reasons for global climate change (Masson-Delmotte et al., 2018). The entire food production system is estimated to contribute 30% of global greenhouse gas emissions (Vermeulen et al., 2012),

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and the FAO stated that meat and dairy production contributed 14.5% of all greenhouse gas emissions globally (Gerber et al., 2013). Agricultural production is responsible for 70-80% (Jägerskog & Jønch Clausen, 2012) of all human water use and 38% (Foley et al., 2011) of land degradation. It is very important to evaluate food systems and diets in terms of sustainability in order to eliminate the serious threat of the mentioned rise in production for climate change and to solve the nutritional problems experienced worldwide (Mason & Lang, 2017).

The concept of sustainable nutrition was first proposed in 1986 by Gussow and Clancy, who argued that sustainability is vital for a healthy diet (Gussow & Clancy, 1986; Johnston et al., 2014). In 2010, the FAO defined sustainable diets as "those diets with low environmental impacts which contribute to food and nutrition security and to healthy life for present and future generations." By adding five equally important principles (environment, health, equity, culture, and economics) to the previous definition, diets that are "protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy; while optimizing natural and human resources" have been defined as sustainable diets (FAO, 2010).

Reducing animal protein consumption, increasing the consumption of vegetable protein instead of animal protein and consuming fruits and vegetables from various sources, choosing seasonal local food, eating sufficiently and avoiding waste by using leftovers, and composting food waste are currently emphasized as the principles of sustainable nutrition in order to significantly reduce global warming, improve global nutritional health, and preserve food resources for the next generations (Guillaumie et al., 2020).

In light of these recommendations, encouraging individuals to embrace sustainable diets will reduce greenhouse gas emissions (Hoek et al., 2017). It has been reported that organic farms have on average 25% lower greenhouse gas emissions than conventional farms (Hülsbergen & Küstermann, 2008; Von Koerber et al., 2017). Short distances of food from farm to table reduce energy consumption and greenhouse gas emissions. Seasonal planting causes lower levels of carbon dioxide emissions because it does not require heating oil like greenhouses or plastic tunnels. Seasonal products that are not produced in heated greenhouses or plastic tunnels often also contain fewer residues such as nitrates and pesticides, which is important for the sustainability of the planet (Von Koerber et al., 2017). Minimally processed foods generally contain higher levels of nutrients with less energy. Processed products often contain high amounts of fat, sugar, and salt; they also include possible food additives such as preservatives, coloring, and flavoring agents. At the same time, food processing requires considerable energy and causes pollutant emissions. Moreover, food processing requires large amounts of virtual water (Stranieri et al., 2017; Von Koerber et al., 2017). Along with all these considerations, it is necessary to develop suitable guidelines for sustainable diet models. As a result of the studies conducted on this subject, it has been reported that the Mediterranean and Nordic diets are suitable for health-promoting and sustainable nutrition (Renzella et al., 2018). In addition, another study showed that the lacto-ovo-vegetarian diet and some plant-based diets are more environmentally sustainable than diets containing foods of animal origin (Esteve-Llorens et al., 2019). Foods with low greenhouse gas emissions (<1 kg CO_2e/kg) include pasta, noodles, bread, and oats; potatoes, onions, peas, carrots, and corn; apples, pears, citrus fruits, plums, and grapes; and sugar. Foods with medium greenhouse gas emissions (1-4 kg CO_2e/kg) include chicken, milk, butter, yogurt, eggs, rice, cereals, and oilseeds; strawberries, bananas, and melons; and cauliflower, mushrooms, broccoli, and green beans. Foods with high greenhouse gas emissions (>4 kg CO_2e/kg) include beef, lamb, and fish (Macdiarmid et al., 2012).

Sustainable nutrition will also reduce nutrient losses and waste (Willett et al., 2019). Approximately one-third of the food produced in the world is wasted (Von Koerber et al., 2017). Reducing food waste and nutrient losses will also reduce greenhouse gas emissions caused by more food production (Hyland et al., 2017). Sustainable nutrition is a concept with health, environmental, social, economic, and cultural subdimensions. Although it is a very current issue, it has been reported that it is not well understood (García-González et al., 2020). There are studies suggesting that a majority of people think that sustainable nutrition is expensive, despite the perception that it is healthy (Dwyer & Drewnowski, 2017; Johnston et al., 2014; Masset et al., 2014). There is a strong relationship between nutrition and purchasing power.

In solving the world's threats to the environment, sustainable nutrition plays a key role. It is very important to evaluate the knowledge and attitudes of university students, who are young adults who will build the future, on this issue. At the same time, the concepts of sustainability and sustainable food production in Turkey have not been explored in the scientific literature. In this study, we aimed to determine sustainable nutrition knowledge and practices of university students studying in Istanbul, which is Turkey's most metropolitan and cosmopolitan city.

Materials and Methods

This cross-sectional study was conducted in April and May of 2020. The population of the study comprised students studying at universities (n=52) in Istanbul. The total number of students constituting this population is 621,549 (306,621 female students and 314,928 male students) (YÖK, 2020). The sample size was calculated as 384 students with 5% precision and 95% confidence interval. All students studying in any faculty of a university in Istanbul were invited to participate in this study. The study was completed with 889 students (674 female, 215 male).

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the University of Health Sciences (protocol code 20/141 and date of approval 24 April 2020). Before completing the questionnaire, students were informed about the purpose of the research and those who agreed to participate were asked to approve the questionnaire.

Study plan and collecting data

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This study was conducted to determine the level of knowledge and practices of university students regarding sustainable nutrition in Turkey's metropolitan city of Istanbul. There is no scale in Turkish for evaluating sustainable nutrition knowledge or practices. For this reason, we designed a questionnaire by reviewing the literature. We tested this questionnaire with 40 volunteering students to evaluate it. As a result, we collected the data with the finalized sustainable nutrition information and practices questionnaire consisting of 3 parts and 50 questions. In addition, we obtained information on the demographics and nutritional habits of the students.

Data collection tool

Demographic Information: Students' age, gender, body weight, height, health status, field of university study, place of residence, and nutrition budget were questioned.

Nutrition Habits: The students were asked about their diet, their preferences, and reasons for their consumption of animal and plant foods, whether they had heard of the concept of sustainable nutrition before, and whether they would change their diet for the environment.

Section 1: Consists of 14 questions on sustainable nutrition knowledge. Each correct answer was given 1 point and wrong answers 0 points. The total possible score is 22 points, referred to as the "knowledge score."

Section 2: Consists of 12 questions on sustainable nutrition practices. Two questions are scored on a 5-point Likert-type scale. The total possible score is 29 points, referred to as the "practices score."

Section 3: Contains 24 questions about purchasing attitudes within the scope of sustainable nutrition. All questions are arranged according to a 5-point Likert-type scale (never = 0 points, rarely = 1, sometimes = 2, generally = 3, always = 4). The total possible score is 96 points, referred to as the "shopping attitude score."

Study data were collected online. Before the online questionnaire was administered, the participants were asked for approval. The link to the questionnaire was sent to the e-mail addresses of the student clubs of all universities in Istanbul and the clubs were asked to share this with students.

Data processing and analysis

All collected data were first examined according to the inclusion criteria. Unsuitable participants were excluded from the study by examining university and department declarations. Three individuals were excluded from the study because they were not students and four individuals because they studied at a university in another province. It was then checked whether all questions were answered completely. At this stage, 81 further individuals were excluded. Those students wrote anything about "nutrition budget". These students were excluded from the study. However, 120 students who wrote either "I don't know" or "I don't want to say". These students were not excluded from the study.

While processing the data, the answers in sections 1, 2, and 3 of the Nutrition Habits Questionnaire were scored and the total score of each section was obtained. The scoring of Likert-type questions was graded, those whose answers were not suitable were given zero points, and suitable answers were given 1, 2, 3, or 4 points, respectively. Correct answers to other questions were given 1 point. Thus, the sustainable nutrition knowledge score (0-22), sustainable nutrition practices score (0-29), and shopping attitude score (0-96) were calculated.

Body mass index (BMI: body weight (kg)/height (m^2)) was calculated according to the height and body weight of the students. BMI values were classified in accordance with the World Health Organization (<18.5 kg/m^2, underweight; 18.5-24.9 kg/m^2, normal; 25.0-29.9 kg/m^2, overweight; ≥30.0 kg/m^2, obese) (WHO, 2020).

Descriptive statistics were analyzed for all variables. The answers in sections 1, 2, and 3 of the Nutrition Habits Questionnaire were scored. Obesity was categorized. When parametric test assumptions were fulfilled, variance analysis was used when comparing the data obtained from more than two independent groups. The Tukey test was used to find the difference between groups with a level of significance if the assumption of variance homogeneity was met. In the comparison of the averages of two independent groups, Student's t-test was used when the assumption of normality was met, and the Mann-Whitney U test was used when it was not. The data obtained from qualitative variables were summarized with number and percentage distributions. All analyses were performed using IBM SPSS Statistics 22 (IBM Corp.) and $p < 0.05$ was considered significant.

Results

This study was completed with 889 students. All of the students were between 18 and 25 years old. Students participated in the survey from 31 universities and 14 different faculties in Istanbul. The highest participation was from faculties of health sciences (29.9%). Most students (41.3%) lived with their families and 75.8% of the students were female. While 10.5% of all students had a diagnosed chronic disease, 86% of those were female students. It was also found that 71.6% of all students had a normal BMI and 13.5% (n = 120) did not specify the monthly money spent on their own food, while 62.2% (n = 889) spent 500 Turkish Liras ($73) per month (Table 1).

Questions were asked about students' opinions on both their diets and environmental problems. The answers for female and male students are shown separately. The number of women participating in the study was approximately three times that of men. For this reason, statistical evaluation between the genders is not shown (Table 2).

The rate of those who had heard about the concept of sustainable nutrition before was 58.27% and the majority of these (81.66%) were female students ($\chi^2 = 23.125$, df = 1, p = 0.001). Female students (n = 200, 48.43%) heard about this concept most in academic/scientific activities, while male students (n = 41, 43.16%) heard about it mostly from social media. The majority of the students had a mixed diet (97.19%), consuming both vegetable and animal foods, and this type of diet was considered to be healthier. Types of vegetarianism practiced by the students were ovo-vegetarianism (n = 4), lacto-vegetarianism (n = 5), pescatarianism (n = 3), and semi-vegetarianism (n = 6). There was no significant difference by gender between those who consumed plant-based foods as

replacements for meat and those who did not ($\chi^2 = 23.125$; df = 1; p = 0.001). The question "Is sustainable nutrition expensive?" was asked and 40.80% of female students answered "I don't know"; this rate was 46.51% among male students (Table 2).

Table 1. Characteristics of students (n=889)

	n	%
Gender		
Female	674	75.8
Male	215	24.2
Faculty		
Health Sciences	266	29.9
Engineering	166	18.7
Spots Sciences	51	5.7
Law	29	3.3
Architecture and Design	46	5.2
Science	60	6.7
Theology	36	4.0
Education	64	7.2
Economics and Administrative Sciences	45	5.1
Social Sciences	39	4.4
Fine Arts	6	0.7
Dentistry	30	3.4
Pharmacy	23	2.6
Medicine	28	3.1
Housing		
Student House	222	25.0
Family House	367	41.3
Dormitory	270	30.3
Other	30	3.4
Diagnosed Chronic Disease		
Yes	93	10.5
No	796	89.5
BMI (kg/m^2)		
Underweight	110	12.4
Normal weight	637	71.6
Overweight	117	13.2
Obese Class I	25	2.8
Budget (Spent to feed) (TL*/month) (n=769)		
<500	553	71.9
500-1000	181	23.5
1001-1500	23	3.0
1501-2000	6	0.8
>2000	6	0.8

*1 Dollar ($) =6.85 Turkish Lira (TL)

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Table 2. Characteristics of the eating habits of students

	Female (n=674)		Male (n=215)		Total (n=889)	
	n	%	n	%	n	%
Have You Heard of the Concept of Sustainable Nutrition?						
Yes	423	62.8	95	44.2	518	58.3
No	251	37.2	120	55.8	371	41.7
Nutrition Style						
Mixed Diet	653	96.9	211	98.1	864	97.2
Vegetarian (all types)	14	2.1	4	1.9	18	2.0
Vegan	7	1.0	-	-	7	0.8
Healthy Eating Model						
Mixed Nutrition	586	86.9	176	81.9	762	85.7
Animal-based Nutrition	15	2.2	30	13.9	45	5.1
Plant-based Nutrition	62	9.2	9	4.2	71	8.0
Purely Herbal Nutrition	11	1.6	-	-	11	1.2
Consuming Meat Substitutes Such as Soya Ground						
Yes	83	12.3	23	10.7	106	11.9
No	591	87.7	192	89.3	783	88.1
Is Sustainable Nutrition Expensive?						
Yes	164	24.3	53	24.7	217	24.4
No	235	34.9	62	28.8	297	33.4
I don't know	275	40.8	100	46.5	375	42.2

The students who consumed foods of animal origin (red meat, chicken, fish, eggs, and milk and other dairy products) were asked the reasons for eating them, and the first three answers to this question among both women and men were the same: liking the taste (71.1% and 73.5% for female and male students, respectively), being healthy (70.2% and 69.8%), and habit (46.0% and 35.8%). In addition, 26.5% of men preferred meat because it is "satisfying."

The average, median, and quartile distributions of quantitative data in the study are shown in Table 3. The mean age and BMI of all students were 21.1 ± 1.7 years and 22.2 ± 6.7, respectively. A total of 120 students answered the question of "What is the average money you spend on nutrition per month?" with either "I don't know" or "I don't want to say." Excluding those students, 769 students spent on average 487.2 ± 391.4 TL and 75% of the students had a budget of 600 TL or less. While the lowest budget specified was 25 TL, the highest budget was 3500 TL. The lowest obtained sustainable nutrition knowledge score was 2 and the highest was 27, and 75% of participants had a score of 20 points or less. While an average of 11.1 ± 2.8 points was obtained for the sustainable nutrition practices score, the highest score was 92 for shopping attitude.

Table 3. Descriptive Related to Quantitative Variables

	n	Mean	Median	SD	Min	Max	Q1	Q3
Age	889	21.1	21.0	1.7	18.0	25.0	20.0	22.0
BMI	889	22.2	21.5	6.7	14.2	190.3	19.5	23.9
Budget	769	487.2	400.0	391.4	25.0	3500.0	250.0	600.0
Knowledge score	889	16.0	16.0	5.3	2.0	27.0	12.0	20.0
Practice score	889	11.1	11.0	2.8	2.0	19.0	9.0	13.0
Shopping attitude score	889	57.0	60.0	16.6	8.0	92.0	48.0	69.0

Table 4 shows the distribution of the answers given by the students to some questions about sustainable nutrition and its practices. In this table, the rate of students who think that local food consumption will contribute to sustainable nutrition is 55.6% and the rate of students who consume products grown in their region is 57.1%. While almost all the students (95.9%) thought that a meat-based diet was not sustainable, the majority (52.5%) did not consume free-range chicken eggs. Also, 9.1% of students consumed red meat every day.

In Table 5, which shows the students' attitudes toward sustainable nutrition while shopping, the rate of those who always use their own shopping bags is 26.2%. The rate of those who always consume seasonal food is 27.4%, and the rate of those consuming ultra-processed food is 7.1%. While 25.3% of the students usually buy local products, 29.1% pay attention to the place where food is generally produced.

Table 4. Frequency distribution of responses by students according to some questions on knowledge and practices in sustainable nutrition

		n	%
What's sustainable nutrition?	Those who do not know	295	33.2
	Those who know	594	66.8
Does the consumption of local foods contribute to sustainable nutrition?	Yes	494	55.6
	No	395	44.4
Is the consumption of seasonal food in the context of sustainable nutrition?	Yes	853	96.0
	No	36	4.0
Does the food production chain (both vegetable and animal) cause an increase in greenhouse gases?	Yes	547	61.5
	No	342	38.5
Does the production of animal food (cattle farms) cause water pollution?	Yes	557	62.7
	No	332	37.3
Which food group has the least environmental impact?	Fruits, vegetables	388	43.6
	Bread and the likes	103	11.6
	Milk and milk products	210	23.6
	Meat and meat products	188	21.2
Which of the following types of meat do you think causes more greenhouse gas emissions?	Bovine meat	615	69.2
	Chicken meat	111	12.5
	Small ruminant meat	44	4.9
	Fish meat	119	13.4
What kind of meat do you consume more in your diet?	I don't eat	11	1.2
	Red meat	377	42.5
	White meat	457	51.4
	Fish meat	44	4.9
Indicate the consumption of red meat (independent of the amount)	No	11	1.2
	1-2 times a week	655	73.4
	1 in 15 days	97	10.9
	Everyday	81	9.1
	1 per month	45	5.1
Do you consume regional food grown in the area where you live?	Yes	508	57.1
	No	381	42.9
Is sustainable nutrition related to animal rights or animal welfare?	Yes	470	52.9
	No	419	47.1
When you buy eggs do you buy free-range chicken eggs?	I don't eat	19	2.2
	Yes	403	45.3
	No	467	52.5
Do you buy fish suitable for the season?	Yes	758	85.3
	No	131	14.7
Do you separate the waste or garbage (plastic, paper, glass, food waste, etc)?	Yes	762	85.7
	No	127	14.3
Which of the following are examples of sustainable diets?			
A diet based on vegetables and fruit, chicken protein, fish and legumes, reduced fat and less sweet.		794	89.3
A diet that does not consume sugary food, nuts are consumed in abundance, fruits are consumed very little. snacks are not made fats and meat products are unlimited.		59	6.6
A diet based on red meat. in which vegetables and fruit are consumed less fats are not restricted, and dessert is free.		36	4.1

A statistically significant difference was found between the male and female group averages in terms of all scores. In terms of shopping attitude score, the difference between the averages of female (58.05 ± 16.95) and male students (53.6 ± 15.08) was found to be statistically significant (t = 3.441, p = 0.001). In terms of practices score, the difference between the averages of female (11.5 ± 2.81) and male students (9.9 ± 2.6) was statistically significant (t = 7.387, p < 0.001). In terms of knowledge score, the difference between the averages of female (16.8±5.07) and male students (13.51 ± 5.12) was statistically significant (t = 8.267, p < 0.001) (Table 6).

There was no statistically significant difference between budget groups in terms of shopping attitude score (F = 0.135, p = 0.874). A statistically significant difference was found

between budget groups in terms of practices score (F = 3.777, p = 0.023). The average score of the group with a budget of >600 TL per month (10.58) was statistically significantly lower than the scores of the groups with average budgets of 250-600 TL per month (11.23) and <250 TL per month (11.21). There was no statistically significant difference between budget groups in terms of knowledge score (F = 2.065, p = 0.128) (Table 7).

There was no statistically significant difference between BMI groups in terms of shopping attitude score (F = 0.737, p = 0.479). There was also no statistically significant difference between BMI groups in terms of practices score (F = 2.607, p = 0.074). A statistically significant difference was found between BMI groups in terms of knowledge score (F = 6.092, p = 0.002). The average knowledge score was statistically significantly lower for the group with above-normal BMIs (14.68 ± 5.06) compared to the average of the group with normal BMIs (16.35 ± 5.37) (Table 8).

Table 5. Distribution of responses from students according to some questions about shopping attitudes

		Never	Rarely	Sometimes	Usually	Always
I check whether the packaging of any product I purchase is recyclable or reusable	n	184	315	229	117	44
	%	20.7	35.4	25.8	13.2	4.9
I usually use my own shopping bag when shopping	n	97	123	172	264	233
	%	10.9	13.8	19.4	29.7	26.2
I read information on the product label	n	66	155	209	226	233
	%	7.4	17.4	23.5	25.4	26.2
I buy local products	n	89	163	302	225	110
	%	10.0	18.3	34.0	25.3	12.4
I pay attention to where the food is produced or grown	n	143	205	259	176	106
	%	16.1	23.1	29.1	19.8	11.9
I buy certified organic food products in my food shopping	n	140	230	273	162	84
	%	15.7	25.9	30.7	18.2	9.5
I buy suitable food for the season	n	66	108	172	299	244
	%	7.4	12.1	19.3	33.6	27.4
I buy ultra-processed packaged food	n	125	218	288	195	63
	%	14.1	24.5	32.4	21.9	7.1
I pay attention to the portion size of the food I take in order not to leave food waste	n	62	123	234	295	175
	%	7.0	13.8	26.3	33.2	19.7
I take care that the products I buy are environmentally friendly	n	104	175	273	221	116
	%	11.7	19.7	30.7	24.9	13.0

Table 6. Gender Comparison in Terms of Evaluation Scores

	Female (n=674)	Male (n=215)	t	p
Knowledge score	16.8±5.07	13.51±5.12	8.267	<0.001
Practice score	11.5±2.81	9.9±2.6	7.387	<0.001
Shopping attitude score	58.05±16.95	53.6±15.08	3.441	0.001

Table 7. Comparison of Budget Groups According to Evaluation Scores

	<250 TL (n=178)	250-600 TL (n=384)	>600 TL (n=207)	F	p
Knowledge score	15.42±5.07	16.03±5.3	16.51±5.32	2.065	0.128
Practice score	11.21±2.79[a]	11.23±2.8[a]	10.58±3.07[b]	3.777	0.023
Shopping attitude score	56.28±17.32	56.81±16.74	56.11±16.93	0.135	0.874

Table 8. Comparison of BMI Groups According to Evaluation Scores

	Below normal (n=110)	Normal (n=637)	Above normal (n=142)	F	p
Knowledge score	15.71±4.66[a,b]	16.35±5.37[a]	14.68±5.06[b]	6.092	0.002
Practice score	11.55±2.77	11.12±2.87	10.73±2.74	2.607	0.074
Shopping attitude score	58.64±16.63	56.87±16.69	56.15±16.33	0.737	0.479

Discussion

Based on our findings, more than half of the students (66.8%) knew about sustainable nutrition. However, they did not have enough information about the scope of sustainable nutrition (health, ecological, social, economic, and cultural subdimensions). At the same time, compared to their knowledge scores, they had poorer practices scores related to sustainable nutrition. As income increased, sustainable nutrition practices scores decreased ($p < 0.05$). Sustainable nutrition knowledge scores of overweight and obese individuals were found to be lower than those of students with normal BMIs ($p < 0.05$). In a previous study, it was found that those with lower nutritional knowledge scores were less likely to adopt the Mediterranean diet and had higher BMIs (Bonaccio et al., 2013). Application of knowledge is as important as the level of knowledge. Therefore, both eating habits and shopping attitudes of the students were scored. The score achieved by 50% of all students for sustainable nutrition practices (11.0) was 37.9% of the total possible score (29.0). This score can be evaluated as quite low. However, the median value (60.0) obtained for the shopping attitude score was 62.5% of the total possible score. The average monthly money spent for nutrition by these students was 487.2 ± 391.4 TL. This budget seems to be insufficient in Turkey, where high inflation of food prices is observed.

University students generally have bad eating habits and do not consume the recommended daily amounts of fruits and vegetables; it has been found that they consume sugar, processed meats, and high-fat and high-calorie foods more often (Blondin et al., 2016). In this study, 51.2% of the students thought that they had a healthy diet and almost all of them (97.2%) were mixed-nutrition diets. The percentage of those who thought that the healthiest diet is mixed nutrition was 85.71%. Meanwhile, 21 students, all female, described their diets as vegetarianism and vegan. Existing food production and consumption patterns are among the main causes of environmental degradation (Ruini et al., 2016). Sustainable nutrition is a concept that includes changes in dietary preferences to reduce excessive consumption and facilitate the transition to nutritious diets with lower environmental impacts, as well as reducing losses and waste in food systems. It has been reported that there are developments in solid waste disposal in Turkey, but the performance is unsatisfactory (Kıyan & İkizoğlu, 2020). Providing adequate nutrition within sustainable nutrition systems is very important globally (Alsaffar, 2016). In the transition from adolescence to young adulthood, young adults have difficulty in making healthy food choices due to increased independence (Stok et al., 2018). In our study, it was the female students who tended to eat healthier. At the same time, the sustainable nutrition knowledge and practices scores of female students were higher than those of male students ($p < 0.05$).

While nutrition directly affects our health, it also affects the environment. It has been determined that the environmental effects of nutrition are not known by young people (Dornhoff et al., 2020; Willett et al., 2019). In our study, the rate of those who thought that foods have no effect on the environment was 48.2% and 71.2% among women and men, respectively ($p < 0.05$). According to this result, students need more information about the relationship between nutrition and the environment, which is based on sustainable nutrition. Moreover, the rate of those who did not consider "climate change as an environmental problem associated with sustainable nutrition" was 62.7%. However, an increase in the average temperature of the world by 1.5 °C disrupts the ecological balance and serious nutrient shortages and nutrient deficiencies, such as cereals containing less Zn, are expected (Alexandratos & Bruinsma, 2012; Masson-Delmotte et al., 2018; Solomons & Schümann, 2017).

It has been stated that older consumers are more sensitive to and dependent on sustainable nutrition, and it has been determined that more insensitive individuals who do not care about the environment include younger individuals, those of male gender, those with lower income or lower education levels, and those with less inclusion in society (Gilg et al., 2005). Participants in the present study are very young (median age: 21 years) and are in serious need of sustainable nutrition education. For example, 40.8% and 46.5% of female and male students, respectively, said "I have no idea" regarding whether sustainable nutrition has an economic dimension ($p < 0.05$). Individual nutritional habits supporting sustainability among university students, who are the consumers and decision-makers of the future, can have a positive effect on their personal and social environments and actively contribute to the sustainable development of the nutrition system (United Nations Educational & Organization, 2014). In this study, it was determined that those who heard about sustainable nutrition had mostly (25.8%) heard about it from academic and scientific activities, such as lectures and conferences. Information will make an important contribution in universities and even at earlier educational levels.

Among all students, only 8.3% thought that a plant-based diet was more beneficial. However, the frequency of students consuming meat every day was only 9.1%. There is important food price inflation in Turkey and meat prices are particularly high. According to the most extensive nutritional study conducted in Turkey, among individuals between 19 and 30 years of age, daily animal protein consumption is 34.1 g among men and 24.5 g among women (T.R.HealthMinistry, 2014). It can be asserted that the economic situation of the students affects their frequency of meat consumption. It is known that red meat has a particularly high environmental impact, and it was determined that 73.4% of the students consumed red meat 1-2 times a week. This situation can be evaluated as positive in terms of sustainability. There was no significant relationship between the incomes of the students and the frequency of meat consumption, but 71.9% of the students who declared their income had a monthly nutrition budget of 500 TL (~$75). Considering the greenhouse gas effects of animal products, it can be said that the carbon footprints of the students are small in this context. The IPCC reported that undeveloped or developing countries have smaller greenhouse gas effects (Masson-Delmotte et al., 2018). However, this is not the case regarding waste reduction; such countries have very limited recycling activities (Umut et al., 2015). For example, in Turkey, there is no activity or obligation for either municipalities or

homes for composting food waste.

Studies have shown that both high school students (Dornhoff et al., 2020) and adults (Macdiarmid et al., 2016) essentially perceive nutrition individually and hardly notice the environmental effects of their own diets. In other words, they are either unaware of the effect of their own nutrition behavior on the global food system or they think that it is very insignificant (Dornhoff et al., 2020; Macdiarmid et al., 2016). In a study conducted in Italy, only 31% of university students thought that their consumption affected the environment (Vecchio & Annunziata, 2013). Studies have shown that women have more sensitive behaviors towards environmental problems and environmental protection (Shivakumara et al., 2015; Xiao & McCright, 2015). In studies conducted with university students, it was found that female students' environmental attitude scores and environmental awareness averages were significantly higher than those of male students (Çabuk, 2003; Şenyurt et al., 2011). In our study, a parallel result was found. In addition, in our study, the question "If you knew that it was more beneficial for the environment, would you change your diet?" was asked, and 89.2% of the female students but only 20.0% of the males answered "Yes."

According to the FAO's 2010 definition, sustainable diets should be protected and respected as the best use of natural resources, biological diversity, and ecosystems (FAO, 2010), and one of the most important criteria in sustainable nutrition is the low impact of nutrition on the environment. In our study, the rate of students who always chose recyclable or reusable packaging when they bought packaged products while shopping was 4.9%. This rate is very low. According to gender, this rate was 5.2% among women and 4.2% among men ($p < 0.05$). However, even though the rate of those who read labels was lower among men than women, the rate of "usually" and "always" reading label information among all students was 51.6%. Regarding the use of local products, although 16.1% of the students never paid attention to the origin of their foods, it was found that 4.0% of female students and 4.2% of males always bought imported food ($p > 0.05$). In the use of packaged products, it is extremely important to separate packages according to their materials. However, in Turkey, there is no law regarding separation at the source. It is necessary to provide training on the importance of disposal of wastes and recycling and recovery.

In the study of Serafini and Toti, it was determined that obesity may have large ecological costs to the environment due to excessive consumption of foods. It is thought that excessive consumption of foods leading to obesity may cause the waste of resources and unnecessary greenhouse gas emissions. Moreover, foods that are eaten beyond biological requirements are considered as metabolic waste. Most of the metabolic food waste is caused by animal products, followed by cereals, pulses and starchy roots, sugar and sweets, and alcohols, respectively. It has been suggested that reducing the frequency of obesity may also reduce greenhouse gas emissions (Serafini & Toti, 2016). In our study, the frequency of being obese and overweight was 2.8% and 13.2%, respectively. In another study (Ayşe & Ali, 2020), the prevalence of obesity and overweight among university students was 4.9% and 18.8%, respectively. These findings are similar to each other. Obesity etiology is based on very complex factors and, accordingly, many disciplines should work together in its treatment. In the future, it can be expected that obesity will be on the agenda more within the scope of sustainable nutrition.

In conclusion, this study is the first to provide data on the knowledge and attitudes of Turkish university students regarding food sustainability from a national and holistic perspective. The results show that there are different levels of knowledge and different attitudes regarding the characteristics of sustainable nutrition. It is also understood that there could be other variables affecting this situation. It is extremely important to help people understand how food affects the environment. In this context, various research efforts, training programs, and political support are needed.

Compliance with Ethical Standards

Conflict of interest

The authors declare no conflict of interest.

Author contribution

Aysun Yuksel conceived, designed, and did statistical analysis & editing of the manuscript. Hulya Yilmaz Onal did data collection and manuscript writing. All authors contributed to and have approved the final version.

Ethical approval

From University of Health Sciences, Hamidiye Scientific Research Ethics Committee, protocol code: 20/141; date of approval: 24.04.2020

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Data availability

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Consent for publication

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Factors affecting home-made food product marketing willingness of women: a case of Gokceada district

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Abstract

In this study, it is aimed to determine the factors affecting home-made food products marketing willingness of women who produce home-made food products in Gokceada district of Canakkale province. The number of farm was determined using a simple random sampling method. The data were collected from women in 155 agricultural farms. The survey was conducted from December 2018 to June 2019. The data were analyzed by descriptive statistics and logit model. This study results showed that the average age of women was 50.9, the education level of 51.3% them was a secondary school and higher and 80.9% of them were married. The logit model results revealed that own agricultural land assets, the status of animal husbandry, continuing home-made food production and home-made food production as the main source of income had a positive effect on home-made food products marketing willingness of women; whereas age, education level, household size, and income level had a negative impact. As a result, the finding of the study is expected to make significant contributions to the production of home-made food products, district economy and rural development.

Keywords: Gokceada, Home-made food, Logit model, Women

Introduction

Turkey has a vital position in terms of food production potential because the cultural richness of Turkey is quite effective in home-made food production. Turkey has developed culinary culture, home-made production styles and a rich local production style in addition to its agricultural product variety (Demirbaş et al., 2006; Kantaroğlu and Demirbaş, 2018). Women living in a rural areas in Turkey have some social roles such as preparation daily and seasonal foods that are necessary for the nutrition of their family as well as housework and child care. These women also contribute to the national economy as well as the family economy by producing dried and processed food for winter. Depending on the culinary culture richness of Turkey, many home-made food products are produced and consumed within this scope. Therefore, the production of home-made food products is important as it is an alternative income generation tool that will be developed for evaluating the labour force potential of women (Kaya, 2011; Korkmaz and Tüfekçi, 2007; Özdemir et al., 2016).

Gokceada, a district of Canakkale, is located northwest of the Aegean Sea and it is the largest island of Turkey. The economy of Gokceada is based on agricultural production and tourism. However, due to the short tourism season in Gokceada district, it can be stated that agricultural production activities constitute the majority of the district's economy. In this district, women in the farms which are mostly constituted by the farmer families

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prefer to produce some home-made food products such as butter, cheese, tarhana, tomato paste whether to consume throughout the year or to contribute the family economy by selling them. Therefore, determining whether rural women gain income from these food products they produce is expected to have an impact on the income of small family farms.

There are many studies on the status of women in rural areas both in national and international literature. The majority of these studies address women's participation in labour force in the rural areas (Bayraktar and Gaytancıoğlu, 2000; Kutlar et al., 2013; Özdemir et al., 2017b), the production of home-made food products (Demirbaş et al., 2006; Pieniak et al., 2009; Köksal, 2014; Özdemir et al., 2017a) and women's entrepreneurship (Anthopoulou, 2010; Sosyal, 2013). When previous studies are examined regarding these issues, it has not been coming across any studies which comprehensively examined the approaches to home-made food production of women living in Gokceada. Therefore, this study is important in terms of determining the profile of women who produce home-made food products in the district and the approaches of them to home-made food production.

In this study, it is aimed to investigate the socio-economic structures of women in the district and to reveal the factors affecting their willingness to market their home-made food products. The findings to be obtained from this study are expected to contribute to the district's economy, rural development, and future studies on this issue.

Material and Methods

The Study Area and Sample Size

To determine the number of agricultural farms in Gokceada, 2017 Farmers Registration System (FRS) database of Gokceada District Directorate of Agriculture and Forestry was used. Within this context, it was determined that the total number of agricultural farms in the district was 567 (Anonymous, 2017). The land size of agricultural farms (own and rent land) registered in the FRS was taken into consideration in the selection of the farms to be surveyed from the sample population. The data were collected from surveys conducted through face-to-face interview technique with women who produce home-made food products in the study area. The surveys were conducted between December 2018 and June 2019 in Gokceada district of Canakkale province. Simple random sampling method was used in the selection of farms (Cochran, 1977).

$$n=\frac{(\sum N_h\ S_h)^2}{N^2D^2+\sum N_h S_h^2} \quad , \quad D^2 = d^2/z^2 \qquad [1]$$

where, n=number of the farms representing the population, N= total number of the farms in the population (567), σ=standard deviation of the population, d= acceptable error limit in population mean (*0.05), = average land asset (10.2 hectares), t= desired confidence level (1.96 for 95%). Thus, the number of farms to be surveyed was determined as 115.

Econometric Model

Descriptive statistics were used to determine the socio-economic characteristics of women. The logit model was used to determine the factors affecting women's willingness to market their home-made food products. Logistic regression is a non-linear model which can be linearization with designed transformations for the binary dependent variable, and dependent variable represents the occurrence or non-occurrence of an event (coded as 1 or 0). The logit model is one of the methods used to estimate the binary dependent variable models (Greene, 2002). In this study, the dependent variable that indicated the marketing situation of women's home-made food products was divided into two response categories. These categories are women who market home-made food products (coded 1= event) and who do not market home-made food products (coded 0=no event) according to the logit model. The STATA statistical analysis program was utilized in the analysis of the data (STATA, 2005). This model is defined as follows:

$$P_i=\frac{1}{1+e^{-(\beta_0+\beta_i X_i)}}=\frac{1}{1+e^{-(Z_i)}} \qquad [2]$$

where, is dependent variable, Pi is the probability of use AI for the i^{th} farmers and it ranges from 0-1, is constant term, =parameter to be estimated, X_i=independent variables. Based on the natural log of this equation (2), the following equation can be written:

$$L_i=\ln\left[\frac{P_i}{1-P_i}\right]=Z_i=\beta_0+\beta_1X_1+\ldots.+\beta_kX_k \qquad [3]$$

McFadden's pseudo R-squared value and the likelihood ratio (LR) were calculated to the goodness of fit of the model and its explanatory power. McFadden's pseudo R-squared value can be as low as 0 but can never be equal to 1, and parameter values between 0.2 and 0.4 contain a reference to an appropriate model fit. The likelihood ratio chi-square (LR chi^2) is defined as -2 (L_0- L_1), where L_0 describes the log likelihood for the constant-only model and L_1 is the log likelihood for the full model with constant and predictors (Greene, 2002). When the LR statistic value of the model is greater than the value of chi-square, the null hypothesis (H_0) is rejected.

Definition of Variables

The dependent variable of the logit model has two response categories. To determine the dependent variable, women were asked whether they market home-made food products or not. Accordingly, these results were combined into a dummy variable; if women do not market home-made food products, this is given the value of 0 (0=no event) and the value of 1 is given if women market home-made food products (1=event). The independent variables included in the model were selected from previous studies (Bayraktar and Gaytancıoğlu, 2000; Pieniak et al., 2009; Özdemir et al., 2017a).

Age is one of the important demographic features affecting the behaviors of the producers since producers' preferences may change depending on their ages. In particular, home-made food product marketing tendencies of women may decrease due to the increase in their ages. For this reason, the willingness of women to market their home-made food products is expected to change depending on the increase in age.

The level of education is also an important factor affecting the producers' production preferences. Because the increase in the education level of the producers may affect the tendency of their production abilities. Therefore, this variable is expected to have an impact on the willingness of women to market home-made food products.

Household size is an effective factor in the decision-making process of producers' participation in the labour force. Since whether the increase or the decrease in the number of individuals in the household may affect the participation of producers in the labour force. Considering the fact that women's marital status is still effective on their participation in the labour force, the household size is expected to have an impact on women's willingness to market home-made food products.

Income level is an important factor in the tendencies of producers to produce a new product or to continue their production. Since especially the tendencies toward production of women who do not have their own income and want to gain economic independence may be higher. Therefore, the income level is expected to have an impact on the willingness of women to market their home-made food products.

Land assets can be seen as an important advantage for producers engaged in agricultural production. Land assets can be seen as an important advantage for producers engaged in agricultural production. Because the fact that women who produce home-made food have their own lands, it may ease their raw material supply. For this reason, land assets are expected to have an impact on women willingness to market their home-made food products.

Animal husbandry can be considered as an influential factor in producers' production activities. It can be said that women engaged in animal husbandry can earn income from this field of activity in addition to their current activities. Therefore, animal husbandry is expected to have an impact on the willingness of women to market their home-made food products.

Continuing home-made food production can positively affect producers' production activities. It is thought that women who produce home-made food products and earn income from this production activity compared to other women will have more tendency to continue home-made food production. For this reason, continuing home-made food production is expected to have an impact on the willingness of women to market home-made food products.

Home-made food production as the main source of income may have an impact on the producers' production activities. In line with what has been indicated, these independent variables were included in the model. It is thought that women whose the main source of income is home-made food production have an higher tendencies to production home-made food products compared to other women. Therefore, home-made food production as the main source of income is expected to have an impact on women's willingness to market their home-made food products.

Results and Discussion

In the study area, the majority of women who produce home-made food were between 46 and 57 years old and the average age of them was 50.9 (Table 1). According to these results, it can be said that the majority of women in Gokceada district is 46 age old and above. In a study conducted in the Thrace region, the majority of women producers were found to be 35 years old and above (Özdemir et al., 2017a). Thus, it can be said that these results are not congruent with those of Özdemir et al. (2017a), which indicated that the age of women producers was middle age and above.

In Gokceada district, the education level of women (51.3%) was a secondary school and above. Njang Che and Sundjo (2018) found that 42% of women participating in labor force in Cameroon were primary school graduate. In a study conducted in the Thrace region, it was determined that 91.2% of women producing home-made food were primary school graduate (Özdemir et al 2017a). In this context, it can be said that the education level of women in the district is higher than that of women producers in Cameroon and less than that of women producers in the Thrace region.

In the present study, 80.9% of women were married. In a study conducted in the Thrace region, the majority of women producers (91.2%) were found to be married (Özdemir et al., 2017a). Njang Che and Sundjo (2018) found that 73% of women producers were married in Cameroon. Thus, it can be said that the ratio of married women producers in the study area is higher than that of women producers in Cameroon and less than that of women producers in the Thrace region.

In Gokceada district, the average household size of women who produce home-made food products was 3.5 persons. According to this result, it is possible to say that the majority of women producing home-made food products in the district are part of the nuclear family structure. Also, it can be said that the average household size of women in the district (3.5 persons) is the same as the average in Turkey (3.4 persons) (TURKSTAT, 2018).

In the study area, the majority of women (49.6%) defined themselves as housewives in terms of occupation. In a study conducted in Turkey, it was found that 69% of women producers who participate in the labour force were housewives (Varol, 2017). Özdemir et al. (2017a) found that 85.4% of women identified themselves as housewives in the Thrace region. Thus, the ratio of women identifying themselves as housewives in Gokceada district is less than those of women producers in the Thrace region and in Turkey. According to these results, it can be said that women in the study area have different occupational groups apart from being a housewife.

In the present study, the monthly income level of women (41.7%) who produce home-made food products was proportionally between €460.9 – €614.4. Özdemir et al. (2017a) found that more than half (56.3%) of women producers had a monthly income between €153.6 – €460.8 in the Thrace region. Thus, it can be said that the monthly income level of women in the study area is higher than that of women producers in the Thrace region.

In Gokceada district, 90.4% of women who produce home-made food products had social security. In a study conducted in Van province, 59% of women working in the rural area had social security (Karakaş et al., 2016). In another study, 36.5% of women were found to have a social security (Soysal, 2013).

Thus, it can be stated that the social security ratio of women in the study area have a higher than those of women producers in studies conducted by Soysal (2013) and Karakaş et al. (2016). In this context, it can be said that women in the district have information about the importance of benefiting from social security.

In the study area, 62.6% of women had less than 25 hectares of land and 91.3% of them had cultivated their lands. In a study conducted in the Thrace region, it was determined that the majority of women producers had 100 decares of land or below and %83.6 of them had processed their lands (Özdemir et al 2017a). In another study in the Thrace region, 55% of women producers were found to have their own land (Özdemir et al 2017b). Thus, it can be said that the findings of the present study are similar to the results of previous studies.

In the present study, 38.3% of women who produce home-made food products were engaged in animal husbandry and 28.7% of them were found to be ovine breeding (goat and sheep). According to these results, it can be said that the number of women engaged in animal husbandry is low and they continue their livestock production in order to obtain additional income.

In Gokceada district, *"pasta, tarhana, tomato paste, marmalade, canned vegetables and jam"* were among the most produced home-made food products by women. However, *"kefir, bulgur, cream and butter"* production were not preferred by women. In a study conducted in the Thrace region, it was concluded that while the most produced products producing by women producers were *"tomato paste, tarhana, couscous and cheese"* respectively, *"bulgur, butter and molasses"* were rarely produced (Özdemir et al., 2017a). In a study conducted in Greece, it was determined that women producers attached the highest importance to the production of sweets, pasta, bread, cheese, salty and spicy meats among the home-made food products (Anthopoulou, 2010). Thus, it can be said that the home-made food products produced by women in Gokceada district are similar to products in the Thrace region while being different from what is mostly produced in Greece.

In the study area, 39.1% of women produced home-made food products for their own consumption only. Women who produce these products for their own consumption only were based on this situation to the low production capacity (66.7%), the inability to find a market to sell (24.4%) and the absence of demand (8.9%). In a study conducted in the Thrace region, it was determined that 83.9% of women producers who make home-made food production produced these products only for own consumption (Özdemir et al., 2017). In addition, they based on their reasons to low production capacity (35.2%), lack of a market to sell (10%) and demand deficiency (%21.5). In a study conducted in Spain, it was determined that women producers produced traditional food for both to consume and to market (Martinez et al., 2014). According to these results, it can be said that the tendencies of women towards home-made food production (commercial) in Gokceada district are higher than those of women in the Thrace region. Also, it can be stated that producing home-made food for women living in the rural areas of Gokceada district, Thrace region and Spain is very important for their own consumption. In addition, it can be said that the most important reason that force women in Gokceada district and Thrace region in order to produce home-made food products only for their own consumption is the low production capacity.

In the present study, 32.1% of women who produce home-made food products for commercial purpose had produced 9 years and longer. Özdemir et al. (2017) found that 37.3% of women producers in the Thrace region had produced home-made food for 2-5 years, 33.9% those had produced for 11 years and longer. According to these results, it can be said that the majority of women producers in Gokceada district and Thrace region tend to produce home-made food for commercial purpose for many years.

In Gokceada district, home-made food production was not the main source of income for 75.6% of women. In a study conducted in Greece, it was revealed that the main source of income for the majority of women producers was not home-made food production (Anthopoulou, 2010). Similarly, in a study conducted in the Thrace region, it was determined that the main source of income of the majority of women producers (93.5%) was not traditional food production (Özdemir et al., 2017). According to these results, the findings of this study are congruent with those of the study conducted in Greece and in the Thrace region. In this context, it can be said that the results obtained from Gokceada district are supported by the findings obtained from Greece and Thrace region.

In the study area, 32.2% of women supplied their raw materials/materials both from their own lands and their own animals. In a study conducted in the Thrace region, it was determined that women producers provided their raw materials/materials required for traditional food production mostly from their own farms (64.6%) (Özdemir et al., 2017). According to these results, it can be said that women of Gokceada district and Thrace region obtain the raw materials required for home-made food production from their own farms or lands mostly.

In the present study, it was found that 90.4% of women tended to continue home-made food production. This situation can be interpreted as an indication that women's approaches to home-made food production are positive. At the same time, this situation can be considered as an indication that women attach importance to transferring the home-made food products approach to future generations.

In Gokceada district, the most important factor affecting the contribution of women in home-made food products to the family economy was insufficient sales opportunities. Also, this was followed by the lack of sufficient workforce, the lack of financial support for production and the inability to promote home-made food products. In a study conducted in the Thrace region, it was determined that the most important factor affecting the contribution of home-made products to the economy was the adaptation to the standards. This was followed by factors such as creating sales opportunities, finding sufficient raw materials and providing financial support for production (Özdemir et al., 2017). According to these results, it can be said that the results obtained from both studies are similar to the results obtained from the current study in terms of some factors.

In the study area, women sold their home-made food

products mostly through neighbour/acquaintance (21.3%), relatives (20.4%), friends (20.1%) and local markets (15%). In a study conducted in the Thrace region, it was found that women producers sold their products to wholesalers or retailers (17.7%), cooperatives (15.7%) and local markets (19.3%). In a study that examines the current situation of women entrepreneurs in rural areas in Turkey, it was determined that 25.4% of women producers sold their home-made food products through their acquaintances (Soysal, 2013). According to these results, it can be said that the majority of women in Gokceada district sell home-made food products through their acquaintances, neighbours, friends and local markets. Thus, it can be said that women should pay more attention to advertisements and promotions related to home-made food products and it is important that they are supported to make their sales using various marketing channels.

Table 1. Definition of variables used in the logit model (n=115)

Variables	Frequency	%	Mean	**SD
Dependent variables				
Home-made food product				
Women marketing this product =0	45	39.1	0.61	0.49
Women not marketing this product =1	70	60.9		
Independent variables				
Age (year)	115	100 0	50.9	10.9
Education level (year)	115	100.0	8.7	4.2
Household size (person)				
0 = <3	28	24.3	0.76	0.43
1 = ≥3	87	75.7		
*Income level (€ month^{-1})				
0= < €460.9	30	26.1	0.74	0.44
1= ≥ €460.9	85	73.9		
Own agricultural land asset (hectare)				
0= No	40	34.8	0.65	0.45
1= Yes	75	65.2		
The status of animal husbandry				
0= Does not do	71	61.7	0.38	0.49
1= Doing	44	38.3		
The status of continuing home-made food production				
0= Does not want to continue	11	9.6	0.90	0.30
1= Wants to continue	104	90.4		
The status of home-made food production as the main income source				
0=The main source of income is not a home-made food	87	75.6	0.24	0.43
1=The main source of income is a home-made food	28	24.3		

*1 Euro=6.51 TRY (Turkish lira) in April 2019, **SD=Standard deviation

Age, education level, household size, monthly income level, own agricultural land asset, the status of animal husbandry and continuing home-made food production and home-made food production as the main source of income were defined as independent variables in the logit model, which is estimated to determine the factors affecting on women's willingness to market their home-made food products. The McFadden's pseudo R-squared value and likelihood ratio (LR) were calculated to test the goodness of fit of the established model and its explanatory power. The LR and chi-square statistic (chi^2) values were calculated as 68.67 and 15.51, respectively. The null hypothesis at 5% significance was rejected because the LR value was found to be greater than the chi^2 value (Table 2). These results revealed that the model is statistically significant and appropriate for the study.

In Gokceada district, it was found that there was a significant and negative relationship between the age of women and their willingness to market their home-made food products. This result shows that the willingness of women's home-made food product marketing decreases depending on the increase in their age. Therefore, it can be said that the willingness of older women to market their home-made food products is lower than younger women. In this context, it can be stated that the tendency of women to continue this activity, which is intense in terms of the manual labour, is expected to decrease depending on the increase in their age. In a study conducted in the Thrace

region, it was found that there was a significant and negative relationship between the age variable and the willingness to market their home-made food products (Özdemir et al., 2017a). In studies conducted in Cameroon and in Pakistan, a significant and positive relationship was found between the age variable and women's labour force participation. In addition, it was stated that older women participating in the labour force were more experienced than young women and young women's goals such as education and marriage were higher than older women (Mujuhid, 2014; Njang Che and Sundjo, 2018). According to these results, the findings obtained from women in Gokceada district are similar to the results obtained from the Thrace region but differ from the findings obtained from the studies in Cameroon and Pakistan. Thus, it is considered that encouraging and supporting younger women for this activity is important for the continuity of home-made food production in Gokceada district. Furthermore, it is expected that this situation has a positive impact on willingness to market their home-made food products.

Table 2. Factors affecting home-made food product marketing willingness of women

Variables	Coefficient	Standard error	z-statistic	p-value>|z| (probability)	Marjinal Effects
Age	-0.1416	0.0405	-3.49	0.001**	-0.0263
Education level	-0.3981	0.1061	-3.75	0.000**	-0.0739
Household size	-2.2674	0.8289	-2.74	0.006**	-0.3069
Income level	-1.7879	0.7032	-2.54	0.011*	-0.2626
Own agricultural land asset	2.0848	0.6335	3.29	0.001**	0.4236
The status of animal husbandry	1.8409	0.6413	2.87	0.001**	0.3033
The status of continuing home-made food production	2.6571	1.0168	2.61	0.004**	0.5810
The status of home-made food production as the main income source	3.9074	1.2066	3.24	0.009**	0.4421
Constant	8.9714	2.9175	3.06	0.001**	
McFadden pseudo R-squared =0.45		log likelihood (L_1)= -42.63842			
log likelihood (L_0)= -76.97272		likelihood ratio (LR)=68.67			
Prob>chi square (chi^2)=0.0000 (Probability)		LR>$chi^2(8)_{(0.05)}$ = 68.67>15.51			

The levels of significance: *p<0.05; **p<0.01

In the study area, it was found that there was a significant and negative relationship between the education level of women and their willingness to market their home-made food products. This result shows that the willingness of women's home-made food product marketing decreases depending on the increase in their educational level. In the study conducted in the Thrace region, it was determined that there was a significant and negative relationship between the level of education and the willingness to market their home-made food products (Özdemir et al., 2017). In a study conducted in Cameroon, it was found that having a low education level of women had a positive effect on the labour force participation ratio and the ratio of participation in the labour market decreased as the education level of women increased (Njang Che and Sundjo, 2018). The findings obtained from women in Gokceada district are similar to the results obtained from the studies conducted in the Trace region and Cameroon. Based on these results, the fact that womens' education level variable can be interpreted in two different ways. The first is that the majority of women have a certain level of education (literate and primary school graduate). The second is that, as women's education level increases, their tendency towards jobs related to their occupational increases.

In the present area, it was found that there was a significant and negative relationship between women's household size and their willingness to market their home-made food products. This result shows that the willingness of women's home-made food product marketing decreases depending on the increase in the number of individuals in their households. In a study conducted in Pakistan, it was found that there was a significant and negative relationship between household size and women's labor force participation (Ejaz, 2007). Accordingly, the results of this study are congruent with those of the study conducted in Pakistan. In Turkey, it is known that whether women are married or not has significant effects on labour force participation ratio and that married women participate less in the labour force. Also, since women who are married and have children have more responsibilities, working or participating in the labour force becomes more difficult for them (Er, 2013). Due to the fact that the number of individuals in the household of women is 3 or more leads to increase their responsibilities such as food, care, laundry, it is thought that their probability of participating in the labour force is hindered. Therefore, women's labour force participation rate is expected to decrease due to the increase in the number of individuals in the household.

In Gokceada district, it was found that there was a significant and negative relationship between the income level of women and their willingness to market their home-made food products. This result shows that the willingness of women's home-made food product marketing decreases depending on the increase in their income levels. In a study conducted in

the Thrace region, it was found that when the income level of women producers increased, their willingness to market home-made food products decreased (Özdemir et al., 2017a). In this context, the results of this study are similar to the findings obtained from the Thrace region. According to these results, it can be stated that the willingness to market their home-made food products of women who want to have their own income in Gokceada district is higher than other women. Therefore, it can be said that the willingness to market their home-made food products of women with low monthly income (<€460.9) or without their own income will increase. This situation also reveals the importance of women's economic independence. Based on this situation, it is expected that encouraging women who do not have their own income for home-made food products will have an impact on spreading this field of activity. Furthermore, it is thought that the production and marketing of home-made food products will gain more importance over time.

In the study area, it was found that there was a significant and positive relationship between the own agricultural land assets of women and their willingness to market their home-made food products. This result shows that the willingness to market home-made food products of women who have own agricultural land is higher than other women. In a study conducted in Pakistan, it was determined that having own agricultural land assets had a positive effect on women's labour force participation (Ejaz, 2007). Thus, the results of this study are similar to the study conducted in Pakistan. Based on these findings, the fact that women have their own lands facilitates the supply of the raw materials necessary for the production of home-made food products and affects the production positively because 91.3% of women who own land cultivate their existing land. Based on this fact, it can be said that women gain income from home-made food products, both by selling their own products and by providing the raw material required for the production of these products Therefore, it can be said that the increase of land assets belonging to women and the cultivation of their existing lands have a positive effect on the women's willingness to market their home-made food products.

In the present study, it was found that there was a significant and positive relationship between the status of animal husbandry of women and their willingness to market their home-made food products. This result shows that the willingness to market home-made food products of women who engaged in animal husbandry is higher than compared to women not engaged in animal husbandry. In a study conducted in Pakistan, it was determined that having an animal presence had a positive impact on women's labor force participation (Ejaz, 2007). Thus, the results of this study are similar to the study conducted in Pakistan. Based on these results, it can be said that women who engaged in animal husbandry in the district provide the raw materials required for home-made food production from this field activity and continue this activity to obtain additional income.

In Gokceada district, it was a significant and positive relationship between the status of continuing to home-made food production of women and their willingness to market home-made food products. This shows that the willingness to market their home-made food products of women who want to continue home-made food production is higher than women who do not want to continue home-made food production. Based on this situation, increasing the tendency of women to continue home-made food production is expected to have a positive effect on increasing their willingness to produce and market these products.

In the study area, it was found that there was a significant and positive relationship between the status of the main source of income of women and their willingness to market their home-made food products. This shows that the willingness to market home-made food products of women whose main income is home-made food production is higher than women whose main income is not home-made food production. Therefore, it is expected that women whose main source of income is home-made food production want to gain more income and show more tendency to continue this field activity to provide more marketing opportunity by promoting these products.

Conclusion

As a result, it is thought that it is important to raise more awareness and encourage young women about this activity compared to older women for the widespread and continuity of the home-made food production tendencies of women in Gokceada district. In addition, women who don't have their income or who want to gain economic independence can be supported about the production of home-made food products. Generally, women who are married and have children have more responsibilities in household. Therefore, working or participating in the labour force is more difficult for them. Hence, it is important to determine strategies that will increase the labour force participation rate of married women with children. Furthermore, women who belongs to nuclear family and has fewer children are more likely to produce home-made food products. Women who have their own agricultural land should be supported to cultivate their agricultural lands and their tendency towards agricultural activities should be encouraged to earn additional income. This situation will increase women's home-made food products marketing willingness. Supporting women, whose main source of income is home-made food production and who tend to continue producing home-made food, has a vital role in eliminating their shortcomings in production and marketing.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

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Not applicable.

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Determination of the preferred two different coccinellid species on different aphid species feeding on broad bean plants

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Abstract

In this study, the orientation of two different predator species [(*Coccinella septempunctata* L., *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae)) to two different aphids [*Aphis fabae* Scopoli and *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae)] was investigated. Y-type olfactometer which was made of glass material with one entrance and two exit openings was used in the study. Different applications were applied to the exit ends of the olfactometer and the orientation of the predators dropped from the entrance end was examined. Individuals passing the marked area (10 cm) on the olfactometer were considered to have turned to that plant. All of the trials were conducted separately for each predator and prey with 10 replications. The counts of the insects advancing on the arms of the olfactometer were made 1, 4 and 8 hours after the release. When looking at the data obtained in the first stage of the study, it was determined that the predator insects mostly gravitate towards the leaves infected with aphids; In the second stage, it was observed that *C. septempunctata* individuals mostly turned towards the side that was contaminated with *A. pisum*, and *H. variegata* individuals mostly turned towards the side that was contaminated with *A.fabae*. When looking at the data obtained from the study, it was determined that the plants damaged by herbivorous insects show an attractive feature for predators. Accordingly, it was concluded that chemicals obtained from broad bean plants damaged by aphids should be analyzed.

Keywords: *Coccinella*, *Hippodamia*, *Aphis fabae*, *Acyrthosiphon pisum,* Olfactometer

Introduction

Living organisms are in constant interaction with each other and with the inanimate environment in which they live (Odum and Barrett, 2005). In this context, arthropods, like all living things, are under the influence of chemicals secreted by the plants in their environment. Accordingly, both herbivores and their natural enemies benefit from these chemicals in finding their food (Bell and Cardé, 1984; Visser, 1986; Roitberg and Isman, 1992; Vet and Dicke, 1992; Cardé and Bell, 1995; Schoonhoven et al., 1998). It is also known that these chemicals are used for defense purposes in plants (Dicke and Vet, 1999; Vet, 1999). When the researches in recent years are examined, it is observed that the reactions of herbivores after the damage on the plant are focused on, and how these reactions affect the herbivores and natural enemies (Price et al., 1980; Turlings et al., 1991; Vet and Dicke, 1992; Thaler, 1999; Kessler and Baldwin, 2001; Becker et al., 2016; Giunti et al., 2016; Lin et al., 2016; Gençer et al., 2017; Silva et al., 2017). In order to achieve positive results in the control of harmful organisms in agriculture, the host and foraging behaviors of the factors used in biological control should be known (Tunca et al., 2011).

Aphids are harmful organisms that cause economic losses around the world. As a result of feeding on the plant, they stop growing and if the population reaches high numbers, they kill the plant on which it is fed. This pest group causes indirect losses in plants due to their secretion of toxic substances and

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carrying virus diseases (Lodos, 1982; Catherall et al., 1987; Kovalev et al., 1991; Elmalı and Toros, 1997; Blackman and Eastop, 2000). Among the hosts of *Aphis fabae* are more than 200 wild plants as well as vegetables, sugar beet, broad beans, beans, potatoes (Völkl and Stechmann, 1998; Barnea et al., 2005; Fericean et al., 2012). Although Acyrthosiphon pisum is a pest of weeds, it also causes damage to beans, lentils, alfalfa, sainfoin and some legumes (Stary, 1970; Ali and Habtewold, 1994).

Coccinellidae is one of the families that are effective in biological control (Khan et al., 2007). *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) belonging to this family, is a species living in the Palearctic region (Central and North Africa, Europe, Arabia, India and China) (Korchefsky, 1932; Horion, 1961). The subject of our study is polyphagous and has been observed especially on aphids that feed on weeds. In addition, species belonging to Aleyrodidae (Hemiptera) and Chaitophoridae (Hemiptera) families are among the groups they feed on (Horion, 1961; Klausnitzer, 1966; Elmalı and Toros, 1994; Aslan and Uygun, 2005; Elekçioğlu and Şenal, 2007). *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), an important aphid predator, is an oval-shaped and 6-8 mm long (Uygun, 1981) and very common species in the palearctic region (Korcschefsky, 1932; Horion, 1961). This predator mostly feeds on aphids; in addition, it has been reported that they are effective on soft bodied insects that cause damage to plants (Ali and Rizvi, 2009).

Beneficial insects are sensitive to chemical aspects of the multitrophic environment, particularly host location (Poppy, 1997) and can learn to associate plant volatiles in the presence of prey (Drukker et al., 2000). Leaves of plants normally secrete small amounts of volatile compounds, but when a plant is damaged by an insect, an increase is observed in the amount of compounds secreted (Reddy, 2012). Beneficial organisms respond significantly to volatile substances released from plants after damage caused by herbivores (Turlings et al., 1990). Predators use numerous clues released by plants alone or when damaged to locate their prey in their natural habitats (Vet and Dicke, 1992). Looking at the studies conducted, it is observed that predator insects use semiochemicals to find their prey (Ninkovic et al., 2001). These volatile chemicals released by plants may differ in different plant and herbivore combinations (Boom et al., 2004). Looking at the studies conducted, it is seen that mass production can be made to use coccinellids against aphids and other harmful organisms. Coccinellids' ability to distinguish odors is effective here. Due to the presence of volatile compounds released by plants due to the feeding of herbivores, coccinellids can distinguish the attacked plant (Dicke, 2009; Heil, 2008).

Considering the researches carried out in the world, the reproductive potential of aphids and their damages on the products are quite high. Farmers prefer intensive chemical control against these pests. This event negatively affects the environment and human health. In order to prevent these negative effects, there are different methods. Accordingly, in this study, predator behaviors of *H. variegata* and *C. septempunctata* (*A. fabae* and *A. pisum*) were investigated.

Materials and Methods

The main materials in the study are two different predators [*Coccinella septempunctata* L., *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae)], Broad bean (*Vicia faba* L.) and two aphids [*Aphis fabae* Scopoli and *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae)]. In the study, all plants, hunts, hunters production and experiments were carried out in Isparta Applied Sciences University, Faculty of Agriculture, Plant Protection Department, Biological Control Research and Application Laboratory.

Production of Plants

The broad bean seeds used in the experiments were planted in 2-liter pots with a soil: peat: perlite mixture at a ratio of 1: 1: 1. After this process, the pod seeds were expected to germinate, after the germination process, the daily maintenance of the plant was made and no chemicals were used in pest controls on the plants. Plant production was carried out in climate cabinets with 27±1 °C temperature and 65±5% proportional humidity and long day lighting (16: 8) conditions.

Culture of Aphids

Aphis fabae and *Acythosiphon pisum* individuals used as food in the study were obtained from mass production in the laboratory. Aphids were transferred to clean plants in separate net cages with the help of a sable brush and their reproduction was achieved. Then, clean plants were left next to the infected plants with the increased aphids and aphids were passed on to the clean plants. This process was carried out as long as the trials continued. All of the aphid production was carried out in climate chambers with 27±1 °C temperature and 65±5 % relative humidity and long day lighting (16: 8).

Providing Predators from Nature

Polyphagous hunters *H. variegata* and *C. septempunctata* were collected from agricultural production areas with aphid damage with the help of a pad, taken to a container with their food, brought to the laboratory and mass production started.

Culture of Predators

H. variegata and *C. septempunctata* individuals who were used as hunters and collected from field conditions in the study were brought to the laboratory and put into mass production. In this process, cages made of plexiglass material covered with tulle on the sides and top are used. In order to make the production easy and fast, hunter individuals were left separately in the cages with aphids used in the experiments and the individuals used in the experiments were obtained from these productions. All of the hunter productions were carried out in climate chambers with 27 ± 1 ° C temperature and 65 ± 5% proportional humidity and long day lighting (16: 8).

Establishment of Trials

In the study, a Y-type olfactometer made of glass material with an entrance and two exit openings was used. In addition, an air pump has been used to keep the air flow in the arms of the olfactometer moving regularly. In the first stage of the trials, a clean plant leaf was placed at one of the exit ends of the olfactometer and a leaf infected with aphids was placed at the other end. Then, 10 hunter individuals were placed at the entrance end of the olfactometer and they were expected to move. In the second stage of the experiments, the plant

contaminated with *A. fabae* was placed on one end of the olfactometer and the plant infected with *A. pisum* on the other end, and the movements of the predator individuals released from the entrance end were observed as in the first stage. Individuals passing the marked area (10 cm) on the olfactometer were considered to have turned to that plant. All of the trials were conducted separately for each hunter and food with 10 replications. The counts of the insects moving on the arms of the olfactometer were made 1, 4 and 8 hours after the release. These experiments were carried out in climate chambers with 27 ± 1 ° C temperature, 65 ± 5% relative humidity and long day lighting (16: 8).

Results and Discussion

In this study, two different predator insects (*C. septempunctata*, *H. variegata*) and two aphids (*A. fabae* and *A. pisum*) were investigated with the help of an olfactometer. In the first stage of the experiment in which the orientation of *Coccinella septempunctata* was examined (1 arm of clean plant and the other plant infected with aphids), it was determined that the predator insect mostly turned towards the arm where the infected plants were found ($p<0.05$). According to the counts made at the end of the 8th hour in this part of the study, the hunter insect preferred the arm with *A. pisum* rather than the arm with *A. fabae* (Table 1). At this stage, two different aphids and a broad bean plant were placed at the two exit ends of the olfactometer and the predator's orientation was investigated. *Coccinella septempunctata* individuals showed more orientation towards the arm with *A. pisum* than the counts made at the end of the 4th hour ($p <0.05$) (Figure 1, Table 2).

Table 1. Amounts of *Coccinella septampunctata* at the different hours to be directed to clean broad bean plant with two different aphids

Coccinella septempunctata	**Infested**	**Cleaned**	**P**	**F**
Application of *Acyrthosiphon pisum* (1 h)	1.8±0.249 a*	0.7±0.153 b	0.001	14.14
Application of *Acyrthosiphon pisum* (4 h)	4.1±0.379 a*	0.7±0.213 b	0.001	61.2
Application of *Acyrthosiphon pisum* (8 h)	8.0±0.258 a*	1.3±0.153 b	0.001	498.78
Application of *Aphis fabae* (1 h)	1.7±0.260 a*	0.6±0.221 b	0.005	10.37
Application of *Aphis fabae* (4 h)	4.4±0.306 a*	0.4±0.221 b	0.001	112.5
Application of *Aphis fabae* (8 h)	7.5±0.307 a*	1.1±0.233 b	0.001	275.5

*Different letters on the same line indicate that there is a statistical difference between the averages according to the Tukey multiple comparison test

Table 2. Amounts of *Coccinella septampunctata* turning to broad bean plant with two different aphids at different times

Coccinella septempunctata	***Aphis fabae***	***Acyrthosiphon pisum***	**P**	**F**
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (1 h)	1.4±0.163 a	1.3±0.213 a	0.714	0.14
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (4 h)	3.5±0.167 a*	4.5±0.224 b	0.002	12.86
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (8 h)	4.4±0.267 a	5.0±0.333 a	0.177	1.98

*Different letters on the same line indicate that there is a statistical difference between the averages according to the Tukey multiple comparison test

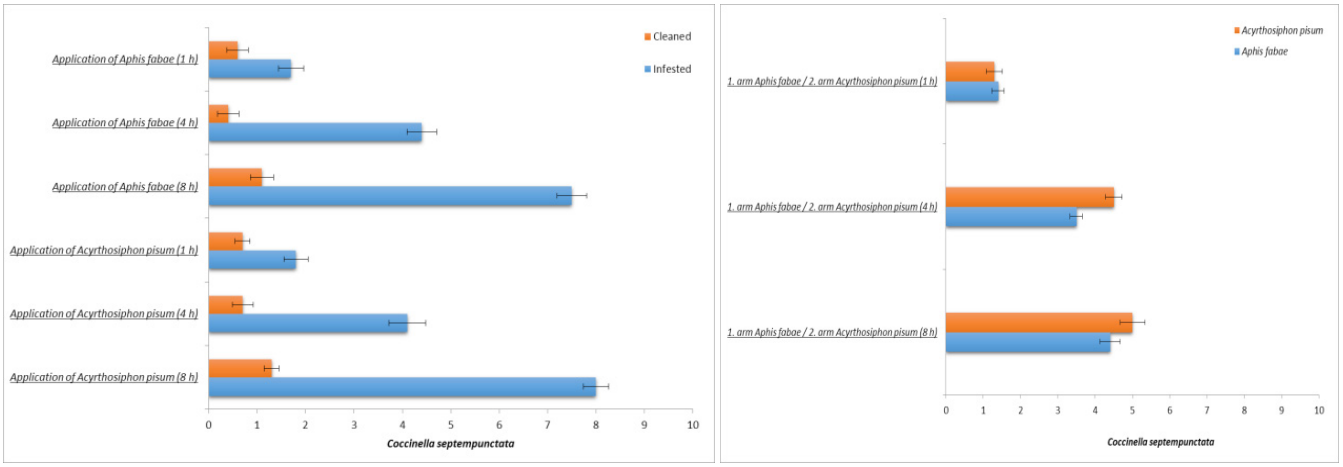

Figure 1. The amount of *Coccinella septempunctata* moving to aphids at different times

In the second stage of the experiment in which the orientation of *Hippodamia variegata* was examined (1 arm of clean plant and the plant infected with the other arm aphid), it was determined that the hunter insect mostly directed towards the arm where the infected plants were ($p < 0.05$). According to the counts made at the end of the 8th hour, the hunter insect preferred the arm with *A. fabae* rather than the arm with *A. pisum* (Table 3). At this stage, two different aphids and a broad bean plant were placed at the two exit ends of the olfactometer and the predator's orientation was investigated. *Hippodamia variegata* individuals showed more orientation towards the arm with *A. fabae* than the counts made at the end of the 4th and 8th ($p < 0.05$) (Figure 2, Table 4).

Table 3. Amounts of *Hippodamia variegata* at different hours to be directed to clean broad bean plant with two different aphids

Hippodamia variegata	**Infested**	**Cleaned**	**P**	**F**
Application of *Acyrthosiphon pisum* (1 h)	1.6±0.306 a*	0.7±0.213 b	0,027	5,83
Application of *Acyrthosiphon pisum* (4 h)	4.1±0.379 a*	0.7±0.213 b	0,001	61,2
Application of *Acyrthosiphon pisum* (8 h)	7.6±0.267 a*	1.1±0.233 b	0,001	336,5
Application of *Aphis fabae* (1 h)	1.6±0.221 a*	0.5±0.167 b	0,001	15,78
Application of *Aphis fabae* (4 h)	4.8±0.249 a*	0.5±0.269 b	0,001	137,53
Application of *Aphis fabae* (8 h)	8.0±0.258 a*	0.7±0.213 b	0,001	474,86

*Different letters on the same line indicate that there is a statistical difference between the averages according to the Tukey multiple comparison test

Table 4. Amounts of *Hippodamia variegata* turning to broad bean plant with two different aphids at different times

Hippodamia variegata	***Aphis fabae***	***Acyrthosiphon pisum***	**P**	**F**
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (1 h)	1.5±0.224 a	1.0±0.255 a	0,16	2,14
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (4 h)	4.5±0.167 a*	3.7±0.213 b	0,008	8,79
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (8 h)	5.4±0.221 a*	4.3±0.260 b	0,005	10,37

*Different letters on the same line indicate that there is a statistical difference between the averages according to the Tukey multiple comparison test

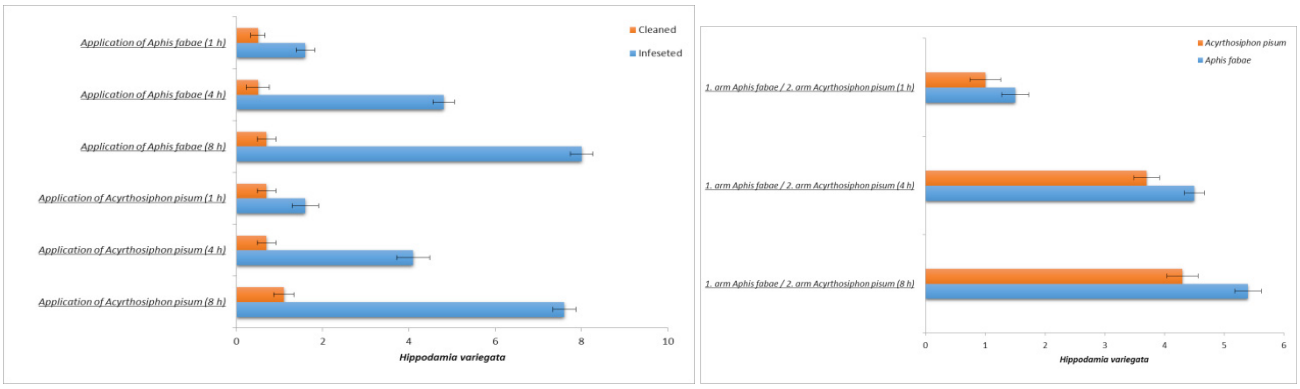

Figure 2. The amount of *Hippodamia variegata* moving to aphids at different times

De Moares et al. (1998) stated that volatile compounds secreted from the plant vary according to the pest and that these compounds are detected by parasitoids. According to the data obtained, when they were damaged by *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) and *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae), which are harmful on some products (cotton, corn and tobacco), the mentioned products secreted different compounds. De Moares and Lewis (1999) determined that the compounds released from cotton and tobacco plants damaged by some herbivores have attractive effects on *Cardiochiles nigriceps* (Vier.) (Hymenoptera: Braconidae) and *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). Llusià and Peñuelas (2001) conducted a study with two apple varieties (Golden Delicious and Starking) damaged by *Pananychus ulmi* Koch (Acarina: Tetranychidae) whether the substances secreted due to the effects of the pests attract predatory mites (*Amblyseius veersoni* Chant and *A. californicus* McGregor). They investigated. In their experiments with the

olfactometer, they observed that the damaged plants attracted 85% of the predatory mites. Lin et al. (2016) examined the orientation of *Propylaea japonica* with an olfactometer. As a result, *P. japonica* has been observed to react to volatile organic compounds from citrus plants damaged by *Diaphorina citri* and *Candidatus liobacter*.

When looking at the studies conducted in recent years, different studies on the compounds released into the environment after herbivores damage plants and how these affect natural enemies stand out (Price et al., 1980; Turlings et al., 1991; Vet and Dicke, 1992; Bernays and Chapman, 1994; De Moares et al., 1998; Dicke et al., 1990; Dicke et al., 1993; Dicke et al., 1994; Geervliet et al., 1994; Mattiacci and Dicke, 1995; Rosenthal and Berenbaum, 1992; Schoonhoven et al., 1998; Thaler, 1999; Kessler and Baldwin, 2001; Becker et al., 2016; Giunti et al., 2016; Lin et al., 2016; Gençer et al., 2017; Silva et al., 2017). When plants are damaged by herbivores, some volatile compounds and secondary metabolites are released as a defense behavior. It has been determined in researches that these compounds secreted have attractive effects on many parasitoids and predators (Kester and Barbosa, 1991; Lecomte and Thibout, 1984; Mattiacci et al., 1994; McAuslane et al., 1991; Paré and Tumlinson, 1999; Steinberg et al., 1993; Udayagiri and Jones, 1992; 1993).

Conclusion

When the data obtained as a result of the study were evaluated, it was determined that both predator insects were directed towards plants damaged by aphids. While *C. septempuntata* individuals, among the predatory insects used in the experiments, mostly turned to the side where *A. pisum* was; It has been determined that *H. variegata* individuals mostly turn to the side where *A.fabae* is located.

Considering the data obtained from the study, it was determined that plants damaged by herbivorous insects show an attractive feature for predatory insects. Accordingly, it was concluded that by analyzing the chemicals obtained from the broad bean plant damaged by aphids, it was concluded that experiments should be conducted to determine to what extent which compound would affect the predator insects.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Total Phenolic Content and Antioxidant Activities of Invasive Erigeron annuus Pers. (Asteraceae) from Different Localities

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Abstract

Erigeron annuus Pers. is a harmful invasive species to natural flora, although it is used in the treatment of indigestion, hepatitis, lymphadenitis, enteritis, and hematuria in traditional medicine. In this study, *E. annuus* samples were investigated in terms of total phenolic content, antioxidant activities and invasive features. Aerial parts of *E. annuus* were collected from Ayder-Çamlıhemşin (Rize), Pazar (Rize), and Trabzon (Turkey). Total phenolic contents, free radical scavenging characteristics against 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and 2,20-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) radicals (ABTS) were analyzed and compared with the collected localities. The invasive feature helps *E. annuus* to adapt everywhere. However, invasion of the plant is not an issue for medicinal applications except the collecting place of the plant. *E. annuus* is a lead accumulator, and the plant is seen on roadsides. Thus, the collecting region of the species should be chosen carefully to not obtain the side effects of heavy metals.

Keywords: Lead accumulation**,** Ecology, TPC, DPPH, ABTS

Introduction

Plant samples found in Neanderthal tombs show that human-plant relationships go back 50,000 years (Solecki, 1972). Human beings have used plants for various purposes in terms of food and medicine. Moreover, ethnobotanical studies are still applied to find new drug active substances. Owing to the studies carried out with plants and their chemical contents, new applications are being developed each day.

Since Reactive Oxygen Species (ROS) cause uncontrollable diseases. The situation occurs when natural by-products of oxygen cause oxidative stress in the brain (Olanow, 1993). On the other hand, antioxidant activities of herbal natural products increase the quality of applications and help prevent chronic diseases (Yu et al. 2003).

In this paper, *Erigeron annuus* Pers. samples were investigated in terms of TPC and antioxidant properties by comparing their invasive properties. While the species is used in traditional Chinese medicine to treat indigestion, hepatitis, lymphadenitis, enteritis, and hematuria (Jo et al., 2013), and as a hypoglycemic agent in Japanese ethnomedicine (Miyazawa and Kameoka, 1979). Besides, various compounds have been isolated from *E. annuus* in scientific studies (Song, et al., 2016; Nazaruk and Kalemba, 2009; Nam et al., 2008; Lis et al., 2007; El-Razek, 2006; Iijima et al., 2003a,b; Hashidoko, 1995), has aldose reductase inhibitory, antioxidant and neuroprotective (Bakar et. al., 2015; Jeong, et al., 2011; Jang et al., 2010; Kim, et al., 2005), anti-inflammatory effects (Yi et al., 2016; Jo et al., 2013; Sung, et al., 2011), anti-cancer (Nazaruk et al., 2014; Réthy, et al., 2007), anti-tumour (Li et al., 2006), antifungal (Kumar et al., 2014), anti-obesity (Choi et. Al., 2019), and anti-diabetic activities as well (Kim et. al., 2009).

Indeed, *E. annuus* is an invasive species native to North

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America and naturalized to Europe, Asia and Australia with the influence of humans (Frey, 2003). However, *E. annuus* is a medicinal plant and is dangerous for the natural flora of countries. More importantly, the species is on the 150 most widespread weed list in Europe. Besides, the plant is one of the most dangerous species in Serbia, Hungary, and Slovakia with rapid development and phenotypic flexibility of the species (Pacanoski, 2017). Furthermore, *E. annuus* is a lead accumulator (Bi et al., 2005) and might be the other reason for the invasive trait of the plant.

Hence, in the present study, antioxidant activities of *E. annuus* collected from three varied localities were investigated. Antioxidant activities were tested with DPPH and ABTS, while TPC was determined by the Folin-Ciocalteu method. Therefore, the study aims to compare the relationship between the spreading properties, TPC and antioxidant activities of *E. annuus*.

Materials and Methods

Reagents and Standards

Folin–Ciocalteu standard, sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), L-Ascorbic acid, and sodium persulfate, ethanol and methanol were purchased from Merck (Darmstadt, Germany).

Plant Material

Aerial parts of *E. annuus* were collected in August 2017 from Northeastern Turkey (Table 1). Plant materials were dried in shadows at room temperature. Plant extracts were prepared by methanol in an orbital shaker for 27 hours. After the evaporation process at 40°C, dried extracts were kept at 4°C in a dark place until analyses.

Table1. Information about the sample area of invasive *E. annuus*

Place	Geographical Location
Pazar	N 41°10'55" E 40°54'11"
Ayder	N 40°57'3" E 41°7'18"
Trabzon	N 40°55'47" E 39°44'1"

Determination Procedure of Total Phenolic Contents (TPC)

TPC of extracts was determined with Folin–Ciocalteu method (Singleton et al., 1999), to 4.0 ml distilled water, 50 µl of the sample, 250 µl Folin–Ciocalteu, and 750 µL Na_2CO_3 was added after 2 h incubation at 25 °C and was measured at 760 nm, the results were calculated as average values of gallic acid equivalent (GAE). Analyses were performed in triplicate.

Analyses of Antioxidant Activities

DPPH·Assay

The samples were estimated on DPPH according to Brand-Williams et al. (1995). DPPH was prepared in 2 mg/25 ml of methanol. Dilutions were implemented with stock solutions of extracts. Diluted plant extracts were mixed with DPPH and put for 30 min at room temperature. The UV absorbance was read at 517 nm. Gallic acid was the control group. The scavenging activity of the DPPH was calculated with the following equation:

DPPH Scavenging Effect % = [(A0 - A1)/A0] x 100

A0 is the control group and A1 is the sample. Analyses were performed in triplicate.

ABTS Assay

ABTS was prepared by 7 mM ABTS and 2.5 mM sodium persulfate and put for 12-16 hours at room temperature in dark. ABTS standard was diluted with ethanol to be absorbance 0.8 to 0.7 at 734 nm in a spectrophotometer. Trolox was used as the antioxidant standard. 10 µL sample was mixed with 990 µL ABTS. The samples were read at 734 nm in spectrophotometer after 30 min incubation at room temperature (Re et al., 1999). Gallic acid and ascorbic acid were used as control groups. Analyses were performed in triplicate.

Statistical analysis

Statistical analyses were applied using SPSS 10.0.1. (SPSS Inc., Chicago, IL). The data were submitted as mean values. Analysis of variance (ANOVA) was implemented by ANOVA procedures.

Results and Discussion

E. annuus samples were collected from the North-Eastern part of Turkey (Rize and Trabzon). The region is specific to temperature differences and precipitation throughout the year (Okcu and Karabulut, 2019). Besides, the average annual temperature of Rize and Trabzon do not increase above 15.2 °C, nor fall below 13 °C (Polat and Sunkar, 2017). However, excessive rainfall causes podzolization in soils of the Eastern Black Sea region. More importantly, Rize and Trabzon provinces demonstrate red-yellow podzolic soil characteristics (Ozyazici et al., 2013). Thus, these soil and climate features are the similarities of studied plant samples.

The TPCs of *E. annuus* samples were shown in Figure 1. In the study, TPC values of the samples were listed as Pazar ≥ Ayder > Trabzon. According to study results, Pazar and Ayder localities were closer to each other than Trabzon. Thus, the study result can be the reason for this feature. Moreover, TPC might change with plant species, applied method, used solvent, ecological conditions, harvesting season and used plant part (Skotti et al., 2014). Nevertheless, in the present study, different TPCs are not influenced by all counted reasons, except ecological factors.

Applications of medicinal plants are the results of their antioxidant activities. Besides, the determination of *in-vitro* antioxidant activities of medicinal plants should be supported with at least two antioxidant activity assays (Schlesier et al.; 2002). Therefore, in the present study, DPPH and ABTS tests were used for *Erigeron* samples. Moreover, Asteraceae members are known for high antioxidant activities (Michel et al., 2020). In the present study, methanol was used to prepare

plant extracts, and the Ayder sample demonstrated the highest activity for DPPH (Figure 2), Trabzon and Pazar samples had close results to each other. Moreover, in the study of Lee and Seo (2006), *E. annuus* has represented potent activities on the peroxynitrite and DPPH radical for DPPH testing with methyl hydrogen peroxide and butanol fractions. In turn, DPPH activities of *E. annuus* samples are elevated. However, the differences in DPPH might be the results of accumulated metals, soil chemicals and stress tolerance of the samples.

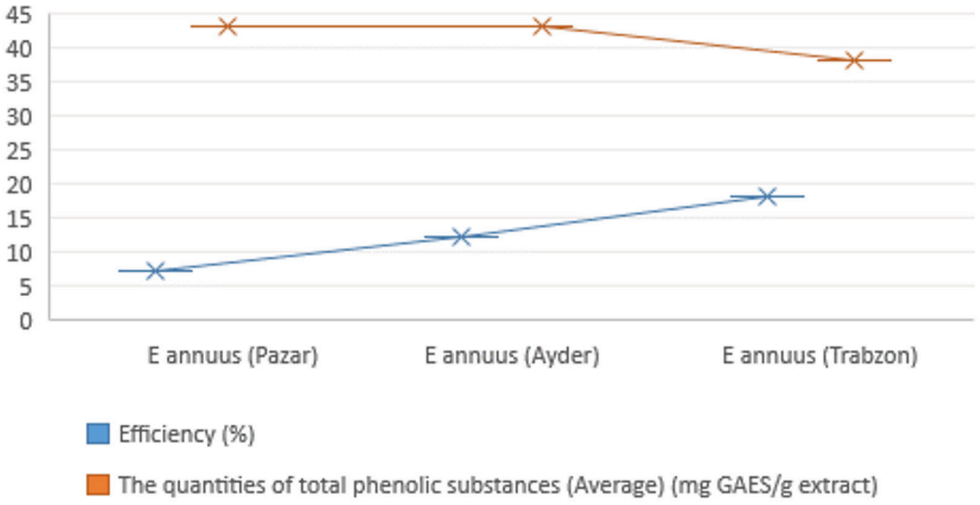

Figure 1. The efficiencies and quantities of total phenolic substances of extracts

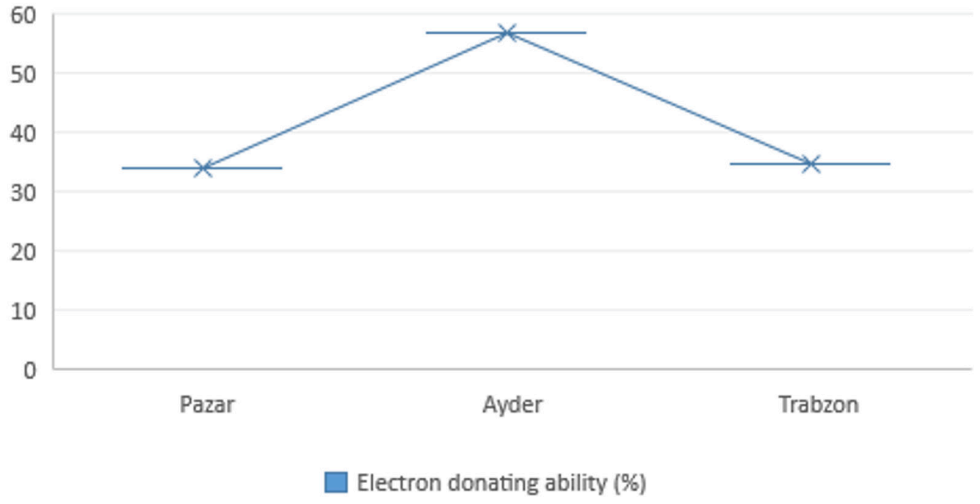

Figure 2. DPPH radical scavenging activities of *E. annuus* crude extracts

According to the ABTS test, three *E. annuus* samples showed the same results equivalent to 3 mM Trolox (Table 2). Moreover, in the study of Jeong et al. (2011), the butanol fraction of *E. annuus* has represented the highest antioxidant activity for the ABTS test. Following the DPPH results, radical scavenging activities of the ABTS experiment might be similar to environmental factors, or different antioxidant activity results may be associated with heavy metal accumulation. Therefore, *E. annuus* demonstrates lead accumulation properties (Bi et al., 2005). Besides, the metal uptake of plants is affected by the metal concentrations of the soil, the cation exchange capacity, the pH of the soil, the plant's organic content and the age of the plant (Annan et al., 2013). Hence, environmental factors and genetic inheritance interact together to adjust the chemical content of plants (Li et al., 2010).

Table 2. ABTS activities of *E. annuus* samples

Extract	Antioxidant radical scavenging activity equivalent to Trolox (mM)
Pazar	3
Ayder	3
Trabzon	3

On the other hand, high phenolic content is associated with high antioxidant activity (Soobrattee et al., 2005). Similar results were determined in the present study, the highest TPCs was observed in Ayder and Pazar samples, but DPPH was the highest in the Ayder sample only, and for the ABTS test all three samples demonstrated similar results. Thus, as similar to the study of Yu et al. (2003), these results reveal accumulation of heavy metal content, soil chemicals and other environmental factors affecting antioxidant properties. However, phenolic contents protect plants against UV radiation (Zhou et al., 2016); and altitude may be associated with the phenolic activity (Guo et al., 2011). Interestingly, in the present study, Ayder samples were collected from the highest altitude, and they ranked first place for TPC and DPPH tests.

Indeed, during the field works, *Erigeron* samples were seen mainly on the roadsides. Besides, road traffic causes a high amount of heavy metal accumulation. Hence, *E. annuus* accumulates heavy metals. Nevertheless, according to the World Health Organization (WHO, 1998), before collecting plants for food or medicinal applications, the localities of plant species should be chosen carefully to not accumulate heavy metals in our body as well.

Conclusion

Lead accumulation feature helps invasion of *E. annuus*, besides, the ecological adaptation of the species supports invasive characteristics for long distances as well. Moreover, *E. annuus* is utilised as a medicinal plant for different applications. Therefore, the invasive feature is not an issue for the medicinal approach, however, local people should be careful about collecting places of the herb. Importantly, the plant should not be collected from heavy traffic regions to not cause the side effects of the species such as heavy metal toxicity. Moreover, the species needs further studies regarding heavy metal accumulation and medicinal applications.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Current perspectives on the Nutrient composition and health benefits of yams (*Discorea* species)

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Abstract

Yams (*Discorea* spp) are food security crops which serve as invaluable food sources of energy, micronutrients and phytochemicals with numerous health benefits. They show a lot of genetic and compositional diversity. New biologically active compounds are being identified from wild yams. This review focuses on the nutrients, and phytochemicals found in yam species and their health benefits. The B group of vitamins, vitamin C, pro-vitamin A; copper; manganese; isoflavones; steroidal saponins; alkaloids; terpenes; and anthocyanidins are found in different species of yams. Wild yams such as *Discorea villosa* and *Discorea hispida* are used in ethnomedicine because of their phytochemical content. Diosgenin, dioscorin, dioscin dihydrodiscorine protodioscin, methyprotodioscin, prosapogenin, epifzelechin, glucopyranoside, phytoestrogen and allantoin are bioactive phytochemicals that may be found in yams. They are valuable as anti-cancer, anti-mutagenic, anti-inflammatory, anti-diabetic, anti-ageing lipid lowering and hepatoprotective agents. Other claims based on traditional uses of yams have not been scientifically substantiated. *Dioscorea* species are likely to contain other unidentified secondary metabolites. *Discorea* species with other health promoting bioactive compounds should be identified; modern techniques should be used to extract yet unidentified compounds in *Discorea* and the food use and health benefits of yam species should be optimized.

Keywords: Yams, Composition, Nutrients, Phytochemicals, Health benefits

Introduction

Yams

Yam is a basic food that is well consumed in the tropical and humid regions of South America, Africa, and South- east Asia (Achi, 2000; Sanusi and Salimonu, 2006; Chukwu, et al., 2007). It is a staple food in many parts of West Africa and contributes significantly to food security in the region (Kouakou, 2010; Amanze et al., 2011). It is cultivated in Africa (West Africa; parts of East, Central and South Africa), Southern Asia (China, Japan and Ocenia), the Caribbean (Mexico and parts of Central America) (FAO, 2008; Polycarp et al., 2012). Yams are used as carbohydrate staples in parts of Africa and Asia (Barton, 2014). Nigeria is a major producer of yams accounting for up to 70% of the global production. *Discorea rotundata*, *Discorea esculenta*, *Discorea cayenensis* and *Discorea alata* are the most widely cultivated species. The yam most commonly bought and consumed in first world nations is White Yam (*Dioscorea rotundata)* followed by Purple Yam (*Dioscorea alata)*.

Diversity of yam species

Yam tubers are native to Africa and Asia: Nigeria, Ghana, Ivory coast, Benin, Ethiopia, Togo,Cameroon,Central African Republic, Chad and Cuba. Yams have genetically diverse species. A lot of research efforts have been geared towards the selection of important species from a variety of wild

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and domesticated species for improved nutrition and health. Obidiegwu and Akpabio (2017) investigated the geography of yam cultivation in Southern Nigeria, while Okoye et al. (2013) investigated the yam consumption patterns in West Africa. Barton (2014) documented the origin and development of yam species. Tando et al. (2015) and Muthania et al. (2013) observed the genetic diversity of Kenyan wild yams; Oben *et* al. (2016) explored the diversity of Cameroonian yams, while Debnath (2019) explored *D. alata* genotypes for better cultivation in India. Mignounia et al. (2002), investigated the isozymic diversity of *D. rotundata* and *D. cayenensis* in Cameroon. Fanyi et al. (2019) even investigated the diversity in the polysaccharides of *D. opposita* genotypes. These genetic diversities also imply variable nutrient and phytochemical composition (Wu et al., 2016). Table 1 summarizes the various yam species and their areas of production.

Table 1. Yam Species Common names and Areas of Production

Common Name	Scientific Name	Area of Production
White yam/White Guinea yam	*Discorea rotundata*	West Africa
Yellow yam/Yellow guinea yam/ Sand paper	*Discorea cayenensis*	Africa: West Africa: West Indies
Trifoliate yam/ African bitter yam	*Discorea dumetorum*	Africa
Water yam/Greater yam/Asiatic yam/purple yam/winged yam	*Discorea alata*	Found in Southeast Asia
Chinese yam/Lesser yam/bitter yam	*Discorea esculenta*	Found in Thailand, China, Southeast Asia,Western Pacific Islands,India, Norhern Australia
Aerial yam/potato yam/Air potato	*Discorea bulbifera*	Grows wild in Africa and Asia: Cultivated in South America: the Carrabean and parts of Southern US.
Asiatic bitter yam	*Discorea hispida*	Asia
Cinnammon yam	*Discorea opposita/Discorea batatas*	Cultivated in China, japan, Korea, Taiwan
Cush-cush yam/Indian yam/Yampee/ Mapuay/Inhame/Tabena	*Discorea trifida*	Northern South America; the caribbean.
Globe yam	*Discorea globosa*	India
Japanese yam/yamatoima/mountain potato	*Discorea japonica*	Japan
Wild yam	*Discorea villosa; D alata; D. batatas; D. composita; D. floribunda; D. hirticaulis; D.japonica, D. macrostachya; D. mexicana, D.opposita; D. tepinapensis*,	China, Korea, Japan India, North America, Canada, Latin America

Varieties of Yams

Although there are many yam varieties, white yam (*Dioscorea rotundata Poir*), is the most favoured yam species in West Africa because of its colour and its highly viscous starch. It is used in pounded yam preparation (which is one of the most popular and prestigious foods of West Africans) (Mozie and Okoro, 1990; Nweke, 2016). It is very palatable and a highly valued crop, worldwide (IITA, 2004). In 2019, 96.5% of global yam production was from Africa; 2.4% from the Americas; 0.7% from the Oceania and 0.4% from Asia (FAOSTAT, 2020). Nigeria is the largest producer from West Africa (>30 million tonnes) followed by Cote d'Ivoire; Ghana and Benin (with about less than 5 million tonnes each) (FAOSTAT, 2020). By 2012, world production of yams was 58.75 million tons and Nigeria produced 38 million tonnes (Verter and Becvarova, 2015). For a long time, yams have provided food for many of the world's poorest and undernourished households, and is generally valued for its numerous varieties and stable yields under conditions in which other crops may fail (Scott *et al.*, 2000).

In Nigeria, many farmers cultivate only white yam. Others cultivate white yam and other yam varieties. *D.rotundata and D. cayenensis* have been used as baseline collections for developing new hybrids. *D.esculenta* does not store as long as other yam species. It does not also contain discorine which is found in other yam species. Japanese yam (mountain potato) contains a large amount of diastase which helps digestion. The term wild yam is used for many wild species. *Dioscorea villosa* is a tuber vegetable, commonly referred to as 'Wild Yam'. In ethno medicine, it is recommended for menopausal and menstrual symptoms. 'Wild Yam' also used to refer to many other species such as *deltoidea*, *versicolor*, *bulbifera*, and *triphylla* (Bhandari *et al*., 2003; Bhandari and Kwabata, 2004a, 2004b, 2005).

Traditional uses of yams

There are many traditional uses of yam tubers and different

methods of preparation. Yam tubers have rough skin (which may be light pink to dark brown). Traditionally yams are used for food and for diverse health problems. 'Fufu' or 'pounded yam', is an African dish, prepared using yam (Nweke, 2016). The powder of Chinese yam is used to make a cake-like mass which is taken with soup, sauce or stew (Zhang et al., 2014). Yams may be eaten boiled, fried or roasted; alone or in combination with legumes, and other foods. In the humid tropical countries of West Africa, yams are one of the most highly regarded foods. In Nigeria, yams have important socioeconomic, cultural and religious functions. Yams are presented during traditional marriages, cultural festivals, burials and religious outings. They are used for traditional ceremonies, indicating the high status given to the food crop. Annual yam festivals are celebrated. In the traditional settings, yams also have a lot of ethno pharmacological applications (Kumar et al., 2017)

Nutrient Composition of Yams.

The composition of yam tubers vary. Yams are very good sources of carbohydrates, the B group of vitamins and Viamin C (Ojo and Ojo, 2009). The USDA estimates of the nutrient composition of yam compared with the nutrient content of *D. opposita* is shown in Table 2a. Many species contain phytohemicals in addition such as isoflavones, saponins (e.g. diosgenin which is a steroidal saponin), phytates, tannins, alkaloids (e.g. dihydrodiscorine), terpenes and carotenoids, isoflavones and proanthocyanidins (Bhandari and Kwabata, 2004a, 2004b; 2005).

Table 2a. USDA Nutrient Composition of Yam and nutrient composition of *Discorea opposita* (Chinese yam)

Parameter	USDA	% RDA	Chinese Yam
Carbohydrate	27.88g	21	5%
Protein	1.53g	3	3%
Fat	0.17g	0.5	0.5%
Dietary fibre	4.1g	11	11%
Minerals			
Sodium	9mg	0.5	0.5%
Potassium	816mg	17	17%
Calcium	17mg	0.5	2%
Magnesium	21mg	5	5%
Iron	0.54mg	7	7%
Vitamins			
E	0.35mg	2	2%
C	17.1mg	28.5	17.1%
K	-	-	2%
A	Beta-carotene 83ug		5%

RDA = Recommended Daily Allowance
Yam (Dioscorea spp.), raw, Nutrition value per 100 g. (Source: USDA National Nutrient data base):Wu et al., 2016

Table 2b. Proximate composition of Discorea dumetorum, Discorea alata and Discorea bulbifera

Parameters	*Discorea dumetorum*	*Discorea alata*	*Discorea bulbifera**	*Discorea hispida*
Moisture (%)	6.10-9.6	6.79	7.16-7.71	15.8-37.8
Ash (%)	2.23-3.79	2.93	2.51-3.97	0.29-1.24
Crude fat (ether extract) (%)	0.50-0.71	1.15	0.37-4.15	1.99-9.36
Crude fibre (%)	1.02-2.03	2.31	1.63-2.45	-
Protein (%)	6.74-9.12	10.27	6.82-9.62	1.13-6.20
Carbohydrates (%)	78.83-79.71	76.55	76.42-77.41	58.3-71.9

Ezeocha and Ojimelukwe, 2012. Egbuonu and Nzewi, 2016.Princewill Ogbonna and Ibeji, 2015. *Values are averages for the green , red and yellow cultivars of *D. bulbifera*.

Nature and Nutrient content of the Chinese wild yam

Dioscorea opposita is the Chinese wild yam which can be eaten raw. It is smaller than the African yam. Chinese Yam (*Dioscorea opposita*) is called "Huai shan" in China. In Korea, it is called "Seyeo" In Vietnam it is called "Khoai mai" In Japan it is called "Nagaimo". It is a swollen cylindrically shaped rhizome which functions as a storage organ that holds nutrient for the plant. The vine of this plant is almost 3 meters long. A thin outer skin covers the white flesh. The fresh yam has a crispy texture and gives out a slippery mucous like

substance similar to ladyfingers. The dried flour from the yam has a powdery texture. It has a taste which is mildly similar to that of potato. The yam is presently grown in China, Korea and Japan. Its nutrients are quite different from many other yams and are listed in Table 2a. Its potassium, iron and dietary fibre contents are relatively high when compared with other tubers. Its carbohydrate content is lower than other yam varieties.

Comparative evaluation of yam nutrients

Table 2a,and b show the compositon of different yam species. Composition of basic nutrients vary with the yam species. Several authors have observed different values for proteins (up to 10%) for *D. alata* and as low as 1% for some varieties of *D. hispida*. Chinese yam has been identified to have up to 11% dietary fibre. It can therefore be observed that composition varies with the yams variety as well as the climatic, soil and agronomic conditions.

Processing methods also affect the proximate composition and nutrient content of yams. Fermentation for about 72 h increased the protein, fat, crude fibre and fat contents but decreased the carbohydrate content of *D. dumetorum* and *D.alata* yam species (Ezeocha and Ojimelukwe, 2012).

Carbohydrate

Yam is a good source of energy; 100 g provides 118 calories. It also contains a lot of complex carbohydrates. Yams contain more than 20% of the daily requirements for carbohydrates. Yam carbohydrates provide energy and promote good mitochondrial function. Yams are also good sources of dietary fibre (species like the Chinese yam contain good amounts of dietary fibre). Yam carbohydrates are found mainly in the form of starch. The amylose/amylopectin ratios of yam starches are higher than the ratio found in other tubers (McPherson and Jane, 1999; Wu et al., 2016). Some yam species like *D. tryphylla* and *D. versicolor* contain relatively low amounts of carbohydrates (17.4% and 25.9% respectively) (Adepoju et al., 2017). *Discorea trifida* contains 30% starch (a waxy starch that lacks amylose) (Guilois et al*.,* 2011; Perez et al*.,* 2011)**.**

Dietary fibre

Some yam species contain high amounts (up to 4.1g/100g) of dietary fibre. Dietary fiber helps reduce constipation, decrease the level of bad (LDL) cholesterol by binding to it in the intestines and lower colon; thereby reducing the risk of cancer by preventing toxic compounds in the food from adhering to the colon mucosa. Additionally, being a good source of complex carbohydrate, it regulates steady rise in blood sugar levels. For the same reason, yam is recommended as low glycemic index and healthy food (Sahu et al*.,* 2020).

Proteins

Yam proteins (about 1.53g/100g- 5.0g/100g) are rich in phenylalanine and threonine but contain only limited amounts of the sulphur containing amino acids (cysteine and methionine) and tryptophan. They are also conjugated proteins that contain phytochemicals and minerals that are essential for good health. The protein is not evenly distributed in the yam tuber. More protein is found near the peel (skin). The quantity of protein found in yams is inadequate to meet dietary needs. Staple yam diets should be supplemented with protein rich foods (Ezeabara and Anona, 2018). Dioscorin is a yam storage protein which is also a bioactive compound.

Vitamins

Different varieties of yams contain variable amounts of vitamins. Soil and environmental management practices also affect the vitamin composition of yams (Udensi et al*.,* 2008)**.** **Vitamins** B_1, B_3, B_5, B_6, C, E, beta-carotene, biotin, and folic acid may be found in good quantities, depending on the yam variety (Ezeocha and Ojimelukwe, 2012). The fresh root also contains good amounts of the antioxidant vitamin, vitamin-C; providing about 29% of recommended levels per 100 g. Vitamin-C plays some important roles. The vitamin C found in many yam varieties helps wound healing, strengthens/boosts the immune system, assists bone growth and is also an anti-ageing vitamin. Vitamin C helps the body to produce enough collagen to maintain the tissues of the digestive tract. It boosts the immune system and protects the body against damage by free radicals. The B vitamins help the body to make red blood cells and make energy from foods through their coenzyme functions. Vitamin B_6 supports biochemical reactions related to healthy immune functions. Vitamin B_6 (pyridoxine) is a coenzyme of amino acid metabolism. Vitamin B_{12} helps in the production of deoxyribonucleic acid (DNA), nerve cells, blood cells, as well as the maintenance of a healthy brain and immune system. It is a co-enzyme required for body detoxification reactions. This vitamin helps to maintain blood flow to the brain. Biotin and vitamin D are required for proper growth of the hair (Ojmelukwe et al., 2005).

Some yam species contain significant amounts of vitamin-A, and β-carotene (Ferede et al., 2010). Carotenes convert into vitamin A inside the body. The carotenes and vitamin A are powerful antioxidants. Vitamin A has many functions like maintaining healthy mucosa and skin, cell differentiation, night vision, growth and protection from lung and oral cavity cancers. The carotenoid rich yam varieties play a crucial role in maintaining vision (Oladeji et al., 2016). They keep the cornea clear; decrease the amount of oil that the sebaceous gland produces and promote hair growth. The pro-vitamin A found in some yam tubers maintains the mucus membranes, assists growth and differentiation, protects the oral cavity and lungs from cancers; prevents night blindness and promotes growth and development. Three genotypes of *Discorea dumetorum* species were found to contian high amounts of vitamin A carotenoids (10.13 µg g^{-1} and 14.00 µg g^{-1}.). Trans beta–carotene–5-8-epoxide was found to be very abundant in this yam species (Oladeji *et al.,* 2016). Three cultivars of *D. bulbifera* (green yellow and red were studied by Princewill Ogbonna and Ibeji (2015) and the average values for vitamin A and vitamin C were 137.27-700.88 IU and 0.04-0.26mg/100g respectively. Discorea caynensis tubers are good sources of pro-vitamin A (Adepoju *et al.,* 2017).

Table 3. Phytochemicals found in Cultivated and wild D. dumetorum

Phytochemical	Cultivated *D dumetorum*	(%)	Wild (edible) *D. dumetorum* (%)	
Fatty acids	Lauric acid	0.56	Linoleic acid	ND
	Myristic acid	2.25	Palmitic acid	10.83
	n-pentadecylic acid	5.59		
	Palmitic acid	21.82[b]		
	cis-oleic acid	10.95		
	Linoleic acid	ND		
Phenols	3,5-Di-t-butyl phenol	1.18	3-Decanone,5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl	1.47
	3,4,4,5-tetramethoxychalcone	ND	3,5-Di-t-butyl phenol	3.38
	13-hexyloxacyclotridea-10-en-2-one	ND	Dihydroxanthin	ND
Sterols	17-(1,5-Dimethylhexyl-10,13,dimethyltetradecahydro-1-H-cyclo-penta[a} phenanthren-3-ol	5.63	Cholesteryl alcohol	ND
			17-(1,5-Dimethyl hexyl-10,13-dimethyl Tetradecahydro-/H-cyclo-penta[a]phenanthren-3-ol	4.47
Aldehydes and ketones	Trans, trans-2,4-decadienal	ND	Vanillyl acetone	0.64
Alcohols	Nonadecatriene-5-14-diol	ND	9,12-Octadecadien-1-ol	
	9,12-octadecadadiene-1-ol	33.52		
	2 Furan menthol	ND		
Hydrocarbons	3-Hexadecene	ND	(1-Methyl-2-piperidinyl) methane	19.16
			Pentadec-1-ene	0.70
			1-Decyl-1-cyclo hexane	ND
Esters	2-Hydroxy-1 (hydroxymethyl)ethyl ester	6.15	2-Hydroxy-1-(hydroxymethyl) ethyl ester	
	Glycerol-1-monolinolate	ND	Methyl Linoleate	
	Beta-monoglyceride	ND	Methyl(7E)-7-Octadecenoate	
	Methyl(9E,12E)-9,12-octadecadienate	ND	Glycerol-1-monolinolate	6.39
	Pthalic acid di (1-hexen-5-yl) ester	ND	Palmitic acid beta-monoglyceride	6.07
Amines	Oleic acid amide	1.12	(2E)-2.7-Dimethyl 2,7-octadien-1 amine	4.14
	Bis (2-Dimethylaminoethyl		Oleic acid amine	1.92
Furan	5-methyl-5-(4,8,12-trimethyltridecyl) dihydro 2(3H)-furanone	ND		-
Alkaloids			Decahydro{1,7} naphthyridine	21.09

Ezeocha and Ojimelukwe, 2012. ND = Not determined.

Minerals

The yam tuber is one of the good sources of minerals such as copper, calcium, potassium, iron, manganese, magnesium, iodine, selenium, zinc and phosphorus. Zinc aids reproduction and protects cells against damage. Copper is a blood forming element and a cofactor for many metabolic reactions. Copper is essential for the production of red blood cells. Some yam species contain significant amounts of manganese (up to 17%). 100 g provides about 816 mg of potassium. Potassium is an important component of cell and body fluids which helps in controlling heart beating rate and blood pressure by countering hypertensive effects of sodium. The body uses manganese as a co-factor for the antioxidant enzyme, superoxide dismutase. Iron is required for red blood cell formation. The B-complex group of vitamins mediates many metabolic body functions. Copper and iron help in the formation of the red blood cells in the human body. Five cultivars of *Discorea hispida* were studied by Saleha et al. (2018). They were found to contain significant amounts of phosphorus (11.7-46.9mg/100g).

Phytochemical composition of some Discorea species.

In addition to nutrients, there are various phytochemicals found in different yam varieties that are beneficial to human health. The quantities of these nutrients and phytochemicals vary with the yam varieties and are influenced by agronomic factors such as soil conditions (pH, available nutrients, organic matter, moisture); the climate (including temperature, precipitation, light intensity), post-harvest handling and storage. *Discorea hispida* samples were found to contain alkanoids and terpenoids. One cultivar also contained phenols and steroids (Saleha *et al*., 2018).

The phytoestrogens help in regulating the female hormone levels. The antioxidant properties of phenolic compounds result from their redox potentials, free radical scavenging activity and singlet oxygen quenching ability (Rice-Evans *et al.*, 1997; Ukom *et al.,* 2014; Ojimelukwe et al., 2017).

Fatty acids, phenols, sterols, aldehydes, ketones, alcohols,hydrocarbons, esters, amines, furans and alkaloids were identified in cultivated and wild varieties of *Discorea dumetorum* (see Table 3). *D. dumetorum* was found to be a rich source of alkaloids (1.75%) and tannins (2.12%) (Ezeocha and Ojimelukwe, 2012).The wild species contained significant amounts of alkaloid D ecahydro-1,7 naphthyridine (21.09%). The naphthyridine derivatives exhibit diverse biological activities such as anti-inflammatory, antimicrobial, antiviral, anticancer, antihypertensive and analgesic activities (Gurjar and Pal, 2019). It also contained high amounts of hydrocarbons (1-methyl-1-2-piperidinyl) methane- 19.6%) and palmitic acid (10.83 %). The cultivated *D. dumetroum* contained large amounts of palmitic acid in their fat (20.82 %); cis oleic (10.95 %) acid as well as the alcohol; 9, 12-octadecadiene-1-ol (33.82 %).

Ethnobotanical Uses of Yams

Yams have numerous medicinal uses in different traditional settings. Its root extracts may be used in treating nephritis; tiredness, poor digestion, poor appetite, weight loss, hyperthyroidism and diabetes (Bone et al., 2000; Chandrasekara and Kumar, 2016). The tuber is used in treating ulcers and boils. The juice of the leaves is useful in treating snakebites and scorpion stings. The tuber acts as an anthelmintic element for removing parasites from the gut (BoneZava et al., 1998). The wild varieties are often associated with a lot of perceived health benefits. Wild yam may be used for the treatment of muscular pains and cramps (such as menstrual pains and premenstrual syndrome); inflammation caused by rheumatoid arthritis; treating symptoms of menopause (Carroll, 2006: Haimov-Kochman and Hochner-Celnikier, 2005; Kelley and Carroll, 2010; Komesaroff et al., 2009). It is also percieved to be useful as anticoagulant; in protecting the walls of arteries and veins; preventing localized abdominal cramps; promoting the secretion of bile; purifying and detoxifying the body; controling afterbirth pain; preventing diverticulosis; controlling hyperlipidaema; managing cough; preventing morning sickness, infertility,osteoporosis, nausea, gall bladder problems; mood swings and retarding the ageing process (Kumar et al., 2017). *Discorea opposita* and *Discorea villosa* have been used as medicine in Asia and beyond. A cream containing 22% and 33% (respectively) of *Dioscorea villosa* decreased diurnal and non-flushing symptoms but was not significantly different than placebo. However, the cream (10% *Dioscorea villosa*) also contained *Linum usitatissmum*, *Perlargonium graveolens*, and *Salvia officinalis* with 10mg active Vitamin E, suggesting that the observed effects should not be attributed to *D. villosa* alone (Kaimel and Kemper, 2009). *Dioscorea villosa, D. opposita, D. hypoglauca, D. composata, D. deltoida, D.parazeri, D. mastrostachya, D. floribunda, D. barbasco, D. macrostachya* and *D barbasco* are considered as medicinal species of yams (Kaimel and Kemper, 2009)

Health benefits of some yam varieties

Discorea villosa is a medicinal yam with a lot of perceived health benefits. Many pharmacologically active compounds have been found in it (Manda et al., 2013; Aumsuwan et al., 2016)). Three furostanol saponins, parvifloside (27.3 mg), methyl protodeltonin (67.1 mg) and trigofoenoside A-1 (18.5 mg) were extracted from *D. villosa* with n-butanol and isolated through metabolomics mining. Further identification of the compounds in the spirostanol-rich extract deciphered four spirostanol saponins: deltonin (31.5 mg), dioscin (7.7 mg) zingiberensis saponin I (15.2 mg) and prosapogenin A of dioscin (3.4 mg). Other biologically active compounds found in *Discorea villosa* are protodioscin, methyprotodioscin, prosapogenin, epifzelechin, glucopyranoside and phytoestrogen.

Anticancer effects of D. villosa

Screening of a large number (more than 300) medicinal herbs, seeds, roots, plankton and fungi for their antitumor effects showed that *Dioscorea villosa* was the most effective product, in inducing apoptosis in the tested tumor cell line. In a neuroblastoma cell line (Neuro-2a), *Dioscorea villosa* ethanoic extract showed an LD_{50} of 19mcg/mL, suggesting that *Dioscorea villosa* may confer anti-cancer effects (Ali *et al*., 2013), but scientific evidence on efficacy and safety are limited (Aumsuwan et al., 2015; 2016). Research to determine other bioactive compounds is still on-going. Diarylheptanoids with diverse pharmacological activities have been found in this yam variety (Dong et al., 2012). .

Management of Type 2 Diabetes

Some yam varieties have been found useful in the management of type 2 diabetes. Adebayo et al. (2012) found that *Discorea* spp modulated the levels and functions of two key enzymes associated with type 2 diabetes in vitro. *D.rotundata* and *D.alata* flours were found to inhibit alpha amylase and alpha glucosidase (key enzymes linked to type 2 diabetes) in a dose dependent manner (1-4mgL^{-1}) (Adedayo et al., 2012). Processing of the yams significantly reduced their inhibitory activities. *D. dumetorum* also has anti-diabetic effect (Undie and Akubue, 1986). Mc Anuff et al. (2003), observed that the Jamaican bitter yam (*D. polygonoides*) steroidal sapogenin extract improved the intestinal morphology of streptozotocin induced diabetic rats thereby improving digestion. Go et al. (2015) was able to establish the antidiabetic effect of *Discorea batatas* in a rat model of streptozotocin –induced diabetic rats. This yam species as well as allantoin which is one of its bioactive constituents modulated the antioxidant activities and lipid profiles by promoting the release of glucagon-like-peptide-1 (GLP-1), thereby improving the functions of the beta

cells to maintain normal insulin and glucose levels Rawat and Palmer, 2013).

Management of Oxidative stress and Hyperlipidaemia

Some researchers have observed the beneficial effect of some yam species on oxidative stress (Boban et al*.,* 2006). The yam phenols were associated with their antioxidant activities (Ukom et al*.,* 2014). The *Discorea* rhizome was found to reduce oxidative stress and artherosclerosis in hyperlipidaemic rabbits (Chang *et al.,* 2005). Son et al. (2007) also observed that diosgenin (a steroidal saponin found in Discorea species) exhibited antioxidative and hypolipidaemic effects on rats fed high amounts of cholesterol. Miyazawa et al. (1996) reported the presence of paneol in *Discorea japponia* which showed anti-mutagenic activity

Some Bioactive compounds/derivatives found in yams and their health benefits

Yams contain biologically active substances, such as diosgenin, dioscin, other steroidal saponins, dehydroapiandrosterone (DHEA) and discoretine (see Table 4). Many wild yam species with significant amounts of the bioactive compounds are bitter.

Biological functions of yam phytochemicals

Table 4. Biological Functions of Phytochemicals found in Yams

Name of Phytochemical	Nature/Mode of action	Yam species	References
Diosgenin	A hydrolysis product of saponins; accelerates keratin protein synthesis to retard skin ageing. Manages cholesterol level; improves lipid profiles and modulates oxidative stress; may be used for the commercial synthesis of cortisone,pregnelonone, progesterone and other steroids; believed to improve gastrointestinal;sensory and sexual functions;influences the secretion of bile.	*D. opposita; D. japponia; D.bulbifera; D.vesicolor;D.deltoidea; D. triphylla*	Lin and Yang, 2008. McLeedy and Chynes, 2001; Teda et al., 2009; Lin et al., 2006; Accatino et al., 1998; Dong et al., 2018
Dioscin (Diosgenin glycone)	Anti-inflammatory, especially for situations related to rheumatoid arthritis; lipid lowering activity; anti tumor activity; regulates blood sugar; has antifungal activity; has membrane disruptive activity	*D. alata; D. opposita; D. persimilis; D. fordii. D. nipponica*	McAnuff et al., 2003; Chang et al., 2005;Manda et al., 2013
Alkaloids (e.g. dihydrodiscorine)	Affects the central nervous system. Used as fish poison.		
Terpenoids			
Phenols		*D. bulbifera; D. versicolor; D. triphylla*	Patel et al., 2015
Saponin	interferes with DNA replication and prevent the multiplication of cancer cells, binds cholesterol		
Dioscorin	dioscorin-(yam protein that has antioxidant & hypoglycaemic properties).	*D. hispida*	Kumoro and Harati, 2015; Hou et al., 2001
Proanthocyanidin	binds physically to cell walls and prevent the adhension of pathogens to human cells)		
Purine degradation product (e.g. Allantoin)	Allantoin improves the secretion and function of glucagon	*D. alata; D. opposita; D. persimilis; D. fordii;* D. batatas	Chandrasekara and Kumar, 2016; Go et al. (2015)

Diosgenin

One major bioactive component of yams is diosgenin, and a variety of molecules which are diosgenin bound to different glycosides. It is commonly found in wild yams. Diosgenin is considered the most important bioactive compound in yam and it is an aglycone. It is a steroid hormone precursor in–vitro. Diosgenin may be used commercially for the synthesis of cortisone, pregnelonone, progesterone and other steroids. Diosgenin enhances DNA synthesis in human 3D skin model and also increases the uptake of bromodeoxyuridine (Meleandy and Clynes, 2001). It interacts with estradiol which is a natural hormone and a precursor for estrogen. It can be used for controlling hyperlipidemia, hyperglycemia, and cardiovascular diseases. It even has immunomodulatory

and antitumor functions. Diosgenin has been found to possess anti-ageing effect. In experiments with a human 3D skin equivalent model (TESTSKIN cells), Diosgenin was found to enhance DNA synthesis in a similar manner as retinoic acid (active form of Vitamin A) at 10nM in human keratinocytes. The action of diosgenin was through a cAMP dependent pathway (which was blocked by an inhibitor of adenyl cyclase) that is independent of both estrogen receptors and GPR30 (Manda et al., 2013). When Diosgenin was administered to ovariectomized mice at 0.01-0.04% of the diet for 20 weeks, the mice had improved skin thickness. Diosgenin is structurally similar to estradiol; the active form of estrogen in the human body. Estrogen in the human body is found in three major forms: estradiol, estrone and estriol. In the liver and small intestines, estrone is converted to estradiol. Its concentration (i.e estradiol) increases after menopause, when the adrenal glands play more pronounced role in hormone synthesis than the ovaries. Estrone and estradiol are metabolized to estriol; their primary urinary product (Lippert et al., 2003). It was also found to induce differentiation of leukemia cells (Ghezali et al., 2014). The status of diosgenin as a phytoestrogen has not been ascertained as it does not interact with estrogen receptors. Wild yam is also rich in saponins and alkaloids. Antioxidant and hypolipidaemic effect; anticancer; interacts with estradiol; influences bile secretion.

Dioscin

Dioscin is a naturally occurring steroidal saponin found in wild yams. Dioscin possesses numerous pharmacological effects. It demonstrates cytotoxic effects through the induction of apoptosis (Cai et al., 2002; Qu et al*.,* 2014)). It has antitumor and antifungal effects (Liu et al., 2012; Choi et al., 2013). It inhibits bone re-sorption and osteoclast differentiation functions (Qu et al., 2014). It has anti-inflammatory, lipid-lowering, and hepatoprotective effects (Guo et al., 2016). It is may be used for the synthesis of diosgenin and other steroidal drugs (Perez et al*.,* 2011). It has also been found to possess nephroprotective, antiviral and cardio protective effects (Yang et al., 2019).

Discoretine

Discoretine is a bioactive compound associated with the effective management of diabetes and is useful for regulating blood sugar. The methanolic extract of *D. dumetorum* showed hypoglycaemic effect in healthy and alloxan diabetic rats when administered intraperitoneally (ip) at 20mg/kg. (Iwu et al*.,* 1990). Discoretine is found in good quantities in *D. villosa*.

Dioscorin

Dioscorin is the major protein found in yam tubers. It can be extracted with water and accounts for about 90% of the extractable proteins in yams. It functions as an alkaloid and possess trypsin inhibitor and immunomodulatory activities. It also has antihypertensive and antioxidant properties (Harijono et al*.,* 2013). It is found in *D. opposita, D. esculenta D. alata, D. japonica,* and *D. batatas.*

Dihydrodiscorine

Dihydrodiscorine is a toxic alkaloid found in some raw yams which has found application as fish poison. Some species of *D. dumetorum* contain dihydrodiscorine isoclines, a heart stimulant (Bevan and Hirst, 1985)

Allantoin

Allantoin has been found to regulate the secretion and function of glucagon**.** Allantoin soothes and protects the skin. It has the ability to heal the skin and stimulate the growth of new tissue. It influences the secretion of glucagon. It promotes wound healing, speeds up cell regeneration, and exhibits a keratolytic effect. It is found in *Discorea opposita*. It may also be found also in *D. alata* and *D. batatas*. Allantoin modulates antioxidant activities by promoting the release of glucagon-like peptide-1 (GLP-1), thereby improving the functions of beta cells of the pancreas to maintain normal insulin and glucose levels (Chandrasekara and Kumar, 2016).

Dehydroepiandrosterone (DHEA)

Dehydroepiandrosterone (DHEA) is a 19 carbon lipophilic endogenous steroid hormone, naturally made by the body through the cholesterol-pregnenolone pathway. It is produced by the adrenal glands, the gonads, and the brain. It improves the well-being of females and enhances the pituitary function of males with impaired pituitary function. It affects the thyroid hormone status thereby affecting the energy balance by increasing metabolic rate and decreasing fat stores. It is related to diosgenin because it is one of the important bioactive compounds that can be made from diosgenin. It can also be synthesized from other sources like geniestein, wild yam, soy and cholesterol. It is believed to be found in some species of Mexican yams. It serves as an indirect precursor to estrogen and testosterone and other steroid hormones. The natural production of this hormone progressively declines at the rate of 2% per year as an individual gets older. It is not clear, whether intake from supplements influences the body levels of the hormone. DHEA is associated with a large variety of pharmacological activities like antidiabetic, anticancer, anti-allergic, control of osteoporosis; control of dementia; anti-ageing; obesity reduction and cardiovascular effects. It is beneficial in autoimmune disorders like lupus erythematosus, immune modulation, muscle building and hormonal problems. Plain DHEA has a breakdown product 7-keto DHEA, which can also be produced from diosgenin (Park et al., 2009). 7-keto DHEA enhances immune function and reduces body fat. Its efficacy is double that of DHEA. DHEA can be converted to testosterone and estrogen, while 7-keto DHEA cannot be converted to these sex hormones.

Other Saponins

Other saponin extracts from wild yams may be used as an anticoagulant and a diuretic for managing inflammation due to rheumatoid arthritis. It is also used to manage muscle pains and cramps. *D hispida* contains significant amounts of saponins. Other perceived health benefits of wild yams are prevention of vaginal dryness in older women, management of pre-menstrual symptoms and menstrual cramps, management of osteoporosis; increasing sexual drive , diverticulosis and gall bladder pain (Taylor, 1997).

Side Effects, Safe Dosage and Toxicity of yams

The cultivated yam species are not associated with any toxicity symptoms. Wild yam is considered safe for consumption by most people, when taken in small quantities.

However, eating large amounts may cause side effects like headaches, nausea, vomiting and digestive disorders. Safe dosage of wild yam depends on person's age and health conditions. Generally people supplement with 425mg wild yam root twice daily, though further studies are needed (Foster, 2000). Aqueous, methanol, and ethyl acetate fractions of *D. villosa* showed a fibrotic effect on the kidneys of experimental animals. They were toxic to renal tubular epithelial cells (NRK52E), suggesting that long term intake can result in fibrosis of the kidney. Feeding rats a diet of 0.72% *Dioscorea villosa* over a period of 28 days, caused mild renal tubulo-interstitial fibrosis although no macroscopic changes were observed. Macrophages were found to accumulate in the glomeruli and interstitial spaces of the liver. Dihydrodiscorine is a toxic alkaloid found in some raw yams which has found application as a fish poison. In animal studies to investigate safety with consumption of *Dioscorea villosa,* no harm was observed after the consumption of 500mg/kg of *D. villosa*, but the oral dose of 2000mg/kg was able to induce hypo activity, piloerection and dyspnoea.

Conclusion

There is a great diversity of yam varieties and although only a few are used for food, many wild varieties are used as medicines in different traditional settings; because of their perceived health benefits. This diversity (and the fact that novel compounds are presently being elucidated from some varieties) suggest that there are unexplored varieties with bioactive compounds, whose metabolic functions are yet to be elucidated. The nutrients and bioactive compounds in yams are responsible for their anticancer, mutagenic; hypolipidaemic; antidiabetic; antioxidant and anti-ageing properties. Studies should be conducted to establish these claims in humans. The traditional use of yams as sex hormone precursor is yet to have scientific backing because diosgenin which is a natural steroidal saponin in yams can only be converted to other compounds (Dehydroapiandrosterone-DHEA; 7 keto DHEA) with this attribute in vitro. This review presents the existing knowledge about the composition and health benefits of yams. Specific bioactive compounds in yams have been directly linked to their health benefits. It highlights the knowledge gaps for adding value to this very important food product for improved food and nutrition security. Other perceived health claims should be scientifically established or debunked.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Effects of Biotin and Ascorbic Acid Applications on Haploid Embryo Induction in Semisolid and Double Layer Nutrient Media in Pepper (*Capsicum annuum* L.) Anther Culture

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Abstract

Generation of homozygous double haploid (DH) lines by androgenesis is a promising alternative to selfpollination programs across generations. Despite the routine use of anther culture in peppers, there are still many bottlenecks and improvements in methodology are required. The aim of this study was to determine the effects of the structure of the nutrient medium (semi-solid and double layer) and the addition of biotin and ascorbic acid to the nutrient media on obtaining haploid embryos by anther culture method. MS (Murashige and Skoog 1962) medium containing 4 mg l^{-1} NAA, 0.1 mg l^{-1} BAP, 0.25% activated charcoal, 30 g l^{-1} sucrose, and 10 mg l^{-1} AgNO3 (silver nitrate) were used as the basal nutrient medium. A total of 8 nutrient media compounds were studied using 0.05 mg l-1 biotin and 0.5 mg l^{-1} ascorbic acid separately or together in semi-solid and bi-layer (double-phase) nutrient media. Solidification of nutrient media was achieved with 7 g l^{-1} agar. The cultured anthers were subjected to high-temperature pre-treatments at 35 °C in continuous dark conditions for 2 days. Then they were taken to a climate chamber at of 25 °C temperature adjusted to 16/8 hour photoperiod. It has been observed that the success of obtaining embryos of semi-solid medium was higher than double-layer medium. The addition of biotin and ascorbic acid to the nutrient medium provided 8.8 fold increases in embryo regeneration compared to the control medium. In the presence of only one of biotin or ascorbic acid in the nutrient medium, the number of embryos increased compared to the control.

Keywords: Androgenesis, Vitamin, Breeding, Pure line

Introduction

Androgenesis (anther or isolated microspore culture), which is the formation of sporophytes from immature male gamete cells in vitro, is the most widely used method for the production of double haploid (DH) lines in vegetable breeding. Fully homozygous lines are produced in a single generation, thus avoiding the large number of selfing cycles required in traditional breeding programs by this method. Studies on anther culture in peppers have shown that embryo induction by androgenesis and the success achieved are under influence of so many factors. Some of these factors are related to the composition and structure of the nutrient medium, growth regulators, genotype and growing conditions of the donor plant, stress pre-treatments, incubation conditions of anthers, microspore development stage, and the conditions during the application of the culture technique (Irikova et al., 2011, Çömlekçioğlu & Ellialtıoğlu, 2018).

Capsicum is considered as one of the recalcitrant species for androgenesis (Heidari-Zefreh et al. 2019, Pinar et al. 2020).Anther culture has been practiced for a long time in

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crops including Capsicum genus, nevertheless, many factors are limiting the frequency of embryogenesis and regeneration in Capsicum. Overcoming the factor limiting success in androgenesis requires extensive studies and new strategies focusing on optimizing in vitro factors and improving current protocols.

The stress treatments to anthers cultures are an essential factor in androgenic response. It has been shown that stress pre-treatments applied to anthers and microspores, inducing the sporophytic pathway, inhibit the gametophytic pathway (fertile pollen development), and increase embryogenic potential (Sanchez et al. 2020). Several pre-treatments protocols have been reported to induce microspore embryogenesis and plant regeneration in different varieties. Pretreatments of anther culture result in an increased androgenic response (Irikova et al., 2011, Perez-Perez 2019, Sanchez et al. 2020). Although stress pre-treatments are necessary to initiate the sporophytic development of microspores, these pre-treatments could also cause the cells to lose their viability.

This study was conducted to investigate the effects of the structure of the nutrient medium (semi-solid and double layer) and the addition of vitamin 7 (biotin) and vitamin C (ascorbic acid) to the nutrient medium on androgenic embryogenesis of pepper.

Material and Method

This research was carried out in the tissue culture laboratory of the Department of Horticulture, Faculty of Agriculture, Eskisehir Osmangazi University (Eskisehir, Turkey). In this study, Diyar F1 capia pepper variety was used as a donor. Donor plant seedlings were planted in 15-liter pots containing peat and grown in an unheated polycarbon greenhouse.

Flower buds were harvested early morning when the corolla and calyx were the same sizes or when the corolla was slightly above the calyx. These buds have anthocyanin production up to 1/3 of the anthers and the anthers mostly contain late uninucleate, mid uninucleate and young binucleate stage microspores (Çömlekçioğlu and Ellialtıoglu 2018).

Sterilization of flower buds; the flower buds were rinsed first with water and then in 70% ethyl alcohol. Then it was kept in 10% commercial bleach (containing 10% sodium hypochlorite) and one drop of Tween-20 solution for 15 minutes. Rinsed 3-4 times with sterile distilled water.

Nutrient media; four semi-solid and four double-layer nutrient media combinations were formed by addition biotin and ascorbic acid together or separately. Table 3.1 shows the nutrient media combinations studied.

Vitamins including MS (Murashige and Skoog 1962) nutrient medium containing 4 mg l^{-1} NAA, 0.1 mg l^{-1} BAP, 0.25% activated charcoal, 30 g l^{-1} sucrose and 10 mg l-1 $AgNO_3$ was used as control treatments. In the experiments, by using 0.05 mg l^{-1} biotin and 0.5 mg l^{-1} ascorbic acid separately and together, semi-solid and bilayer media were created and a total of 8 nutrient media compounds were studied. Both the solid and the liquid layer of the bilayer nutrient medium contained the same components. Solidification was achieved with 7 g of l^{-1} agar for the semi-solid medium and the solid phase of the double-layer medium. The sterilization of the media was carried out by keeping in an autoclave at 121 °C for 15 minutes under 1.2 atm pressure. Obtained embryos were transferred to hormone-free semisolid MS media.

The filaments were carefully cut with the help of a scalpel and forceps inside a laminar flow cabinet, and the anthers removed from the buds were placed on the nutrient medium as the dorsal surface in contact with the medium without immersion in it.

The sterile Petri dish (7 cm) was used for semi-solid media and a 100 ml glass jar was used for double-layer media. Ten anthers obtained from 2 buds were planted in each petri dish (or glass jar). The edges of the petri dishes, which have been planted, were covered with parafilm.

Incubation; anthers planted on media were subjected to continuous dark conditions and high-temperature application at 35 ºC for 2 days. Then the cultures were taken to a climate chamber adjusted to 25 ºC temperature and 16/8 hour photoperiodic arrangement with the light intensity of approximately 1000 lux.

Experiment design and statistical analyses; the study was carried out with 4 replications and 5 Petri dishes (or glass jar) per repetition. Ten anthers obtained from two flower buds were planted in each dish (200 anthers were used for each medium). Experiments were arranged in a completely randomized design. The data were subjected to analysis of variance (ANOVA) using the TARIST (Açıkgöz et al., 2004) statistical software The least significant difference (LSD) test was used to separate of differences in means.

Table 1. Nutrient media studied and structure and their contents.

Medium		Content
Semi solid	M 1 (CONTROL)	MS + 4 mg l^{-1} NAA, 0.1 mg l^{-1} BAP, % 0.25 Activated charcoal, 30 g l^{-1} Sucrose, 10 mg l^{-1} $AgNO_3$, 7 g l^{-1} Agar, pH 5.6-5.8
	M2	M1 + 0.05 mg l^{-1} Biotin + 0.5 mg l^{-1} Ascorbic Acid
	M3	M1 + 0.05 mg l^{-1} Biotin
	M4	M1 + 0.5 mg l^{-1} Ascorbic Acid
Double layer	M5	M1
	M6	M2
	M7	M3
	M8	M4

Results and Discussion

The effect of ascorbic acid and biotin in the culture medium to optimize the production of anther-derived haploid embryos of the pepper was investigated in this experiment. The total number of embryos obtained, the number of embryos per bud and per 100 anther (Table 2) showed significant differences ($P \leq 0.01$) according to the nutrient media and contents.

A total of 9 embryos were obtained from the M1 medium that was studied as a control in this experiment. On the other hand, the highest number of embryos (80 embryos) developed from M2 medium containing both 0.05 mg l^{-1} biotin and 0.5 mg l^{-1} ascorbic acid. In the presence of only one of biotin or ascorbic acid in the nutrient medium, an increase in embryo formation was achieved compared to the control medium. This increase was found higher in the presence of only biotin (M3) than in the presence of ascorbic acid (M4). 13 and 11 embryos developed from semi-solid M3 and M4 media, respectively. Biotin and ascorbic acid added to MS media significantly affected embryogenesis positively. In this study, semi-solid media were generally more successful than double-layer media.

Biotin containing M7 (11 embryos) was found to be more successful than others double-layer media. Embryo regeneration could not be achieved in M5 medium. A total of 125 embryos from semi-solid media and a total of 31 embryos from double-layer media regenerated. The number of embryos obtained from semi-solid media increased by 403% (fourfold) compared to double-layer media.

Table 2. Total Number of Obtained Embryos, Embryos per Bud and Embryos per 100 Anther Obtained by the Media.

Media		Total Embryo Number	Embryo Number per Flower Bud	Embryo Number per 100 Anthers
Semi-solid	M1	9.0 bc	0.9 bc	3.6 bc
	M2	80.0 a	8.0 a	32.0 a
	M3	13.0 b	1.3 b	5.2 b
	M4	11.0 b	1.1 b	4.4 bc
Double-layer	M5	0.0 c	0.0 c	0.0 c
	M6	5.0 bc	0.5 bc	2.0bc
	M7	11.0 b	1.3 b	4.4 bc
	M8	5.0 bc	0.5 bc	2.0 bc
	LSD $P \leq 0.01$	1.88	0.94	3.76

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

Between 0.0 and 8.0 embryos per flower bud were obtained. It was determined that the highest performance was obtained from M2 medium with 8.0 embryo number per flower bud.

The embryos obtained from the media studied were also calculated as the number of embryos per 100 anthers. The highest embryo ratio was obtained from the M2 with 32 embryos/100 anthers. The addition of biotin and ascorbic acid to semi-solid media provided approximately 8.8 fold higher number of embryos compared to the control (M1) medium.

In the presence of only biotin or only ascorbic acid in the nutrient medium, the number of embryos increased by 4.4 and 2.2 fold, respectively, compared to the control. In double layer M6 and M8 media, 0.55 decrease was observed in the rate of embryos obtained compared to semi-solid M1 media. The embryo could not be obtained from dual-phase medium (M5) without biotin and ascorbic acid. The highest rate of embryos was obtained in nutrient medium (M7) containing ascorbic acid in double-layer media.

Even if all the necessary conditions of the plant are provided in anther culture, the structure of the nutrient medium content is very important in reaching the result. The semi-solid medium was found to be more successful in terms of embryo induction than the dual phase medium.

In this study, the addition of biotin and ascorbic acid to the nutrient medium together created synergy and improved embryo productivity. Vitamins, known for their antioxidative properties, increased haploid embryo development. It has been determined that antioxidants such as biotin and ascorbic acid are necessary in anther culture to improve the haploid embryogenesis of pepper. A higher embryo was obtained from the medium to which biotin was added (M3) compared to the medium to which only ascorbic acid (M4) was added. In this case, a more positive effect of biotin on obtaining embryos in anther culture compared to ascorbic acid was determined.

Androgenic embryogenesis is based on the ability of microspores, after exposure to various stresses, to differentiate and deviate from normal pollen development into sporophytic development and transform into a whole plant.

Microspores can be encouraged to deviate from their gametic development and transition to embryogenesis by in vitro specific stress applications (Shariatpanahi 2006, Hosp et al.2007, Seguí-Simarro and Nuez 2008; Munoz-Amatriain 2009, Rodriguez-Serrano et al.2012, Ahmadi and Ebrahimzadeh, 2020, Perez-Perez 2019).

Certain stresses such as low or high temperature applications, osmotic stress and mutagenic substances, carbohydrate or nitrogen starvation, drought stress, gamma irradiation, oxidative stress, low atmospheric pressure have been reported

to play a vital role in microspores reprogramming microspores in many plant species (Irikova et al., 2011, Shahinul Islam 2012, Sanchez et al.2020).

Exposing the anthers in the culture to high temperature pre-treatment (35 °C) is the most commonly used stress condition in pepper. Cheng et al. (2020) reported that stress is considered to be the inducer for microspore embryogenesis, heat stress is indispensable in pepper androgenesis and green plants could not be obtained without heat pretreatment.

However, the applied stress conditions are important factors that negatively affect the viability of microspores in the early stages of culture. The sudden increase in the levels of intracellular reactive oxygen species (ROS) of microspores exposed to a high-temperature pre-treatment causes the death of the microspores (Zur et al. 2009, Gill and Tuteja, 2010, Varnier et al., 2009).

While high ROS level causes a decrease in microspore viability, it also affects the metabolism in many microspore cells that survive, triggers the transition to sporophytic development, and changes microspore development (Shariatpanahi et al. 2006, Zur et al 2009, Ceyhan and Aktaş, 2020).

Reactive oxygen species are natural byproducts of cellular metabolism but can be harmful to the cell if not detoxified. However, vitamins can activate defense reactions against oxidative stress (Zur et al. 2009).

Vitamins are considered important components that induce plant cell growth, and their role as an antioxidant has also been reported (El sharabasy 2019). With the addition of ascorbic acid, the removal of reactive oxygen species is achieved to a great extent (Becker et al. 2014).

Plant cells grown in vitro can synthesize essential vitamins in insufficient quantities. For this reason, culture media are often supplemented with vitamins to increase growth. Therefore, endogenous or exogenous antioxidants are thought to increase the efficiency of embryogenesis by increasing the viability of microspores. As ascorbic acid is an antioxidant, it provides tolerance to osmotic and oxidative stress in plants. Ascorbic acid reduces oxidative stresses in the plant and ensures the survival of microspores. This situation has a positive effect on the increase of embryos obtained. It has been reported that ascorbic acid increases embryogenesis by increasing microspore viability, whether it is found in the plant endogenously or when applied exogenously (Habibi et al. 2009, Hoseine et al., 2013, Zeng et al., 2015).

One of the most important functions of B vitamins is that they play a role as a cofactor in enzyme-catalyzed reactions and contribute to many metabolic activities in plants. Vitamin B tolerates osmotic and oxidative stresses in plants (Roje, 2007).

Considering the importance of biotin in plants, a significant increase in the rate of embryos was detected In this study by the addition of biotin to nutrient media compared to the control group, and it was determined that biotin had a positive effect.

Al-Khayri (2001) found that the effects of biotin and thiamine concentrations on callus growth and somatic embryogenesis vary according to species and doses and have a positive effect on embryogenesis. Ozsan and Onus (2017) reported that the best androgenic results were obtained from media containing biotin and folic acid among MS media which different types of B vitamins (biotin, folic acid, and cobalamin) were added.

DH methods have great importance in plant breeding and agriculture. Breeding new varieties is an activity that requires a long time, but also requires high labor and cost. Increasing embryo productivity in anther culture can reduce the duration and cost of plant breeding and increase the use of DH technology in breeding programs.

As a result, it has been determined that it is important to increase the tolerance of microspore cells against oxidative stress caused by the stress pre-applications applied to anthers, and adding vitamins to the culture medium due to their anti-oxidant properties is a factor that increases the success. The necessity of using which vitamin at which dose against different stress pre-applications with different genotypes should be determined experimentally and further studies should be conducted.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Natural parasitism of maize stemborers, *Sesamia* spp. (Lepidoptera: Noctuidae) eggs by *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) in Southeastern Turkey

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Abstract

Maize, *Zea mays* L., is currently among the leading cultivated crops in Turkey. Several lepidopteran stemborer species especially, *Sesamia nonagrioides* Lefèbvre and *S. cretica* Lederer (Lepidoptera: Noctuidae) may cause high maize yield losses. This study is the first to report parasitism of *Sesamia* spp. eggs by *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) in Southeastern Turkey. During our extensive 3-years region-wide survey program (2018-2020), we collected *Sesamia* spp. eggs laid between leaf sheets and cobs/stalks. *T. evanescens* adults hatched from the host eggs collected from two locations in Diyarbakır: one in the central county (L10) and the other in Bismil county (L11). The discovery efficiency, parasitism efficiency, and parasitoid impact indexes of *T. evanescens* were 77.5%, 38.92%, and 32.87% in L10 and 50.0%, 33.45%, and 12.42% for L11, respectively. *Sesamia* spp. eggs collected from other counties of Diyarbakır province and other provinces such as Batman, Mardin, and Şanlıurfa did not yield in *T. evanescens* emergence. To our best knowledge, this study is the first to report the presence of *T. evanescens* in Southeastern Turkey. Possible factors involved in the restriction of *T. evanescens* are discussed. Special attention should be devoted to increase the potential of conservative biological control strategies to support egg parasitoids.

Keywords: Discovery efficiency, Diyarbakır, Egg parasitoid, Parasitism efficiency, Parasitoid impact

Introduction

Maize, *Zea mays* L. currently ranks among the most widely sown crops in Turkey with an approximate annual production of 6.000.000 tons (TUIK, 2020). In the southeastern region of Turkey, maize is either sown as the main (first) or the second crop by the majority of large-scale farmers. Maize yield is under high suppression by insect pests especially, lepidopteran stemborers.

Sesamia spp. are leading multivoltine maize stemborers in Turkey. The presence of *Sesamia* spp. in Southeastern Turkey was reported ~50 years ago which was restricted to some counties in which maize cultivation was performed by smallholders (Adıgüzel and Ergül, 1969). These pests may infest all maize growth stages and organs except plant roots (Bayram, 2003). *Sesamia* spp. larval feeding on maize plants in earlier growing stages causes dead heart and the host plant is eventually dead. Their larvae can tunnel into maize stems and cobs resulting in high yield losses reaching up to 100%. The nitrogen and protein uptake of grains are also reduced after larval feeding (Bayram, 2003). In addition, the higher abundance and feeding performance of *Sesamia* spp. are positively correlated with maize disease, *Fusarium* species producing toxic compounds like fumonisin (Avantaggiato et al. 2002).

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The control of *Sesamia* spp. under field conditions is mostly based on insecticide practices performed by farmers in Turkey and 2-3 sprays are recommended when the abundance of the pests exceeds the economic threshold (Bayram, 2003). Insecticide practices may not provide a successful control of *Sesamia* spp. after the larvae enter the host plant stems and cobs. Furthermore, many natural enemies inhabiting maize could be negatively affected by insecticide treatments. Egg parasitoids seem to be the best candidate natural enemies to both prevent the entrance of larvae into maize stems/cobs and provide a unique solution to environmental concerns.

The egg parasitoids parasitizing *Sesamia* spp. eggs mainly belong to two Hymenopteran families, Scelionidae and Trichogrammatidae. For example, the presence of *Telenomus busseolae* has been reported for many countries in Europe, Africa, and the Middle East being recovered from *Sesamia* species and *Busseolae fusca* (Lepidoptera: Noctuidae) (Gahan 1922, Nixon 1935, Bedford, 1935, Moutia and Courtois, 1952, Harris, 1962, Hafez et al, 1977, Scheibelreiter, 1980 Fergusson, 1983). In addition, some *Trichogramma* species were reported to parasitize *Sesamia* spp. eggs under natural conditions in African and Mediterranean countries (Nagarkatti and Nagaraja, 1977; Sertkaya et al.,1999; Sertkaya and Kornoşor, 2002). However, we were unable to locate any study reporting *Trichogramma* species in Southeastern Turkey, especially in maize fields. *Trichogramma* species are polyphagous and can parasitize many insect pests. Surveys targeting the detection of native *Trichogramma* spp. populations in maize fields could suggest useful information on biological control programs in other crops.

The main purpose of the current study is to report the presence and the natural parasitism of *T. evanescens* on *Sesamia* spp. eggs in Southeastern Turkey maize fields between 2018 and 2020. Possible factors involved in the restriction of *T. evanescens* spatial distribution in maize fields are discussed.

Materials and Methods

Survey locations

Regular samplings were performed in maize fields in Diyarbakır, Mardin, and Şanlıurfa Provinces while irregular sampling efforts were devoted to other Southeastern provinces namely Adıyaman, Batman, Siirt, and Şırnak where *Sesamia* spp. egg specimens were collected between 2018-2020 (Figure 1). The summers in the region remain warm during a long-lasting period resulting in semi-arid climatic conditions which are climatically classified as Csa (Köppen, 1936).

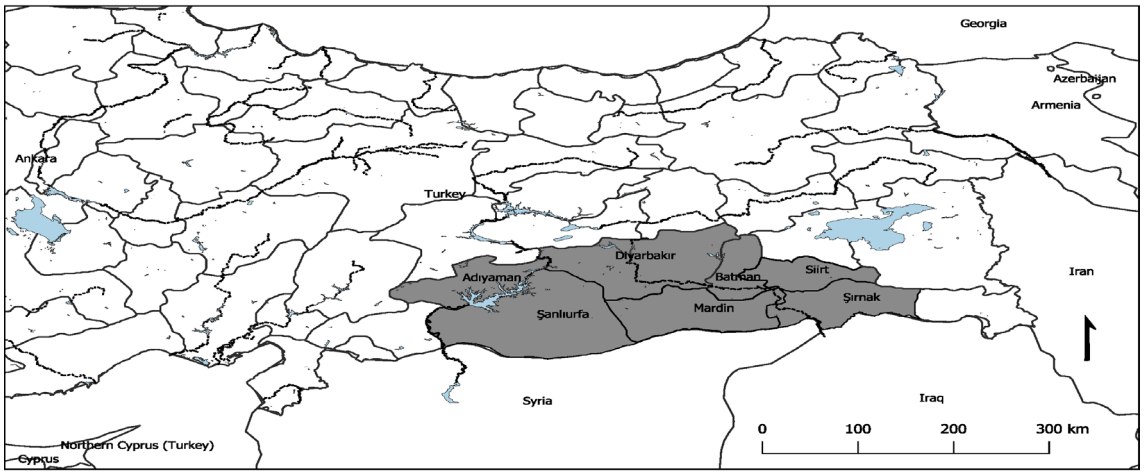

Figure 1. Map of Southeastern Turkey provinces surveyed for the presence and parasitism efficiency of *T. evanescens* during study.

Field sampling procedure

Samplings were performed starting from earlier maize growth stage "leaf development" to "end of fruit development" between maize growth stages: 13-79 according to BBCH scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie, Weber and Bleiholder, 1990; Lancashire et al., 1991). Fields were randomly chosen for samplings. In each field, a total of 100 plants were checked, randomly distributed among 20 sampling spots (5 adjacent plants per spot), representing 1 ha area as a survey unit (Bayram and Tonğa, 2016, Tonğa et al., 2021). The distance between spots was not less than 5 meters. Each plant was carefully inspected from root collar to top of the plants since *Sesamia* spp conceal their eggs between maize leaf sheets and stems/cobs. *Sesamia* spp. eggs were put into glass tubes (1 × 10 cm), labeled and protected via cotton stopper until transferred to the laboratory. A climate chamber was set to 26 ± 1 °C, 60 ± 5 RH, 16:8 L:D condition for incubation process of *Sesamia* egg batches. The incubated host eggs were daily checked until the emergence of the parasitoid adults completed. *Trichogramma evanesces* adults were fed with a 20% honey solution in the same tubes for at least 24 hours. The Mediterranean stem borer, *Sesamia nonagrioides* (Lepidoptera: Noctuidae) eggs were provided after feeding and mating. Thus, the laboratory populations

of *T. evanescens* were multiplied for further examinations. Specimens were morphologically identified by Prof. Dr. Gennaro Viggiani (Institute for Sustainable Plant Protection, Italy) and molecularly characterized by Dr. Nicolas Ris and Dr. Sylvia Warot (Institute Sophia Agrobiotech, France).

Statistical Analysis

Trichogramma evanescens adults emerged from the hosts eggs collected from two sampling locations from Diyarbakır province (central and Bismil counties) in 2019. Since no parasitism by *T. evanescens* was observed in 2018 and 2020, these years were discarded from analysis as well as other plant growth stages in 2019. *T. evanescens* is facultative gregarious and, several parasitoid larvae can develop in a single host egg. Natural parasitism of *T. evanescens* on *Sesamia* spp. eggs was assessed employing the parasitism indexes proposed by Bin and Vinson (1990).

The discovery efficiency expresses the number of discovered egg batches (in each location) divided by the total number of sampled egg batches and is presented as simple percent raw data. The parasitism efficiency represents the number of host eggs parasitized by *T. evanescens* divided by the total number of eggs in discovered egg batches (1).

The parasitoid impact was calculated as the number of parasitized eggs divided by the total number of host eggs (2).

$$Parasitism\ efficiency\ (\%) = \frac{the\ number\ of\ host\ eggs\ parasitized\ by\ the\ parasitoid}{the\ total\ number\ of\ host\ eggs\ in\ the\ egg\ batches\ discovered\ by\ the\ parasitoid} \times 100 \text{---------(1)}$$

$$Parasitoid\ impact\ (\%) = \frac{the\ number\ of\ host\ eggs\ parasitized\ by\ the\ parasitoid}{the\ total\ number\ of\ host\ eggs\ in\ the\ collected\ egg\ batches} \times 100 \text{----------(2)}$$

The arcsin square root transformed data of the parasitism efficiency and the parasitoid impact were subjected to analysis of variance (ANOVA) via "*car*" package (Fox and Weisberg, 2019). Differences between percent parasitism of different counties were compared with Tukey multiple comparison tests using "*lsmeans*" package (Lenth, 2016). All statistical analysis was performed in the statistical software environment R (v-4.0.4) (R Core Team, 2021).

Results

Sesamia spp. egg samplings yielded in no parasitism by *T. evanescens* in Southeastern Turkey in 2018 and 2020. *T. evanescens* adults hatched from eggs collected in central (L10) and Bismil (L11) counties of Diyarbakır in the second survey year (2019) only (Table 1, Figure 1). Therefore, *T. evanescens* was not recorded in any surveyed provinces in this study except Diyarbakır (Table 1, Figure 1).

In total, the number of *Sesamia* spp. eggs collected in L10 and L11 were 3109 (from 40 plants) and 789 (from 8 plants) while the number of *T. evanescens* adults hatched host eggs were 1022 (from 31 plants) and 98 (from 4 plants) respectively. The number of *Sesamia* spp. eggs from other locations are not presented due to no parasitism by *T. evanescens*.

There were statistical differences between the parasitism efficiency of *T. evanescens* and its impact on *Sesamia* spp. eggs among surveyed locations ($F_{efficiency}$ = 51.97, df= 11, P < 0.001; F_{impact} = 8.52, df= 11, P < 0.001). The discovery efficiency, parasitism efficiency, and parasitoid impact of *T. evanescens* were 77.5%, 38.92%, and 32.87% in L10 whereas 50.0%, 33.45%, and 12.42% in L11, respectively. The parasitism efficiency of *T. evanescens* did not differ between L10 and L11 while its impact was higher in L10 when compared with L11.

Discussion

This study is the first to report the presence and natural parasitism of *S. nonagrioides* and *S. cretica* eggs by *T. evanescens* in Southeastern Turkey. The incubation of their eggs confirmed the emergence of *T. evanescens* from eggs collected from two locations in Diyarbakır province namely central (L10) and Bismil counties of Diyarbakır province (L11). *Sesamia nonagrioides* and *S. cretica* stand among the most destructive maize yield-reducing organisms in Southeastern Turkey. The morphological identification of their eggs at the species level is difficult. Due to their simultaneous presence in Southeastern Turkey maize fields, the parasitism of *T. evanescens* on *Sesamia* spp. eggs under field conditions is evaluated at the genus level. The natural parasitism of *Sesamia* spp. eggs by *T. evanescens* is calculated based on three different variables i.e., the discovery efficiency, the parasitism efficiency, and the parasitoid impact for these two locations (Bin and Vinson, 1990).

The discovery efficiency referring the searching ability of *T. evanescens* on *Sesamia* spp. eggs under field conditions represents the raw data simply dividing the number of discovered egg batches (considering all eggs on a plant as a single batch) to all egg batches in each location. Thus, *T. evanescens* were able to discover 77.5% of *Sesamia* egg batches (oviposited plants) in L10 while 50% of egg batches were discovered by the parasitoid in L11.

As another calculated variable, the parasitism efficiency of *T. evanescens* was not statistically different between two locations, L10 and L11 and the parasitoid was able to exploit 38.92% and 33.45% of the discovered host eggs in both locations respectively. The parasitism efficiency was calculated based on the number of *Sesamia* spp. eggs exploited by *T. evanescens* rather than the number of parasitoid adults. Therefore, in both locations, the parasitoid was not capable of exploiting all host eggs in a discovered egg batches because of several reasons. For example, depending on temperature, *Sesamia* spp. eggs may normally hatch 4-8 days in the

Table 1. The discovery efficiency, the parasitism efficiency, and the parasitoid impact of *T. evanescens* on *Sesamia* spp. eggs collected in Southeastern Turkey Provinces.

Location	Sampling date	Province	County	Latitude	Longitude	Discovery efficiency (%)	Parasitism efficiency (%)	Parasitoid impact (%)
L1	10.07.2019	Diyarbakır	Bismil	37.860000	40.880556	0	0a	0a
L2	30.07.2019	Mardin	Nusaybin	37.095833	41.339722	0	0a	0a
L3	07.08.2019	Mardin	Mazıdağı[a]	37.487778	40.447222	0	0a	0a
L4	27.08.2019	Mardin	Mazıdağı	37.503889	40.448889	0	0a	0a
L5	27.08.2019	Mardin	Mazıdağı[a]	37.487778	40.447222	0	0a	0a
L6	11.09.2019	Şanlıurfa	Ceylanpınar	36.970278	39.984444	0	0a	0a
L7	18.09.2019	Diyarbakır	Silvan	37.127222	41.222778	0	0a	0a
L8	18.09.2019	Batman	Batman	38.039167	41.178611	0	0a	0a
L9	27.09.2019	Diyarbakır	Diyarbakır	37.890113	40.275188	0	0a	0a
L10	02.10.2019	Diyarbakır	Diyarbakır	37.850556	40.451944	77.5	38.92±3.27b	32.87±3.95c
L11	02.10.2019	Diyarbakır	Bismil	37.840556	40.518333	50.0	33.45±7.61b	12.42±5.36b
L12	08.10.2019	Mardin	Nusaybin	37.093889	41.184167	0	0a	0a
			F				51.97	8.52
			df				11	11
			P				<0.001	<0.001

[a] Same location sampled in different sampling dates. *Sesamia* spp. eggs were found in both sampling dates.
Means followed by different letters in each column depict differences between sampling locations.

postoviposition process (Orang et al., 2014; Sedighi et al., 2017). Therefore, the host egg acceptance by the parasitoid is possible for a time period shorter than hatching. The parasitism capacity of a female Trichogrammatid parasitoid is limited to at most 25 eggs per day (Muli et al., 2010.). Because *Sesamia* spp. egg batches are relatively large, it seems quite normal that parasitism efficiency of *T. evanescens* remained in the levels revealed by this study. In field conditions, several female *T. evanescens* may discover a host egg batch which is expected to result in higher parasitism. However, the facultative gregarious reproduction behavior by *T. evanescens* on host eggs of *Sesamia* spp. size of which is larger than parasitoid female leads self and conspecific superparasitism that limits the host egg exploitation during parasitism in nature (van Dijken and Waage, 1987; Doyon and Boivin, 2005).

Trichogramma evanescens impact on *Sesamia* spp. varied and was higher in L10 when compared with L11 with 32.87% of parasitized host eggs in L10 while only 12.42% of host eggs were parasitized in L11. A possible and maybe the most important exploitation limiting factor for *T. evanescens* is the simultaneous host egg batch exploitation by the specialized parasitoid species of *Sesamia* spp., *Telenomus busseolae* (Hymenoptera: Scelionidae) (unpublished data). Possible competition between two egg parasitoid species would likely restrict the parasitism performance of the non-specialist. Therefore, *T. evanescens* may not be able to exploit large egg batches while they could contribute to suppressing the pest infestation by exploiting the host eggs partially.

Trichogramma evanescens is a minute parasitic wasp and plays significant roles in the control of lepidopteran pest populations on different crops many of which are cultivated in Southeastern Turkey during a growing season. This suggests that *T. evanescens* could easily locate different host species on crop plants throughout the growing season. The parasitoid successfully established its population parasitizing *Sesamia* spp. eggs in two maize fields in Southeastern Turkey. Probably, the highest level of *Sesamia* spp. eggs at the end of the season resulted in the successful host location by the parasitoid. Another possible reason to explain successful establishment of the parasitoid could be the non-chemical agricultural practices conducted in *T. evanescens*-present maize fields and surrounding cultivated lands.

The presence and natural parasitism capability of *T. evanescens* was reported for many host species from different regions of Turkey. For example, Sertkaya (1999), reported natural parasitism of *Sesamia nonagrioides* eggs in the Mediterranean region by *T. evanescens*. Another important maize pest, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) egg parasitism in the Black Sea region, the Marmara region, and the Mediterranean region was associated with *T. evanescens* emergence (Özdemir, 1981; Özpınar ve ark., 1996; Öztemiz, 2007). However, to our knowledge, the presence of *T. evanescens* in Southeastern Turkey was not reported previously. Further research is required to reveal the natural parasitism capacity of Trichogrammatid species on important lepidopteran pests infesting cultivated host plants, for example, *O. nubilalis, Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), *Earias insulana* (Boisd.), *Helicoverpa armigera* (Hübner), *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae).

The spatial distribution of *T. evanescens* parasitism on the eggs of *Sesamia* spp. was restricted to two relatively close

locations (~8 km between). Many factors may be involved in the restriction of *T. evanescens* parasitism on *Sesamia* spp. in Southeastern Turkey. For example, in general, maize fields require 3-4 times insecticide treatments in Southeastern Turkey due to *Sesamia* spp. infestation while these two maize fields were not chemically treated in 2019. Another factor could be the presence of other cash crops cultivated in short-scale farming in surrounding lands that are not chemically treated as well. The chemically untreated crops constitute a good habitat for the above-mentioned lepidopteran pests accordingly for their generalist egg parasitoid, *T. evanescens*.

Conclusion

Trichogramma evanescens adults emerged from *Sesamia* spp. eggs collected from Southeastern Turkey maize fields. Further research programs such as monitoring the natural parasitism performance of this parasitoid species on different host species under field conditions and augmentative biological control should be performed to suppress lepidopteran crop pests in the region. In addition, removing the factors that negatively affect the natural parasitism by egg parasitoids i.e. reducing pesticide usage may help improve conservative biocontrol programs.

Compliance with Ethical Standards

Conflict of interest

The author declares no conflict of interest associated with this study

Author contribution

This study is a part of Ph.D. thesis of Adil Tonğa who designed and performed the study, analyzed the data, and wrote the manuscript in consultation with Prof. Dr. Ahmet Bayram. Both authors read and approved the manuscript. The authors confirm that the data of this study are original and have not been published or in consideration for publication elsewhere.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Effects of grafting on growth, root morphology and leaf physiology of pepino (*Solanum muricatum* Ait.) as affected by salt stress under hydroponic conditions

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Abstract

In this study, grafted and ungrafted pepino (*Solanum muricatum* Ait.) plants were tested under different saline conditions. The nutrient solution experiment was conducted within October – November 2016, by employing the technique of Deep-Water Culture (DWC) in an entirely operated automatically climate chamber found in the Plant Physiology Laboratory of Erciyes University, Agriculture Faculty, Kayseri, Turkey. Plants were examined under three various salt levels (i.e., 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) by growing them in a 8 liter pots loaded constantly in an aerated Hoagland solution. The study was organized with completely randomized block design through three repetitions. The climate chamber study was performed to investigate effects of salt stress on plant growth, shoot- root fresh- dry weights, photosynthesis, leaf area formation, chlorophyll content of leaf (SPAD), leaf and root electrolyte leakage, total length of root, volume of root, and diameter of root in grafted and ungrafted pepino plants. The results showed that shoot growth, root morphological and leaf physiological responses were considerably (p<0.001) influenced by various levels of salt conditions at the nutrient solution. Increased salt level of the nutrient solution decreased significantly root and shoot growth, area of leaf, photosynthetic activity of both grafted and ungrafted plants. Irrespective of being grafted, significant declines were observed in shoot fresh weight (23.6%, 52.1%), root fresh weight (24.8%, 52.8%), leaf area (21.3%, 51.9%), shoot dry weight (24.3%, 53.0%), root dry weight (15.4%, 45.1%), SPAD (5.7%, 18.7%), photosynthesis rate (24.6%, 42.1%), total root length (6.7%, 16.4%), and root volume (3.8%, 5.8%) of pepino plants under 4 dS m^{-1} salt applications and 8 dS m^{-1} salt applications, respectively. Grafting promoted growth of plant in pepino plants under both control and saline conditions, furthermore it was noticed that under saline conditions biomass production of both grafted and ungrafted ones were significantly depressed. Grafted plants produced 54.1%, 43.0% and 9.6% higher shoot fresh weight; 52.0%, 42.0% and 12.8% higher root fresh weight; 52.5%, 40.7% and 8.7% higher leaf area; 60.0%, 46.6% and 11.1% higher shoot dry weight; 68.8%, 36.0% and 29.3% higher root dry weight; 19.9%, 9.2% and 8.2% higher SPAD; 8.0%, 5.1% and 10.8% higher photosynthesis rate; 8.6%, 3.6% and 6.6% higher total root length; 3.1%, 6.7% and 2.4% higher root volume than ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, correspondingly. Overall, our study showed that the effectiveness of grafting with respect to expansion of plants growth and development under salinity. Grafting was demonstrated to be an effective mean to achieve this goal.

Keywords: Nutrient solution, Pepino, Root volume, Salt stress, *Solanum muricatum*

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Introduction

Pepino (*Solanum muricatum* Ait.) a vegetable belonging to the Solanaceae family, it is innate to Andean regions of Columbia, Peru, Chile from South America, it was domesticated in pre-Hispanic times. It is, on the other hand, grown in several tropical and subtropical areas (Prohens et. al., 1996; Huyskens-Keil et. al., 2006), and it was an essential crop throughout the periods of the Inca Empire. It is a similar vegetable as phylogenetically with potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* L.). Pepino stalks root with ease, and it is vegetatively propagated by small cuttings. Ripening of pepino dulce fruit is characterized by a climacteric (El Zeftawi et al., 1988); develop parthenocarpic fruit (Hermann, 1988). The fruit is consumed fresh or cooked. When ripe it has a muskmelon-resembling flavor, tender, and juicy but vary greatly in shape, color, size (Hermann, 1988), and also in sugar (Dennis et al., 1981) SSC (El-Zeftawi et al., 1988; Hermann, 1988), and pH. The fruits are often exotically colored. Within, they are a little watery with pleasant flavor, but usually not over-sweet. At its finest, pepino fruit is soft and juicy and is utilized as a dessert fruit. They are high in vitamin C (45-70 mg/100 g) (Hermann, 1988; Morley-Bunker, 1983; Redgewell and Turner, 1986), K (>1000 mg kg^{-1}), and are considered more selenium than highly ordinary fruits and vary greatly in flavor (Dennis et al., 1981; Hermann, 1988), in many cases leaving an off-flavor sensation. Currently, little is known about pepino crop, as the crop remains underexplored or unexploited. In countries such as New Zealand, the USA (California), and Chile, pepino is being grown under contemporary and regulated conditions, but not on a wide scale. During the 1990s and first years of 2000s, Spain has been a reference of breeding and marketing of pepinos (Rodriguez-Burruezo et al., 2011). Pepino is one of the latest introduced vegetables to Turkey. In recent times, the pepino crop has developed an increasing interest in Turkey owing to its flavor, nutritional value, and attractive appearance. It is also known as a medicinal plant in the country. Pepino production has been rising in the last years in Turkey (Cavusoglu et al., 2009; Yalcin, 2010).

Salinity stress inhibits growth and expansion of the plant, causing osmotic stress and yield loses and might be toxic to plants (Mass and Nieman, 1977; Levitt, 1980). In addition, Na and chloride ions may inhibit absorption of essential minerals (Levitt, 1980). Defeating salt stress problems is crucial for future agriculture to meet the food needs of future populations. One feasible method to encourage salt stress tolerance by physical means is grafting of a productive scion on a salt stress-tolerant rootstock. Vegetable plants were first grafted in the late 1920s by Japan and Korea, where watermelon was grafted onto gourd rootstocks to enable for continuous cropping in fields that were prone to Fusarium wilt soil-borne disease (Davis et al., 2008). It also serves as a revolutionary procedure for the proper propagation of fruit-bearing vegetables including cucumber, tomato, eggplant, bean, and melon in Korea, Mediterranean region, Japan, and in Europe (Pogonyi et al., 2005). Grafting with vigorous rootstocks improves pest and disease resistance, yield, increases the nutrients uptake and the mineral content of plant aerial portion, drought, and cold tolerance, growth, fruit quality, tolerance to high and low temperatures and salinity tolerance as emphasized for several crops including tobacco, melon, watermelon, and tomato (Sarabi et al., 2017; Ulas et al., 2019; Ulas et al., 2019a, b).

Up to now, several studies connected to pepino propagation have been conducted throughout the world in countries including Italy (Vincenzoni, 1988), China (Luo, 1994), Spain (Seidel, 1974), Netherlands (Welles, 1992), France (Peron et al., 1989), the United States (Maynard, 1989), Turkey (Ercan and Akilli, 1995; Yalcin, 2010), Israel (Pluda et al., 1993), and Korea (Joo et al., 1990). However, to our knowledge, no detailed study concerning the grafting effect on growth, root morphology and leaf physiology of pepino plants under salt stress at hydroponic growth systems has been performed in Turkey in literature. Thus, this research is purposed at assessing impacts of grafting on shoot growth and root morphological and leaf physiological responses of pepino plants as affected by salt stress under hydroponic growth condition.

Materials and Methods

Plant material, treatments, and experimental design

A hydroponic experiment was conducted between October - November in 2016 for six weeks by using an aerated deep-water culture (DWC) technique in a fully automated climate chamber in the Plant Physiology Laboratory of Erciyes University, Faculty of Agriculture, central Anatolia in Turkey. For the vegetation period, the average day/night temperatures were 25/22 °C, the relative humidity was 65–70% and about 350 µmol m^{-2} S^{-1} photon flux was supplied in a photoperiod of 16/8 h of light/dark regimes in the controlled growth chamber. Pepino *(Solanum muricatum* Ait. seedlings were used as plant materials by an agricultural company. The pepino variety Miski, which is the most grown variety under greenhouse and field conditions in Turkey. The pepino seedlings were obtained in multipots filled with a mixture of peat (pH: 6.0-6.5) and perlite (2v:1v). The seedlings were grafted by "tube grafting method" described by Lee (1994), while ungrafted pepino seedlings were used as control plants. After grafting, plants were healed and acclimatized in the tunnel covered with double-layered plastic film and shade cloth in the climate chamber for one week. In order to prevent grafted plants from wilting by the excessive transpiration and to enhance healing, the tunnel was closed for the first three or four days of the healing and acclimatization period. For the next three or four days, the opening and closing of the tunnel were done depending on the conditions of grafted plants and the growth room. This was done for the acclimatization of grafted plants to environmental conditions outside the tunnel. The grafted plants stayed at the healing and acclimatization period totally for ten days. After the end of healing and acclimatization, grafted plants were transplanted to 8 liter plastic pots after roots were washed from growth media. Two plants were grown in each pot filled with 8 liter nutrient solution (modified Hoagland). During hydroponic study only distilled water with analytical grade (99% pure) chemicals contained were used. The solution was continuously aerated by an air pump to supply sufficient dissolved oxygen (8.0 mg/ L). The experiment was conducted

with three different salt levels (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}). Each pot was filled with 8 liter cultivation solution that was aerated by an air pump. The nutrient solution contained 1.5 mM calcium nitrate ($Ca(NO_3)_2$,) 250 µM monopotassium phosphate (KH_2PO_4), 500 µM potassium sulfate (K_2SO_4), 325 µM magnesium sulfate ($MgSO_4.7H_2O$), 50 µM sodium chloride (NaCl). Micronutrients were 80 µM iron (Fe) (III)-ethylenediaminetetraacetic acid (EDTA)- sodium (Na), 0.4 µM manganese sulfate ($MnSO_4$), 0.4 µM zinc sulfate ($ZnSO_4$), 0.4 µM copper sulfate ($CuSO_4$), 8 µM boric acid (H_3BO_3), 0.4 µM sodium molybdate (Na_2MoO_4). Solutions were replaced completely every week in the first two weeks. The experiment was in a completely randomized block design with three replications and six plants in each replication.

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

Grafted and ungrafted pepino plants were harvested by cutting them into the leaf, stem, and roots for the fresh weight determination at the end of the experiment. After measuring the fresh weights of each shoot and root fraction, samples were dried separately in paper bags in a ventilated oven at 70 °C for 72 hours. Shoot: root ratio was calculated from the dry weight measurements.

Root Morphological Measurements

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

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

Leaf Physiological Measurements

At the hydroponic experiment, some of the leaf physiological parameters were determined destructively at final harvest, while some of them were measured non-destructively prior to the final harvest. The leaf chlorophyll index (SPAD) was determined non-destructively by using a portable chlorophyll (SPAD) meter (Minolta SPAD-502). During the growth period, SPAD readings were performed on 3rd and 4th week of the vegetation period at the center of the leaves on the fully expanded youngest leaf of whole plants for each treatment. The leaf area of the plants was measured destructively during the harvesting process by using a portable leaf-area meter (LI-3100, LI-COR. Inc., Lincoln, NE, USA). Total leaf area was recorded in centimeter square (cm^2).

The leaf-level CO_2 gas exchange (µmol CO_2 m^{-2} s^{-1}) measurements were done non-destructively in controlled growth chamber by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). The leaf photosynthesis measurement was performed on the most recent fully expanded leaves, using four replicate leaves per treatment on 3rd and 4th week of the vegetation period. All gas exchange measurements were carried out between 09:00 and 12:00 HR.

Leaf and Root Electrolyte Leakage Measurements

Electrolyte leakage (EL) in leaves and roots was determined as described by Lutts et al. (1995). EL measurements were done on the youngest fully expanded leaves between 11:00 and 15:00 hr every 48 hr using three replicates per treatment. 1 cm leaf disks were excised from young fully expanded leaves using a cork borer. To remove surface contamination, leaf samples were washed 3 times with distilled water and then placed in individual stoppered vials containing 10 mL of distilled water.

EL determination in plant roots was done by taking fresh root tips (2 cm in length) from each treatment at the final harvest. The samples of root and leaf were incubated at room temperature (25 ◦C) on a shaker (100 rpm) for 24 h. The electrical conductivity of the bathing solution (EC1) was read after incubation. The same samples were then placed in an autoclave at 120 ◦C for 20 min and a second reading of the EC (EC2) was made after cooling the solution to room temperature. The EL was expressed as: EL= (EC1)/ (EC2) × 100.

Statistical Analysis

Statistical analysis of the nutrient solution experiments data was performed using SAS Statistical Software (SAS 9.0, SAS Institute Inc., Cary, NC, USA). A two-factorial analysis of variance was performed to study the effects of genotype or grafting combination and salt and their interactions on the variables analyzed. Levels of significance are represented by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and ns means not significant (F-Test). Differences between the treatments were analyzed using Duncan's Multiple Test ($p < 0.05$).

Results and Discussion

Shoot and Root Biomass Production and Partitioning

Shoot fresh weight, root fresh weight, leaf area of the grafted and ungrafted plants examined under different salt applications (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) were presented in Table 1. Fresh weight of shoot, fresh weight of root and leaf area were significantly influenced by grafting, various salt applications, and grafting × salt interaction. Weak growth and development of plants were recorded in grafted and ungrafted pepino as salt level was rised in nutrient solution. Therefore, significant declines were observed in fresh weight of shoot (23.6%, 52.1%) and root (24.8%, 52.8%) and leaf area (21.3%, 51.9%) of pepino plants under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1} (control) salt application, respectively. To date, several studies have indicated that salinity causes many types of harm, including growth inhibition, physiological disorders, and yield and quality reduction; nevertheless, the degree of salt impairment has been indicated to be reliant on cultivar, salinity level, salinity span, and plant developmental stage (del Amor et al., 1999). Fresh weight of shoot, fresh weight of root, dry weight of shoot, dry weight of root reduction under salt application has been observed in melon (Del Amor et al., 1999; Rouphael et al., 2012), watermelon (Colla et al., 2006; Yetisir and Uygur, 2010), cucumber (Colla et al., 2013), tomato (Gong et al., 2013), and eggplant (Yasar et al., 2006) under salt stress

conditions.

Grafted plants had a greater fresh weight deposition. They produced significantly higher shoot fresh weight under both 4 dS m^{-1} (93.43 g $plant^{-1}$) and 8 dS m^{-1} (52.0 g $plant^{-1}$) salt applications comparing with ungrafted plants. Grafted plants produced 54.1%, 43.0% and 9.6% higher fresh weight of shoot than ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively. Root fresh weight was observed to be comparatively more in grafted ones than in ungrafted ones under 1 dS m^{-1} (control) salt application, though there was no substantial difference occurred among grafted and ungrafted pepino under 4 dS m^{-1} salt application, as well as under 8 dS m^{-1} salt application. Grafted plants significantly had higher root fresh weight under both 4 dS m^{-1} (19.16 g $plant^{-1}$) and 8 dS m^{-1} (10.88 g $plant^{-1}$) salt applications than ungrafted plants. Grafted plants produced 52.0%, 42.0% and 12.8% higher fresh weight of root than the ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively. In our study, grafting had encouraging impacts on pepino growth under control and salt stress conditions. Inhibitions of shoot growth in ungrafted plants are due to decline in leaves number, deposition of shoot fresh weight and shoot dry weight, extension of shoots and a decrease in the area and size of the leaf (Flexas et al., 2004; Zhang et al., 2009). Our outcomes were in line by research of grafted plants such as pepper (Penella et al., 2016; Al Rubaye et al., 2020), melon (Ulas et al., 2019a) and watermelon (Ulas et al., 2020) in a saline state.

The area of leaf measurements for different salt applications at the final harvest indicated that grafted ones had a greater leaf area than ungrafted ones. Though there were no substantial variations observed amongst grafted and ungrafted pepino under 4 dS m^{-1} salt application. Grafted plants produced 52.5%, 40.7% and 8.7% higher leaf area than the ungrafted plants under different salt applications (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}), respectively. Results obtained confirms with previous studies demonstrating that ungrafted watermelon leaf area showed significant improvement as grafting with two different commercial rootstocks, (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) and Macis [*Lagenaria siceraria* (Mol.) Standl.] under salt stress and hydroponic conditions (Yamac, 2017). Decline in leaf was due to accumulation of ion in the leaves, especially the old leaves (Greenway and Munns, 1980). Wignarajah et al. (1975) underscored that salt application limited leaf expansion in beans, and it was mainly as a result of cell division inhibition instead of cell expansion. Under salinity, decline in leaf area was seen on grafted watermelon (Colla et al., 2006; Yamac, 2017) and grafted pepper (Al Rubaye et al., 2020).

Table 1. Fresh weight of shoot, fresh weight of root and leaf area formation of grafted and ungrafted pepino at hydroponic system under salinity (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1})

Salt levels	Shoot fresh weight (g $plant^{-1}$)		Root fresh weight (g $plant^{-1}$)		Leaf area (cm^2 $plant^{-1}$)	
	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted
1 dS m^{-1}	81.80 a	126.00 b	17.24 a	26.18 b	1867 b	2846 a
4 dS m^{-1}	65.30 a	93.43 b	13.48 a	19.16 a	1542 a	2169 a
8 dS m^{-1}	47.46 a	52.00 b	9.64 a	10.88 a	1087 b	1182 a
Significance						
Salt	***		***		***	
Graft comb.	***		***		***	
Salt X Graft comb.	***		***		***	

Values indicated by various letters are substantially distinct from genotypes inside columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Dry weight of shoot, dry weight of root, and root to shoot ratio of the grafted and ungrafted plants examined under various salt applications (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) were presented in Table 2. Dry weight of shoot, dry weight of root, and ratio of root to shoot were substantially ($p < 0.001$) influenced by grafting, changing levels of salt, and interaction of grafting × salt. Irrespective of the grafting, adverse effects were observed and recorded in growth and development of both grafted and ungrafted plants as salt application increases. Therefore, significant declines were observed in shoot dry weight (24.3%, 53.0%), and root dry weight (15.4%, 45.1%) of pepino plants under 4 and 8 dS m^{-1} of salt applications comparing with 1 dS m^{-1} (control) of salt application, respectively. Decline in dry weight of shoot and, dry weight of root were observed also in many crop species including tomato (Dasgan et al., 2002), pepper (Chartzoulakis and Klapaki, 2000), melon (Sivritepe et al., 2005), eggplant (Chartzoulakis and Loupassaki, 1997), strawberry (Awang et al., 1993), broad bean (de Pascale and Barbieri, 1997), and cucumber (van der Sanden and Veen, 1992) under salt application.

Grafted plants produced significantly higher shoot dry weight under both 4 dS m^{-1} (9.54 g $plant^{-1}$) and 8 dS m^{-1} (5.24 g $plant^{-1}$) salt applications comparing with ungrafted plants. Grafted plants produced 60.0%, 46.6% and 11.1% higher dry weight of shoot than the ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1}, and 8 dS m^{-1} salt applications, respectively. Khah et al., (2006) studied grafting effects have on tomato growth and yield in greenhouse and open-field conditions, 'Big Red' tomato was self-grafted (used both as scion and rootstock) and ungrafted served as control, and two hybrid tomatoes

'Primavera' and 'Heman' were selected as rootstocks. Grafted plants with rootstocks were more vigorous than ungrafted ones in the greenhouse as well as in the open field. On the other hand, higher plant height, stem fresh-dry weight, and leaves fresh-dry weight were recorded in self-grafted plants than in ungrafted control under both conditions of cultivation. The obtained results were in line with those of Yamac (2017), who underscored that salt stress under saline and hydroponic conditions decreased shoot dry weight in grafted and ungrafted watermelon. The combined osmotic stress impact could be the major cause of reduction of growth (Greenway and Munns, 1980), which is detrimental to plants in growth stage and uptake of ion (Dumbroff and Cooper, 1974). Penella et al. (2016) exhibited that grafted pepper plants were less susceptible to salt stress than the ungrafted ones, with respect to photosynthesis and subsequently growth and yield. To date, in several greenhouse crops grown under hydroponic conditions, the ameliorative grafting impact on plant growth under salt application has been entirely consistent with other studies in tomatoes and melons (He et al., 2009; Rouphael et al., 2012; Yamac, 2017).

Root dry weight was highly influenced by salt application in grafted and ungrafted plants. Dry weight of root of the grafted plants were substantially greater than ungrafted ones under 1 dS m^{-1} (control) salt application, though no considerable variation was observed among grafted and ungrafted pepino under 4 dS m^{-1} salt application, as well as under 8 dS m^{-1} salt application. Regarding dry weight of root, grafted plants produced significantly higher root dry weight under both 4 dS m^{-1} (1.79 g $plant^{-1}$) and 8 dS m^{-1} (1.13 g $plant^{-1}$) salt applications comparing with plants that were not grafted. The grafted plants produced 68.8%, 36.0% and 29.3% higher root dry weight than the ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively (Table 2). Regarding root-to-shoot ratios, no significance difference was reported among ungrafted and grafted plants under various salt applications. These results confirm with studies conducted by other researchers, which indicated that, under salt stress conditions, grafted pepper plants showed higher significance in root dry mass than ungrafted plants. In grafted plants, higher root development, independent of salt stress, might be the cause of more photosynthetic rate regulated in grafted plants (Penella et al., 2016). Improvement in crop growth and performance was observed when plants were grafted onto strong rootstocks with numerous root hairs and higher root length which facilitates additional water and mineral uptake from soil and transfer them to plant aerial parts (Yarsi and Sari, 2006).

Table 2. Dry weight of shoot, dry weight of root, and root to shoot ratio of grafted and ungrafted pepino at hydroponic system under salinity (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1})

Salt levels	Shoot dry weight (g $plant^{-1}$)		Root dry weight (g $plant^{-1}$)		Root to shoot ratio (g g^{-1})	
	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted
1 dS m^{-1}	8.15 b	13.03 a	1.36 b	2.30 a	0.17 a	0.18 a
4 dS m^{-1}	6.50 b	9.54 a	1.31 a	1.79 a	0.20 a	0.19 a
8 dS m^{-1}	4.72 b	5.24 a	0.88 a	1.13 a	0.19 a	0.22 a
Significance						
Salt	***		***		***	
Graft comb.	***		***		*	
Salt X Graft comb.	***		***		***	

Values indicated by various letters are substantially distinct from genotypes inside columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Leaf Physiological Development, Photosynthetic Activity of Leaves, Leaf and Root Ion Leakage

The results of leaf chlorophyll content (SPAD), photosynthesis, leaf and root ion leakage at final harvest of grafted and ungrafted pepino grown under various salt levels (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) were shown in Figure 1. Obtained data clarify that chlorophyll content of leaf was considerably ($p < 0.001$) influenced by grafting, various salt applications, and grafting × salt interaction. Leaf chlorophyll content was considerably reduced by rising salt level of the nutrient solution. Regardless of grafting, reductions in comparable shoot- root dry weight under salinity resulted in a significant reduction in chlorophyll content (5.7%, 18.7%) of pepino plants under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1} (control) salt application, respectively. This explains the reason why the production of shoot- root dry weight (Table 1 and 2) of grafted and ungrafted plants were negatively influenced by hydroponically saline condition, because biomass production and yield of crops are extremely contingent on leaf area formation and activities of leaf photosynthesis (Hirasawa and Hsiao, 1999). The inhibition of growth in several salinity-focused plant species is most often related to a decline in photosynthetic ability (He et al., 2009). Decreased photosynthesis due to increased salinity may be due to a number of causes, like smaller conductance of stomatal and depression in certain physiological activities associated with carbon uptake and fixation, or a combination of the two (Flexas et al., 2004; Zhang et al., 2009).

Grafted plants produced significantly higher leaf chlorophyll content under both 4 dS m^{-1} (66.2 SPAD) and 8 dS m^{-1} (56.8 SPAD) salt applications comparing to ungrafted plants. Grafted plants produced 19.9%, 9.2% and 8.2% higher SPAD than the ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively. Content of chlorophyll,

that has a directly effect on leaves photosynthetic activity, is a significant index of photosynthetic potential and can be regarded as a source of food and energy. Trinchera et al. (2013) documented a relatively greater concentration of chlorophyll pigments in grafted plants comparing with ungrafted control plants. The data is also in accordance with those of Yamac (2017), who concluded that salinity diminished leaf chlorophyll contents in grafted and ungrafted watermelon at the hydroponic conditions.

Photosynthesis was influenced significantly ($p < 0.001$) by different levels of salt and grafting. A sharper drop in the rate of photosynthesis occurred because of salt application at grafted and ungrafted plants. Ungrafted and grafted plants receiving a 1 dS m^{-1} (control) salinity displayed maximum photosynthesis rate than 4 or 8 dS m^{-1} salt applications. Irrespective of graft combinations, significant declines were observed in photosynthesis rate (24.6%, 42.1%) of pepino plants under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1} (control) salt application, respectively. Bethke and Drew (1992) considered the rationale for reduction in photosynthesis at salt-stressed pepper plants and proposed that photosynthesis reductions are largely non-stomatal, biochemical level and strongly associated with concentration of both Na^+ and Cl^- in plant leaves. Grafted plants produced significantly higher photosynthesis rate under both 4 dS m^{-1} (9.1 µ mol CO_2 m^{-2} s^{-1}) and 8 dS m^{-1} (7.2 µ mol CO_2 m^{-2} s^{-1}) salt application comparing by ungrafted ones. Grafted plants produced 8.0%, 5.1% and 10.8% higher photosynthesis rate than the ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively. Though, there were no substantial variations observed among grafted and ungrafted plants under 1 dS m^{-1} and 4 dS m^{-1} salt applications. These findings are corresponding to those of Penella et al. (2015) stated that salinity affects photosynthesis negatively as slightly at grafted pepper plants than ungrafted plants. Similar results were observed at pepper genotypes under salt stress conditions by Chartzoulakis and Klapaki (2000). In another study, in grafted watermelon plants, photosynthesis level was decreased as salt level increased in the nutrient solution (Yamac, 2017).

Regarding leaf ion leakage, it was substantially ($p < 0.001$) affected by different levels of salinity; though not grafting, and grafting × salt interaction. On the other hand, root ion leakage was significantly ($p < 0.001$) affected by grafting and various salt applications, though not grafting × salt interaction. Regardless of grafting mixture, an enhancement in salt content of nutrient solution resulted in a significant increase in leaf (17.1%, 36.7%) and root (20.3%, 48.9%) ion leakage of salt-treated pepino plants (4 dS m^{-1} and 8 dS m^{-1}) comparative to controls (1 dS m^{-1}) (Figure 1C and 1D). These are typical reactions of plants that generally express tolerance strategies which can be seen in studies of melon (Kuşvuran, 2010; Sarabi et al., 2017), rice (Lutts et al., 1996), cucumber (Mumtaz-Khan et al., 2013), pepper (Kaya et al., 2001), sugarbeet (Ghoulam et al., 2002), barley (Perez-Lopez et al., 2008), okra (Saeed et al., 2014), watermelon (Li et al., 2017) and tomato (Kaya et al., 2001). Grafted plants produced significantly higher leaf and root ion leakage under both 4 dS m^{-1} (28.15, 39.05) and 8 dS m^{-1} (33.72, 48.44) salt applications comparing with ungrafted plants. Similar results were observed under saline conditions at grafted plants such as cucumber (Colla et al., 2013), watermelon (Yamac, 2017), melon (Ulas et al., 2019a), tomato (Ulas et al., 2019b), and pepper (Penella et al., 2016; Al Rubaye et al., 2020).

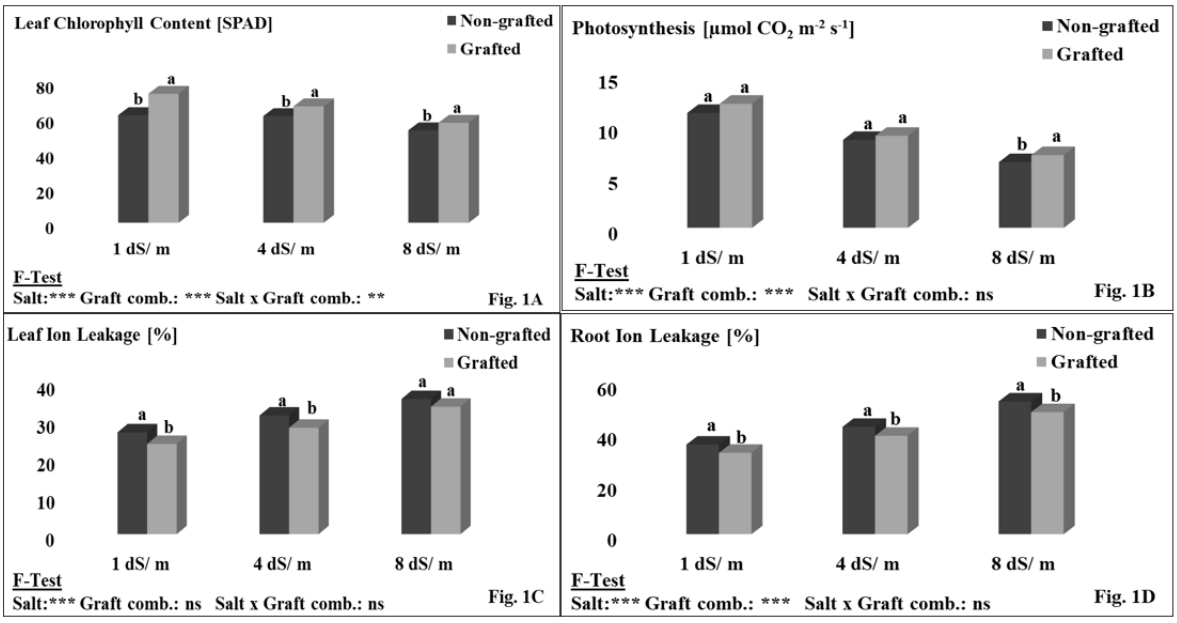

Figure 1. Leaf chlorophyll content (SPAD) (A), leaf photosynthesis (B), leaf (C) and root ion leakage (D) of grafted and ungrafted pepino at hydroponic system under salinity (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}). Values indicated by various letters are substantially distinct from genotypes inside columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Root Morphological Development and Root Architecture

At the completion of grafting experiment, root morphological development and architecture of grafted and ungrafted plants tested at different salt levels (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) were presented in Table 3. Total root length, and root volume were considerably ($p < 0.001$) influenced by grafting, various salt applications, and grafting × salt interaction. Irrespective of grafting, significant declines were observed in total root length (6.7%, 16.4%), and root volume (3.8%, 5.8%) of pepino plants under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1}, respectively. Average root diameter was influenced substantially ($p < 0.001$) by grafting, different levels of salt, but not the interaction of grafting x salt. Average root diameter significantly increased (1.5, 6.2%) under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1}, respectively. Grafted plants produced significantly higher overall root length (8602.08 cm $plant^{-1}$, 7818.52 cm $plant^{-1}$), volume of root (5.60 cm^3 $plant^{-1}$, 5.38 cm^3 $plant^{-1}$), diameter of root (0.28 mm $plant^{-1}$, 0.3 mm $plant^{-1}$) under both 4 dS m^{-1} and 8 dS m^{-1} salt applications comparing with ungrafted plants, respectively. The grafted plants produced higher total root length (8.6%, 3.6% and 6.6%), root volume (3.1%, 6.7% and 2.4%) than ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively.

Salt stress decreases transpiration and respiration, and also water absorption and root growth (Dölarslan and Gül, 2012). Like our research, Rastgeldi (2010) found that salinity induced decrease in total root length according on pepper genotypes comparing with control plants. In another study, the rootstocks experienced increment or decrement effects in root biomass depending on genotypes under salinity and drought (Penella et al., 2017).

Table 3. Total root length, total root volume and total root diameter of grafted and ungrafted pepino at hydroponic system under salinity (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}).

Salt levels	Total root length (cm $plant^{-1}$)		Total root volume (cm^3 $plant^{-1}$)		Average root diameter (mm)	
	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted
1 dS m^{-1}	8690.88 b	9441.09a	5.55 a	5.73 a	0.29 a	0.28 b
4 dS m^{-1}	8307.34 b	8602.08a	5.25 b	5.60 a	0.29 a	0.28 b
8 dS m^{-1}	7331.37 b	7818.52a	5.25 b	5.38 a	0.30 a	0.30 b
Significance						
Salt	***		***		***	
Graft comb.	***		***		***	
Salt X Graft comb.	***		**		ns	

Values indicated by various letters are substantially distinct from genotypes inside columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Conclusion

In conclusion, increasing salt level had a considerable negative impact on shoot growth, root morphological and leaf physiological responses of grafted and ungrafted plants. Although this negative impact was obvious in ungrafted plants than grafted plants. Irrespective of grafting, significant declines were observed in shoot fresh weight (23.6%, 52.1%), root fresh weight (24.8%, 52.8%), leaf area formation (21.3%, 51.9%), shoot dry weight (24.3%, 53.0%), root dry weight (15.4%, 45.1%), SPAD (5.7%, 18.7%), photosynthesis rate (24.6%, 42.1%), total root length (6.7%, 16.4%), and root volume (3.8%, 5.8%) of pepino plants under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1} (control) salt application, respectively. Grafted plants produced 54.1%, 43.0% and 9.6% higher shoot fresh weight; 52.0%, 42.0% and 12.8% higher root fresh weight; 52.5%, 40.7% and 8.7% higher leaf area; 60.0%, 46.6% and 11.1% higher shoot dry weight; 68.8%, 36.0% and 29.3% higher root dry weight; 19.9%, 9.2% and 8.2% higher SPAD; 8.0%, 5.1% and 10.8% higher photosynthesis rate; 8.6%, 3.6% and 6.6% higher total root length; 3.1%, 6.7% and 2.4% higher root volume than ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively. Grafting encouraged plant growth in pepino plants under both control and salinity, also, significant decline in plant biomass under salinity was found in grafted and ungrafted pepino. Grafting technology has demonstrated to be a rapid alternative approach for conferring resistance to biotic and abiotic stresses, promoting plant vigor, increasing plant growth, and developing pepino production. Definitively, the use of grafting is a significant integrated approach for more developed cultivation forms, like hydroponics especially under salt stress conditions. As a next step, the same experiment may be performed with different Solanaceae rootstock genotypes to observe morphological, physiological, and biochemical responses among scion (pepino)/rootstock combinations under different salt treatments.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Influence of Different Priming Materials on Germination and Alpha-Amylase Enzyme of Hybrids Sorghum (*Sorghum bicolor* L. Moench. x *Sorghum sudanense* Staph.) Seeds

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Abstract

Germination is considered a critical step in the development cycle of the plant. But no information is available regarding seed priming with Putrescine, Jasmonic acid, Kinetin, Potassium Nitrate (KNO_3), Salicylic acid at a time in the aged and non-aged seeds of sorghum cultivars. However, to know the effect of seed priming with the aforesaid chemicals in the aged and non-aged seeds of sorghum cultivars on the germination rate, germination vigour and alpha-amylase activity, research was conducted under laboratory condition, at Field Crops Central Research Institute, Ankara, Turkey. Aged and unaged seeds of Sugar Grazer II and Digestivo hybrid silage sorghum cultivars were used as the seed material of the experiment. Putrescine, Jasmonic acid, Kinetin, KNO_3 and Salicylic acid were used as priming chemicals. The results revealed that aged and un-aged seeds of the cultivars showed different responses to similar chemical and KNO_3 application to un-aged seeds had a positive effect on germination rate and alpha-amylase, Whereas Jasmonic acid and Putrescine applications had a positive effect on the aged seeds. Priming with putrescine, KNO_3 and jasmonic acid showed the best results in the experiment.

Keywords: Alpha-Amylase, Germination, Priming, Seed

Introduction

Seed germination forms the basis of plant formation as the period of strongest life activity in all life cycles of a plant. During this period, the metabolism of fats, proteins and carbohydrates provides energy for seedling growth (Shang and Zhao, 1993). These three major nutrient reserves are specifically hydrolysed by amylases, proteases and lipases, respectively (Barros et al., 2010; Di Girolamo and Barbanti, 2012). The carbon resources required for the growth of the embryo are obtained by the breakdown of starch. Starch is catalysed by the amylase enzyme family and broken down as a result of reactions (Sun and Henson, 1991). α-amylases are endo enzymes that can produce shorter chains called border dextrins, glucose and maltose by breaking random α- (1, 4) bonds within their polysaccharide chains. β-amylases are exohydrolases that hydrolyze α- (1, 4) bonds in polysaccharides (Catusse et al., 2012). The activities of soluble sugars and amylase enzymes are positively associated with seed germination and seedling viability (McDonald, 2000). The seed, whose metabolic activity is minimized by drying, gradually loses its germination rate and strength by being exposed to physiological and biochemical changes during improper storage conditions and prolonged storage period. As a result of aging in the seed, changes in the macromolecular structures of the enzymes that need to operate for seed germination occur in thin structure, partial curl or non-curl, condensation of polymers, lipid peroxidation through free

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radicals, enzyme inactivation, reduction of protein amount, disintegration of cell integrity and genetic damage. Problems in the activities of enzymes cause a decrease in the respiratory potential and ATP formation, resulting in a decrease in the amount of nutrients that must be provided to the seed during the germination process, and result in a decrease in the germination rate (Narayana Murthy and Sun, 2000; Goel et al., 2002; Lehner et al., 2008). Preliminary imposition of priming on seeds allows the seed to partially hydrate, causing metabolic activities to begin, increasing the activity of biochemical mechanisms required in the repair of cell damages and activation of antioxidants in the defence system. However, it does not allow root formation (Shrivastava et al. 2014). In addition, priming increases the activity of enzymes such as α-amylase, which have an important role in the germination of the seed, causing the compounds in the endosperm to break down in a shorter time and accelerates the induction of enzymes that catalyze the transfer of these degraded compounds (Di Girolamo and Barbanti, 2012). Positive effects of jasmonic acid and salicylic acid application on germination power were observed by Ebrahim Pour Mokhtari and Emeklier (2017) in hybrid sorghum, Buyukcingil (2007) in grain sorghum, and Canakci (2010) in barley. Sorghum seeds generally lose their biological properties in a short time. This situation affects plant genetic characteristics in storage conditions. Since sorghum seeds need very well prepared seedbeds for production. If planting is done in poorly prepared soils, germination and emergence rates will be very low. On the other hand, sorghum seeds should be preserved in very good conditions, as the seed vitality rapidly disappears. In this study, the effects of priming with different chemicals on the aged and unaged seeds of two different hybrid sorghum varieties (Sugar Grazer II and Digestivo) were investigated to evaluate the germination and α-amylase activity.

Materials and Method

Location and Materials

This research was conducted under laboratory conditions at Field Crops Central Research Institute, Ankara-Turkey. Aged and unaged seeds of Sugar Grazer II and Digestivo hybrid silage sorghum (*Sorghum bicolor* L. Moench. x *Sorghum sudanense* Staph.) cultivars were used as the seed material of the experiment. Five chemicals Putrescine (4 mg/L), Jasmonic acid (1 mg/L), Kinetin (10 mg/L), KNO_3 (25 mg/L) and Salicylic acid (4mg/L) were utilized for this study.

Treatments and Design

The experiment is consisted of three factors viz. a) Two cultivars (i. Sugar Grazer II, and ii. Digestivo), b) Two seed ages (aged and unaged), and c) Six priming (i. No priming-control, ii. Priming with Jasmonic acid, iii. Priming with Kinetin, iv. Priming with Potassium Nitrate ($KNO_{3)}$, v. Priming with Putrescine, and vi. Priming with Salicylic acid). The experiment was laid out with RCDB and three replications.

Experimentation

For laboratory experiments, seeds were utilised for each treatment at the end of the priming session. This study was used five chemicals (Putresin (4 mg/L), Salicylic acid (4 mg/L), Kinetin (10 mg/L), KNO_3 (25 mg/L) Jasmonic acid (1 mg/L)). Chemicals used in the study were provided by Merck and Sigma Aldrich Chemical Co. (Anonymous, 2014). Depending on the variety, Sugar Grazer II variety was kept for 8 hours, Digestivo variety was kept for 6 hours. After the priming period was over, the seeds were washed with distilled water, dried in Petri dishes for 24 hours at room temperature, and then made ready for planting. The seed aging process was determined according to Matthews (1980) and Zamani et al. (2010). The seeds (100 x 3 = 300) were placed in germination cabinets with a temperature of ± 1°C in special germination containers, adjusted to 25°C as the standard germination temperature and left to germinate.

Data collection

Germination Rate and Germination Percentage

According to ISTA rules, the seeds germinated on the fourth day were counted, and the average of these values calculated as percentages was determined as the germination rate. Germination percentage (%): Seedling vigour is determined as seeds that germinated on the 10th day according to ISTA rules (Anonymous, 2011).

Alfa-amylase

Starch was extracted by using the method of Singh *et al*. (2009). Sorghum grain (100 g) was immersed in 200 ml of NaOH (0.25%, w/v) at 5°C for 24 h. The soaked grains were washed and ground with an equal volume of water using a blender for 3 min. The mixture was filtered through a 200-mesh screen. The remainder on the sieve was rinsed with distilled water. Grinding and filtering were repeated on this material. After rinsing, the remainder on the sieve was discarded. The filtered liquid was allowed to stand for 1 h. The filtered liquid was centrifuged at 760 × g for 10 min. The grey colored froth, top protein-rich layer was removed using a spatula. Water was added again to re-suspend the sample, and centrifugation was done for 5 min. washing and centrifugation process was repeated several times until the top starch layer was white. The starch was dried for 24 h at 40°C. After each sample of starch is extracted, for measuring alpha-amylase activity, the resultant mixture for raw starch digesting activity contains 0.3 g of raw starch, 30 ml deionized water, 5.5 ml enzyme solution (2.5 units ml^{-1}) and 4 ml of 0.1 M acetate buffer (pH 5.0). The resultant mixture was incubated for 1 h at 30°C. The absorbance of reaction mixture filtrates was analyzed in the spectrophotometer. The absorbance of the supernatant was 620 nm. The absorbance was converted to α-amylase units using a commercial α-amylase preparation with known activity.

Statistical Analysis

Statistical analyses were subjected to variance analysis using the JMP 10 program according to the Random Plots Factorial Trial Design. The significance of the differences was evaluated with the F test, and the grouping of the means was made according to the LSD 5% test.

Results and Discussion

In the study, five different chemicals of putrescine, jasmonic acid, kinetin, KNO_3, salicylic acid were applied to the seeds of two hybrid sorghum varieties (Sugar Grazer II and Digestivo) as aged and unaged conditions and compared with the control (not primed) seeds.

Table 1. Analysis of variance on the germination rate, germination vigor and alpha-amylase effect of two sorghum cultivars as seed aged and unaged conditions

Source	df	Germination rate	Seedling vigour	Alpha-amylase
Cultivar	1	1.0538	0.7149	69.6343***
Seed age	1	0.8785	0.097	11.9432**
Priming	5	22.3048***	13.518***	36.4122***
Cultivar × Seed age	1	6.9343*	0.2396	469.2659***
Cultivare×Priming	5	4.6155**	1.941	64.2289***
Seed age×Priming	5	2.4036	2.5588*	65.9455***
Cultivare×Seed age×Priming	5	6.8339***	0.4107	26.7269***
CV (%)		21	28	28

*, **, *** 5%, 1%, 0.1% significant, respectively

Germination Rate

The results of the variance analysis performed with data on the germination rate of the priming in the seeds of two sorghum hybrid varieties are given in Table 1. Cultivar x seed age interactions were found significant at 5% level, cultivar x priming at 1% level, and cultivar x seed age x priming interactions at the 0.1% level. The results of the variance analysis on the alpha-amylase of the primed seeds are given in Table 1. Seed age was found significant at 1% level and other features were found significant at the 0.1% level.

The germination rate of the un-aged seeds of the Sugar Grazer II variety varied between 86.33 and 95.00%. The highest germination rate was observed in seeds treated with Jasmonic acid, and the lowest in control seeds. In addition, seeds treated with Kinetin and control seeds were in the same group in terms of the lowest germination rate. The germination rate of aged seeds of the Sugar Grazer II variety varied between 84.00 and 98.33%. The highest germination rate was determined in seeds treated with Jasmonic acid followed by Putrescine, and the lowest germination rate was determined in control and Kinetin seeds. The germination rate in the unaged seeds of Digestivo variety varied between 83.00 and 98.00%. The highest germination rate was obtained from seeds treated with KNO_3, and the lowest germination rate from control seeds. The germination rate of aged seeds of Digestivo varied between 89.00 and 93.67%. The highest germination rate was obtained from seeds treated with Putrescine, and the lowest germination rate from control seeds (Table 2).

When we compare the responses of the aged and unaged seeds of both varieties to different chemicals in terms of germination speed characteristics, the aged and un-aged seeds of the Sugar Grazer II variety showed the highest germination rate as a result of the pre-treatment with Jasmonic acid (Figure 1). Jasmonates priming has a supportive role in morphological and physiological changes and provided the highest germination rate. The highest germination rate showed in the pre-application with KNO_3 in the unaged seeds and with Putrescine in the aged seeds of the Digestivo variety. Due to the changes in seed compounds as a result of the aging process, seeds showed different reactions to different chemicals and showed different results. The different reactions between the two cultivars are related to the physiological characteristics of the seeds of the cultivars, and this result is expected. Our findings regarding the germination rate are showed similarities with the results of Tekin and Bozcuk (1998) in sunflower, Çavuşoğlu (2007) in barley for the application Putrescine; Madakadze et al. (2000) in millet, and Buyukcingil (2007) in grain sorghum for application of KNO_3.

Table 2. The average germination rate values of aged and normal seeds of sorghum varieties of different chemical applications

	Unaged		Mean	Aged		Mean
	Digestivo	Sugar Grazer II		Digestivo	Sugar Grazer II	
Control	83.00	86.33	84.67	89.00	84.00	86.50
Jasmonic acid	93.33	95.00	94.17	93.67	98.33	96.00
Kinetin	97.00	86.33	91.67	90.00	96.00	93.00
KNO_3	98.00	93.67	95.84	92.67	92.33	92.50
Putrescine	92.00	94.00	93.00	93.67	98.33	96.00
Salicylic acid	93.67	88.00	90.84	91.67	87.67	89.67
Mean	92.83	90.56		91.78	92.78	
Unaged /aged means	91.69			92.28		

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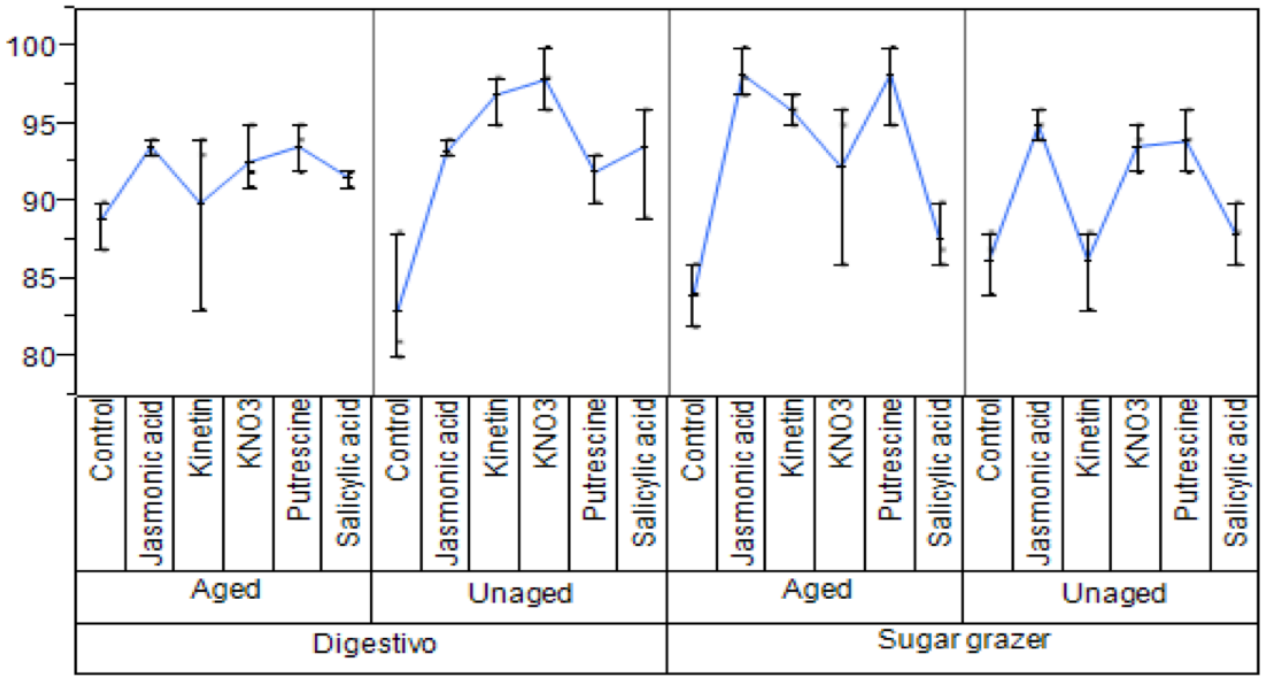

Figure 1. Germination rate values of aged and normal seeds of two sorghum varieties of different chemical applications

Germination Percentage (Vigor)

The results of variance analysis regarding the effects of priming on the germination vigor of the seeds of two different sorghum hybrid varieties are shown in Table 1.Statistically, only seed age x priming interactions were found significant at the level of 5%, the differences among the primings were significant at the 0.1% level, and differences between the varieties were found insignificant.

In terms of germination vigor, the germination power of the un-aged seeds of the Sugar Grazer II variety varied between 87.67 and 95.67%. While the highest germination vigor was determined in seeds treated with Jasmonic acid followed by Salicylic acid, and the lowest germination power was seen in control seeds. The germination power of the aged seeds of the Sugar Grazer variety varied between 87.67 and 97.67%. However, the highest germination power was determined in seeds treated with Putrescine, and the lowest germination vigor was determined in control seeds. The germination vigor of the non-aged seeds of the Digestivo variety varied between 86.67% and 96.67%. The highest germination vigor was determined in seeds treated with Salicylic acid and KNO_3, and the lowest germination vigor in control seeds. The germination strength of the aged seeds of Digestivo varied between 89.00 and 95.00%. The highest germination power was determined in seeds treated with Jasmonic acid, and the lowest germination vigor in control seeds (Table 3). When we compare the responses of aged and non-aged seeds of both varieties to different chemicals, the aged and unaged seeds of the Sugar Grazer II variety showed the highest germination vigor as a result of pre-treatment with Jasmonic acid (Figure 2). Jasmonic acid changed the morpho-physiological properties and showed the highest germination power. The non-aged and aged seeds of the Digestivo variety showed the highest germination vigor as a result of the pre-treatment with Salicylic acid and KNO_3. Ebrahim Pour Mokhtari and Emeklierin (2017) obtained the highest germination rate with the application of Jasmonic acid in both aged and un-aged seeds in hybrid sorghum varieties. Buyukcingil (2007) stated that the application of Jasmonic acid and Salicylic acid in grain sorghum provides positive effects on germination vigor. Our study showed similarities with the results obtained by Canakci (2010) in the application of Salicylic acid in barley, Xu et al. (2011) with the application of Putrescine in tobacco seeds.

Alpha-amylase in the aged seeds of the Sugar Grazer II variety varied between 0.006 and 0.057. Nonetheless, the highest alpha-amylase value was obtained in the seeds treated with Putrescine, and the lowest was found in the seeds treated with control and KNO_3. Alpha-amylase in the un-aged seeds of the Sugar Grazer II variety varied between 0.006 and 0.011. The highest alpha-amylase was determined in seeds treated with Putrescine, while the lowest alpha-amylase was determined in control seeds. The germination strength of the non-aged seeds of the Digestivo variety ranged from 0.003 to 0.005. Alpha-amylase in the aged seeds of the Digestivo variety varied between 0.005 and 0.021. The highest alpha-amylase was determined in the seeds treated with Salicylic acid, and the lowest in the seeds treated with Kinetin (Table 4 and Figure 3). The most activity of the α-amylase enzyme was obtained by Asadi Danalo et al. (2018) as a result of the priming process with 3 mM Spermidine in *Borago officinalis* plants. According to the results obtained by Lee and Kim (2000), the priming in aged seeds of rice plant increased the

total sugar content in the seed and the activity of the α-amylase enzyme, resulting in an increase in the germination rate. It has been determined by many researchers that the increase in the activity of the alpha-amylase enzyme increases germination speed and strength, allowing seeds to germinate under a wider range of environmental conditions (Mukasa *et al*., 2003; Basra *et al*., 2005; Sathish and Sundareswaran, 2010; Dehghanpour Farashah *et al*., 2011).

Table 3. The average germination percentage (vigor) values of aged and normal seeds of sorghum varieties of different chemical applications

	Unaged			Aged		
	Digestivo	Sugar Grazer II	Mean	Digestivo	Sugar Grazer II	Mean
Control	86.67	87.67	87.83	89.00	87.67	87.67
Jasmonic acid	93.00	95.67	94.00	95.00	97.33	96.50
Kinetin	92.33	90.33	91.83	91.33	92.33	91.33
KNO_3	96.67	94.33	94.83	93.00	92.67	93.50
Putrescine	92.00	95.00	92.50	93.00	97.67	96.33
Salicylic acid	96.67	95.67	94.83	93.00	91.67	93.67
Mean	92.89	93.11		92.39	93.22	
Unaged /aged means	93.00			92.81		

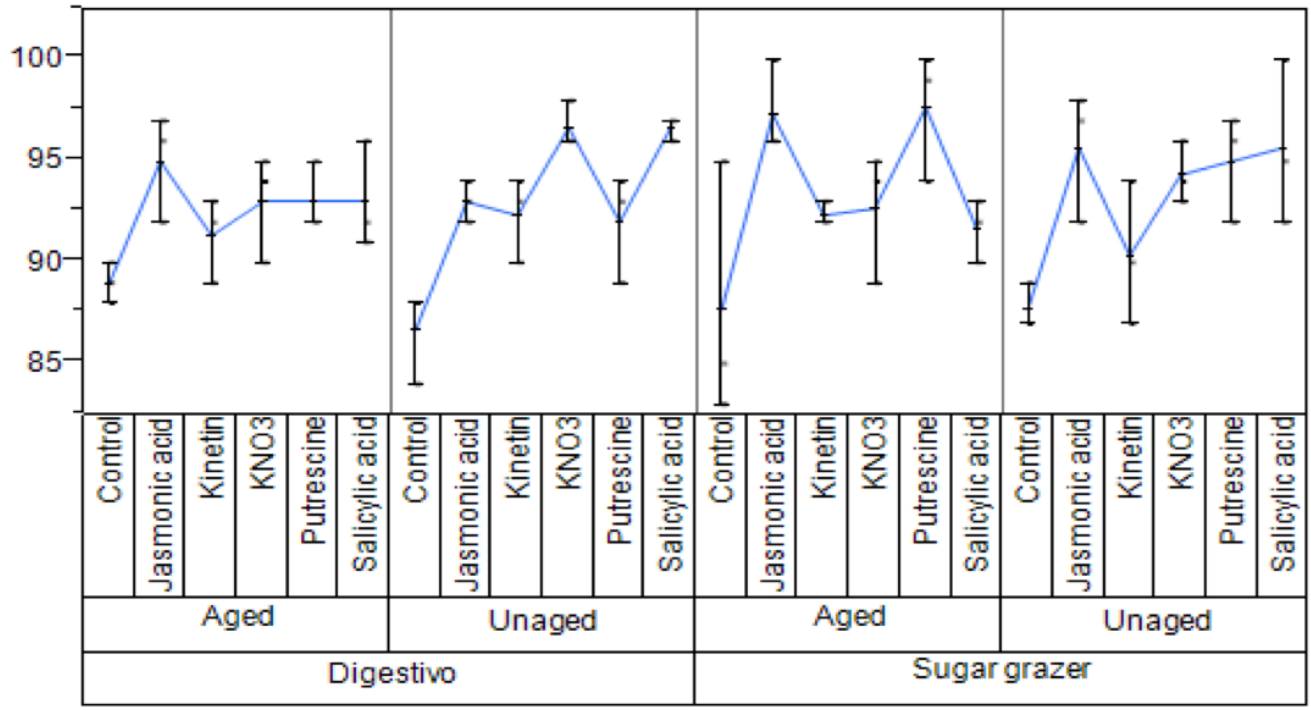

Figure 2. Germination percentage (vigor) of aged and normal seeds of two sorghum varieties of different chemical applications

Table 4. The average alpha-amylase values of aged and unaged seeds of sorghum varieties of different chemical applications

	Unaged			Aged		
	Digestivo	Sugar Grazer II	Mean	Digestivo	Sugar Grazer II	Mean
Control	0.003	0.01	0.007	0.006	0.006	0.006
Jasmonic acid	0.027	0.007	0.017	0.014	0.042	0.028
Kinetin	0.057	0.002	0.030	0.005	0.010	0.008
KNO_3	0.058	0.006	0.032	0.011	0.006	0.009
Putrescine	0.012	0.001	0.007	0.009	0.057	0.033
Salicylic acid	0.058	0.011	0.035	0.021	0.024	0.023
Mean	0.036	0.006		0.011	0.024	
Unaged /aged means	0.021			0.018		

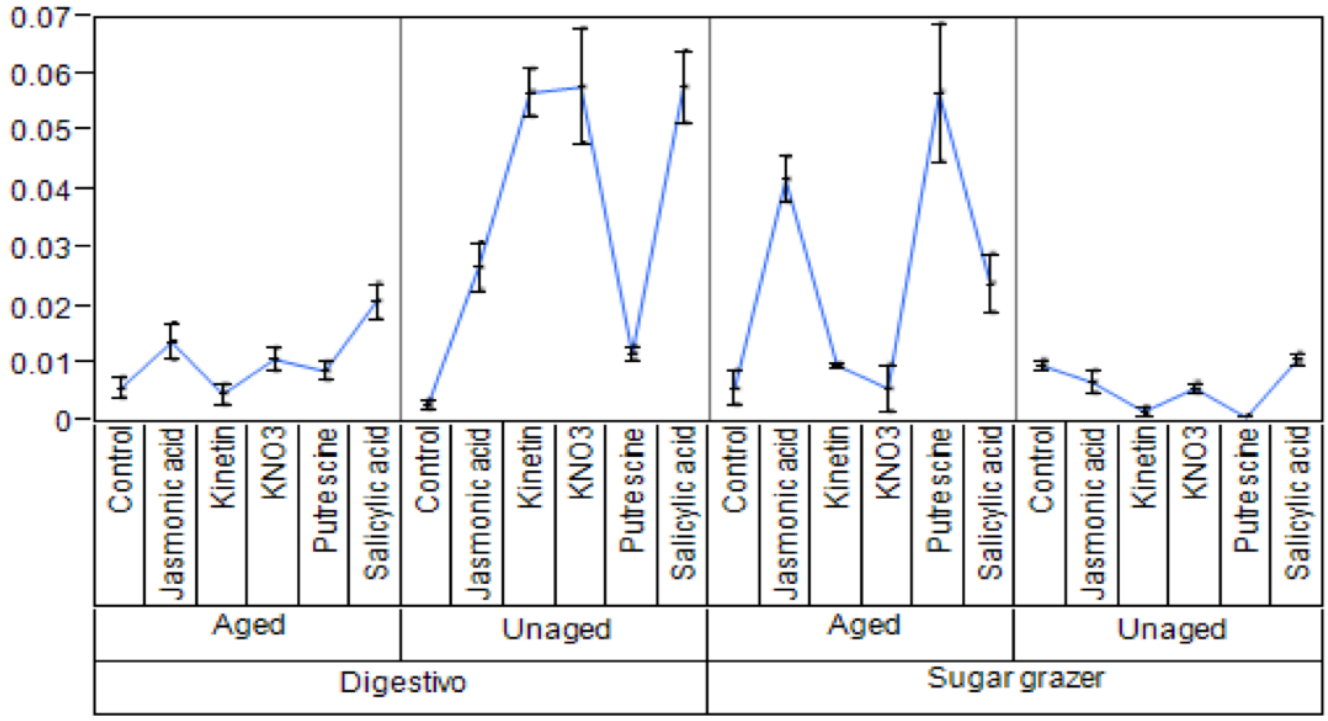

Figure 3. The Alpha-amylase of aged and unaged seeds of two sorghum varieties of different chemical applications

Conclusion

Priming with Putrescine, Jasmonic acid, Kinetin, KNO3 and Salicylic acid remarkably changed the germination rate, germination vigour and enzyme activities in the seeds of the aged and non-aged seeds of sorghum varieties. Therefore, aged and un-aged seeds of the cultivars showed differential responses to similar chemicals. Application of KNO_3 to the un-aged seeds had a positive effect on germination rate and alpha-amylase, whereas Jasmonic acid and Putrescine applications had a positive effect on the aged seeds. Priming with Purescine, KNO_3 and Jasmonic acid showed the best results in the experiment.

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

Negar Ebrahim Pour Mokhtari designed the study and collected the data. Ferhat Kizilgeci made the statistical analysis and wrote the original draft of the article. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Effect of Different Silicone Sources and Concentrations on *in vitro* Micro Propagation of 140 Ru Grape Rootstock

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Abstract

Silicon, which is widely used in different fields, has been used in plant production in *vivo* and *in vitro* studies in recent years. Especially in *in vitro* studies, it is seen that its effect on plant growth and development has been examined. In this study, the effect of three different silicon sources and their four concentrations on micro-propagation of 140 Ru grape rootstocks was investigated. In the study, as explants one-node micro cuttings of rootstock and MS (Murashige and Skoog) as the nutrient medium were used. 1 mg L^{-1} BA (Benzyl Adenine) at the stage of obtaining shoots from cuttings and in the rooting stage, 1 mg L^{-1} IBA (Indole Butyric Acid) were added to the nutrient medium. At both stages, 0 (Control), 0.5, 1.0 and 2.0 mg L^{-1} doses of potassium, sodium and calcium silicate were added to the nutrient medium. Explant viability and mortality rate, shooting rate, plant length, node number, shoot fresh and dry weight, chlorophyll content (SPAD), root number, root length, root fresh and dry weight were examined to determine the effect of the applications. The variance analysis of the study was carried out according to the Two-Way Completely Randomized Experimental Design. According to the results, among the silicon sources, the highest shooting rate (84.40%) was found in the medium containing sodium silicate. The highest shoot fresh and dry weight (0.178 g and 0.026 g, respectively) and root fresh and dry weight values (0.213 g and 0.023 g, respectively) were obtained from potassium silicate. While the number of roots was 2.98 in the medium containing potassium, it was determined as 2.91 in the medium containing calcium silicate. Media containing 1 mg L^{-1} silicate was found to be more successful than 0, 05, 2 mg L^{-1} concentrations. The highest values recorded at the concentration were 4.49 cm in plant length, 7.44 in node number, 0.183 g and 0.028 g in shoot fresh and dry weight, respectively, 28.37 in SPAD value and 3.27 in root number. As a result of the study, it is concluded that adding 1 mg L^{-1} concentration of potassium, calcium and sodium silicate to the nutrient medium can be used in future studies related with in micro propagation.

Keywords: Grapevine, *In vitro*, Potassium silicate, Sodium silicate, Calcium silicate

Introduction

Plant tissue culture is the process of culturing plant cells, tissues and organs isolated under sterile conditions in suitable nutrient mediums (Babaoglu et al., 2001). The content of the nutrient medium and the culture conditions affect the growth and development of plant cells, tissues, and organs *in vitro*. Generally, organic nutrients, sugar, plant growth regulators and solidifier agents are added into plant tissue growth culture (Mansuroglu and Gurel, 2001; Sivanesan and Park, 2014).

Tissue culture is an effective technique used in micro propagation of herbaceous and woody plants. Micro propagation can be described as obtaining of the new plants from the plant parts in artificial nutrition culture and aseptic conditions. Micro-propagation of grapevine with tissue culture

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is widely performed worldwide. Shoot apical meristems and axillary buds are used as explants for grapevine genotypes *in vitro* culture (Gray and Benton, 1991; Diab et al., 2001; Banilas and Korkas, 2007; Alzubi et al., 2012). All grapevine genotypes give similar reactions for certain medium. Reaction degree shows momentous changes according to the genotype, culture medium and hormone. That's why, the most suitable micro-propagation protocol must be created for every genotype. Recently, it has been observed that studies have been carried out to be increased the success rate by adding different silicon (Si) sources to the nutrient medium in micro-propagation (Martins et al., 2019; Montovani et al., 2020).

According to some researchers, although silicon is not among the essential elements for plants (Ma, 2004; Soares et al., 2008), there are also studies reporting that many plants benefit from this element (Epstein, 1994) and that silicon plays a beneficial role in stimulating the growth and development of many plant species (Avestan et al., 2016; Sahebi et al., 2016; Costa et al., 2021).

According to Kadlecova et al. (2020), although silicon is an abundant compound in soil, it is rarely used in plant nutrient medium. Based on its known physiological role and studies done so far, it is observed that silicon has good potential to improve the growth properties of *in vitro* cultivated plants. However, before applying it in practice, it is necessary to determine the optimal application conditions by research, taking into account the fact that plant responses to different chemicals added to the nutrient medium may vary by species and even varieties.

It is submitted that adding silicon into plant tissue medium grows the morphogenetic potential of the plant cells, organs, and tissues. Many of the beneficial effects of silicon are attributed to accumulation of silicon on the cell walls of the roots, leaves and bodies (Ma and Yamaji, 2006)

It was reported, that adding silicon into culture medium increases the hardness of the cell wall and mechanical strength and decreases the transpiration by increasing hemicellulose and lignin content of the plants that are reproduced *in vitro* (Camargo et al., 2007). In many of studies, it was shown that silicon application is increased plant growth and yield under the biotic and abiotic conditions (Artyszak et al., 2015; D'Imperio et al., 2015; Hartly, 2015; Muneer and Jeong, 2015; Xu et al., 2015; Yin et al., 2016). Considering the reality that the effect of different silicates added to the nutrient medium may change according to plant species and even varieties. That's why, optimal application conditions should be re-evaluated for each case.

Under the satisfactory *in vitro* studies for addition of silicon, the plants relating to the orchid (Soares et al., 2011; Soares et al., 2012; Montovani et al., 2020), begonia and violet (Lim et al., 2012), clove (Manivanna et al., 2018), banana (Asmar et al., 2013a and 2013b; Asmar et al., 2015) and apple plants (Reezi et al., 2009; Avestan et al., 2016) are available.

Despite the effects described in various species, a limited number of studies (Mozafari et al., 2018) have been found examining the effects of adding Si to the culture medium on the micro-propagation of grapevine. Therefore, the study was planned to determine the effect of 0.5, 1.0 and 2.0 mg L^{-1} concentrations of potassium (K_2SiO_3), sodium (Na_2SiO_3), and calcium silicate ($CaSiO_3$) added as a silicon source to the Murashige and Scoog (MS) medium (Murashige and Skoog, 1962) in *vitro* on the micro-propagation of 140 Ru grapevine rootstock and aimed to eliminate the lack of information on this subject.

Materials and Methods

This study was conducted in Tissue Culture Laboratory of Department of Horticulture, Faculty of Agriculture, University of Cukurova in 2019.

Material

In the study, one node micro cuttings taken from actively growing shoots of 140 Ruggeri grapevine rootstocks grown in the Research and Application vineyard of Cukurova University, Faculty of Agriculture, Department of Horticulture were used.

Methods

Culture media preparation

After adding 30 g L^{-1} sucrose and silicates, additionally for shoot growing stage 1 mg L^{-1} BA (Benzyl Adenine) and for rooting stage 1 mg L^{-1} IBA (Indole Butyric Acid) was added to MS culture medium (Ducheva Biochemie, M0222.0050). pH of the medium was adjusted using 1N HCL and 1N KOH as 5.8 and then 8 g L^{-1} agar was added into media. Nutrient medium prepared was dispensed into the test tubes in diameter 2.5 cm and in length 15 cm as 10 mL. After the tubes were sterilized in autoclave at 121 ^oC and 1.05 kg cm^{-2} pressure for 15 minutes, they left to cool in a sterile cabinet.

Explant preparation and shoot obtaining

The tops of the shoots of 10 cm in active vegetation period were taken. After that, they were brought to laboratory and leaves on them were cleaned out and divided into one-node-cuttings These cuttings were disinfected for 20 minutes with using a solution containing 5% sodium hypochlorite (commercial bleach) and 1-2 drops of Tween 20 (Merck, 9005-64-5) (Mese and Tangolar, 2019). Afterwards, the explants were rinsed down in sterile cabinet 3 times with distilled water. After that, these explants were planted in the test tubes (25 mm x 150 mm) containing 10 mL MS medium that supplemented to 0, 0.5, 1.0 and 2.0 mg L^{-1} concentrations of K_2SiO_3 (Alfa Aesar, 1312-76-1), $CaSiO_3$ (Alfa Aesar, 10101-39-0) and Na_2SiO_3 (Sigma, 6834-92-0) and 1 mg L^{-1} BA for shoot obtaining stage of the experiment. These node explants were kept in the tubes for 4 weeks period.

Rooting of shoots

In the shoot obtaining stage, the shoots reached to the length of 2 cm and created 2-3 leaves were taken. These shoots were planted in the test tubes containing 10 mL MS medium that consists of 0, 0.5, 1.0 and 2.0 mgL^{-1} potassium, calcium and sodium silicate and 1 mg L^{-1} IBA concentrations. The shoots were cultured in the tubes for 45 days.

Culture conditions

Cultures were placed in a growth room with a temperature of 25 ± 1 ° C, a photoperiod of 16 hours, a luminous exposure of 3000-4000 lux (11000-15000 watts. m^{-2}). Lighting was provided by Cool daylight type TLD 36 w/54 fluorescent lamps.

Phytotechnical analyses

In the phase of shoot obtaining from the one-node microcutting, the explant viability, mortality and shooting rate were determined. These variables were calculated as follows:

Viability rate (%) = (no. of survived explants)/(no. of cultured explants) x 100

Mortality rate (%)= (no. of dead explants)/ (no. of cultured explants) x 100

Shooting rate (%) = (no. of sprouted explants)/(no. of survived explants) x 100

In the rooting phase of experiment, the number of node (or leaf) and root (n) were determined by counting. The length of shoots and roots (cm) were measured with a ruler.

Shoot and root fresh weights (g) were weighed, and then after drying in an oven at 65°C for 48 hours, their dry weights (g) were recorded by a digital scale.

The chlorophyll index (SPAD readings) was measured with a chlorophyll meter (SPAD 502, Konica Minolta, Osaka, Japan). The readings were determined on the two middle leaves of each plant.

Experimental design and statistical analysis

The research was planned according to the completely randomized experimental design of 3 replicates with 10 plants per repeat. The first factor was consisted of 3 Si sources (K_2SiO_3, $CaSiO_3$ and Na_2SiO_3);the second factor was composed of 4 concentrations (0, 0.5, 1.0 and 2.0 mg L^{-1}) of Si sources. Analysis of variance was performed to the obtained data using JMP (v.800, SAS Institute Inc., USA) statistical program based on SAS. For determination of different groups, LSD (Least Significant Different) was used at 0.05 level ($P \leq 0.05$). Angle transformation values were used for the variance analysis of the values obtained as percentages.

Results and Discussion

In the shoot obtaining stage, different Si sources had no significant effect on explants viability and mortality rates of 140 Ru rootstocks (Table 1). The highest shooting rate (84.40%) was obtained from sodium silicate and the lowest rates were in potassium and calcium silicate (72.12% and 73.86%, respectively) treatments. The effects of silicon doses were not significant on viability, mortality and shooting rates of explant. Interaction was not significant, either. The findings obtained at this phase, in which the roots were not formed, yet, suggested that the presence of roots is necessary for the silicon to be effective. The thought that silicon is an ineffective element on plant growth and development cannot be clearly supported at this stage in our opinion (El Fadl and Reda, 2014).

In the rooting stage of the research, the sources of silicon were not found important on shoot length and number of nodes. In spite of that, the effect was found to be significant in point of the plant fresh and dry weight values (Table 2). The highest shoot fresh and dry weight values were obtained from the medium containing potassium silicate (0.178 g and 0.026 g, respectively) and sodium silicate (0.159 and 0.024 g, respectively); the lowest values were obtained from media containing calcium silicate (0.139 g and 0.021 g, respectively) considering these properties. It is seen in Table 2 that the media containing 1 mg L^{-1} silicon concentration were more successful. In this concentration, the highest values were determined in shoot length 4.49 cm, in node number 7.44, in fresh and dry weight 0.183 g and 0.028 g, respectively.

No significant difference was found between the silicon sources in terms of SPAD index. In evaluation between silicon concentrations, the highest SPAD index (28.37) was taken from the medium containing 1 mg L^{-1} (Table 3).

Table 1. The effect of different silicon sources and concentrations on explant viability, mortality and shooting rate.

Sources of variation	Viability rate (%)	Mortality rate (%)	Shooting rate (%)
Si Source			
K_2SiO_3	95.02	4.98	72.12 b[x]
$CaSiO_3$	94.14	5.86	73.86 b
Na_2SiO_3	92.85	7.15	84.40 a
LSD 5%	NS	NS	7.36
p	0.244	0.764	0.023
Si Concentrations (mg L^{-1})			
Control	95.27	4.73	73.16
0.5	91.06	8.94	83.72
1.0	94.23	5.77	76.31
2.0	95.45	4.55	73.98
LSD 5%	NS	NS	NS
p	0.755	0.241	0.227
Interaction			
LSD 5%	NS	NS	NS
p	0.484	0.895	0.117

[x] Means within columns followed by the same letter/letters do not differ significantly from each other at $P \leq 0.05$ by by LSD multiple range test.
NS: Non Significant

Table 2. Effects of different silicon sources and concentrations on shoot growth and development.

Sources of variation	Shoot lenght (cm)	Node number (n)	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
Si Source				
K_2SiO_3	3.85	6.43	0.178 a[x]	0.026 a
$CaSiO_3$	3.61	6.75	0.139 b	0.021 b
Na_2SiO_3	3.90	7.14	0.159 ab	0.024 ab
LSD 5%	NS	NS	0.023	0.004
p	0.109	0.265	0.008	0.026
Si Concentrations (mg L^{-1})				
Control	3.49 bc	6.67 ab	0.151 b	0.023 b
0.5	3.35 c	6.17 b	0.143 b	0.022 b
1.0	4.49 a	7.44 a	0.183 a	0.028 a
2.0	3.82 b	6.81 ab	0.157 ab	0.023 b
LSD 5%	0.34	1.00	0.027	0.068
p	<0.0001	0.097	0.028	0.027
Interaction				
LSD 5%	0.59	1.73	0.046	0.007
p	<0.0001	0.204	0.005	0.005

[x] Means within columns followed by the same letter/letters do not differ significantly from each other at P≤0.05 by by LSD multiple range test. NS: Non Significant

Table 3. The effect of different silicon sources and concentrations on SPAD index

Sources of variation	SPAD index
Si Source	
K_2SiO_3	25.80
$CaSiO_3$	23.77
Na_2SiO_3	26.07
LSD 5%	NS
p	0.109
Si Concentrations (mg L^{-1})	
Control	22.76 b[x]
0.5	24.81 b
1.0	28.37 a
2.0	24.91 b
LSD 5%	2.72
p	0.003
Interaction	
LSD 5%	4.70
p	0.323

[x] Means within columns followed by the same letter/letters do not differ significantly from each other at P≤0.05 by by LSD multiple range test. NS: Non Significant

Contrary to our study, it has been stated by many researchers that the difference was important in the effect of silicate sources on plant height and number of nodes. Among these researchers, Asmar et al. (2011) tested the 1 g L^{-1} concentration of sodium, potassium, and calcium silicate in MS medium in the *in vitro* rooting stage of the Maçã banana plant, and it was observed that adding sodium silicate to the nutrient medium increased the shoot length and the fresh and dry weight of the shoots. Asmar et al. (2015) were determined in another study of them that 1 g L^{-1} concentration of calcium silicate which was applied in MS Medium in the *in vitro* culture, has increased the content of leaf chlorophyll of banana plants. It is seen that these results are in accordance with our data. Dias et al. (2017) have taken into culture Antorium's node explants with different sodium silicate concentrations (0.0, 0.5, 1.0 or 2.0 mg L^{-1}) in Pierik medium. They were determined that partly as same as our studies, the number of leaf and plant dry weight have increased in the plants in the medium added 0.5 mg L^{-1} and 2.0 mg L^{-1} sodium silicate. While in the same study (Dias et al., 2017), the highest chlorophyll a and b values were observed

in plants with 2.0 mg L^{-1} sodium silicate added, 1.0 mg L^{-1} concentration gave higher chlorophyll value in all three silicon sources. Rodrigues et al. (2017) in their study in sweet potato with 0.0, 0.5, 1.0 and 2.0 mg L^{-1} concentrations of potassium, calcium and sodium silicate, the maximum number of leaves (7.0 pieces) was determined. The longest shoots (4.02 cm) were determined from media containing 1 mg L^{-1} sodium silicate, and the highest plant fresh weights were determined in media containing potassium and calcium silicate (0.263 g and 0.284 g, respectively). While Soares et al. (2011) also reported that the addition of 5.0 mg L^{-1} K_2SiO_3 and 20.0 mg L^{-1} Na_2SiO_3 to modified Knudson C medium increased shoot length and number of shoots in orchid plants, Manivannan et al. (2018) observed that potassium silicate was more effective than calcium silicate on clove plant cultured *in vitro* in MS environment containing three different concentrations (0, 1.8 or 3.6 mM) of potassium and calcium silicate. El Fadl and Reda (2014) reported that in the dry date Bartamuda, fresh and dry weight increased in the presence of potassium and sodium silicate. Avestan et al. (2016) have obtained in their study on MM106 apple rootstock the highest plant fresh and dry weight and chlorophyll level from the mediums containing 100 mg L^{-1} nano-silicon. Lim et al. (2012) have reported that the highest plant fresh and dry weight and chlorophyll content in the varieties of begonia and violet were obtained from respectively 200 mg L^{-1} and 100 mg L^{-1} potassium silicate application. Braga et al. (2009) have determined that in their study in which they added 1 g L^{-1} doses of calcium, sodium and potassium silicate to MS medium in the micro propagation of strawberry plant, the fresh and dry weight of the shoots increased in sodium silicate presence, and adding silicate increased chlorophyll level. In the study of Soares et al. (2012), it was determined that the orchid plants have shown more growing in the artificial light application with 0.5 and 2.0 mg L^{-1} calcium silicate. As to in our studies, it was determined that 1.0 mg L^{-1} calcium silicate gives better result than the other concentrations. The idea has formed that the different results from this study and the studies mentioned above is probably due to the plant species studied and interaction of silicon sources.

In the studies of Martins et al. (2019) it was showed that the use of silicon was a good alternative to improve the photosynthetic pigment content and growth of plants propagated *in vitro*. SPAD values obtained in this study support the results of the researchers.

When the three different silicon sources used in the study were compared, the effects of the applications on the number of roots and the root fresh and dry weight were found to be significant. The highest root number was in the medium containing potassium (2.98) and calcium silicate (2.91). There was no significant difference between silicon sources in terms of their effects on average root length. In the root fresh and dry weight, potassium silicate (0.213 g and 0.023 g, respectively) was taken first place, calcium silicate (0.187 g and 0.019 g, respectively) second place, and sodium silicate (2.10 g, 0.152 g and 0.016 g, respectively) third place. The effect of concentrations used in the research was not significant on root length and fresh and dry weight of the roots. The highest root number value was seen in 1 mg L^{-1} dose (Table 4).

Table 4. Effects of different silicon sources and concentrations on root growth and development

Sources of variation	Root number (n)	Root length (cm)	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})
Si Source				
K_2SiO_3	2.98 a[x]	5.52	0.213 a	0.023 a
$CaSiO_3$	2.91 a	5.54	0.187 ab	0.019 ab
Na_2SiO_3	2.10 b	5.38	0.152 b	0.016 b
LSD 5%	0.51	NS	0.047	0.004
p	0.003	0.936	0.041	0.016
Si Concentrations (mg L^{-1})				
Control	2.18 b	5.84	0.182	0.019
0.5	2.67 b	5.19	0.179	0.018
1.0	3.27 a	5.80	0.197	0.021
2.0	2.53 b	5.09	0.177	0.019
LSD 5%	0.58	NS	NS	NS
p	0.007	0.441	0.863	0.584
Interaction				
LSD 5%	1.01	NS	0.094	0.008
p	0.002	0.212	0.0004	0.001

[x] Means within columns followed by the same letter/letters do not differ significantly from each other at P≤0.05 by by LSD multiple range test. NS: Non Significant

Lim et al. (2012) were also not found significant in their studies between the effect of potassium silicate at a concentrations of 0, 100, 200 or 300 mg L^{-1} added to the MS medium *in vitro* on the root length of the begonia plant. Contrary to our results, when Soares et al. (2011) cultured 1 cm long orchid seedlings by adding sodium silicate (0, 5, 10, and 20 mg L^{-1}) and potassium silicate (0, 5, 10, and 20 mL L^{-1}) and Knudson C medium in all possible combinations, they determined, that the maximum no. of roots and root length were realized in the medium added 20 mg L^{-1} sodium silicate and 5 mL L^{-1} potassium silicate. It is thought that the difference between our study and the findings of these researchers is due to quite high doses they used. Contrary to our data, Dias et al. (2017) have observed an increase in the root number of Anthurium plants in the nutrient medium supplemented with 1.0 mg L^{-1} sodium silicate. Soares et al. (2008) , who had taken results in contrary our findings, determined that there was a decrease in root fresh and dry weight of orchid plants with the increase in sodium silicate concentration. The fact that the silicon concentrations used here were higher than the concentrations used in this study and that the plant species were different indicates that the success changed according to the plant species and the source and concentration of silicon used. Mitani et al. (2005) also reported that the silicon accumulation in shoots varies significantly between plant species.

Conclusion

As result of the study, it has been determined that silicon may be added as supplementary source to the culture medium in the micro-propagation of grapevine. The results obtained regarding the different properties examined in our study showed that 1 mg L^{-1} concentration of potassium, followed sodium silicate to the nutrient medium during *in vitro* micro-propagation of the grapevine can be added successfully to increase plant quality. In subsequent studies, the trying of different silicon sources and concentrations would be beneficial to determine the reactions of other grapevine species and varieties.

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

This study was derived from the Master Thesis of Sawsan Qasim LATEFF.

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

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Not applicable.

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Determination of Erodiblity (USLE-K) Status of Suruc Plain Soils Before Transition to Irrigated Agriculture within the Scope of Southeastern Anatolia Project

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Abstract

Erosion is a major problem that limits vegetative production, adversely affects the ecosystem and causes soil losses that are difficult to recover. This study was carried out with the aim of determining the erosion status and some soil properties of the soils of Suruc plain in Sanlıurfa province. Soil properties; It has been determined that the pH is slightly high, the soils are non-saline, the organic matter is medium and the soil is clayey. Expresses the change of data by the coefficient of variance (CV). The lowest variability (CV = 2.45) in the soils of Suruç plain is the other parameters, respectively, the clay ratio (CV = 19.27) medium level, silt ratio (CV = 20.64) medium level, organic matter (CV = 27.14) medium level and sand ratio. (CV = 27.34) varies moderately. It was determined that the soil properties showing the highest variability were lime (CV = 37.06), EC (CV = 38.89) hydraulic conductivity (CV = 695.41). According to the correlation coefficient of soil erosion (USLE-K) parameter (r=0.99**) and P <0.01 level, it was determined that there is a very important relationship. The high coefficient of variation of some soil properties revealed the need for more sampling in the area. According to the frequency table, the erosive degrees of the soils are determined to be 10% very little, 26% less, 56% medium and 8% strongly erodable soils. In the Suruc plain, which will be opened to irrigation within the scope of the Southeastern Anatolia project, instead of traditional irrigation methods, more suitable irrigation systems should be chosen according to the soil characteristics and the slope of the land.

Keywords: Erosion, USLE-K, Soil Properties, Suruc, Sanliurfa

Introduction

Erosion is one of the most important soil problems, which occurs as geological or natural erosion in the process that begins with erodiblity the surface of rocks and soils, limiting the sustainable productivity of soils and restricting the growth of plant (Cassol et al., 2018). Soil erosion emerges as a global problem as one of the most important causes of land degradation in a global sense, which is directly affected by climate, topography, land use and geomorphological characteristics (FAO, 2017). The erosion of soils is caused by the degradation of the aggregated structure of the soils by the surface flow of water in the soil or by the effect of heavy rainfall (Renard et al., 1997). Erosion in general; Landslides pose serious socio-economic threats in the watersheds, reducing agricultural productivity, land loss in sloping lands, and the extinction of species in nature (Hu et al., 2019). In addition to the economic damage caused by erosion in agricultural area, it poses a serious ecological threat on the waters due to the sedimentation of rivers, lakes, streams and seas in coastal regions (Garcia et al., 2016). The K factor used in the determination of universal soil loss (USLE) is an important erosion index used to determine the susceptibility of soils to water erosion. In determining the

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K factor, it enables the determination of annual soil loss caused by the annual rainfall (Zhang et al., 2008). The rapid increase of the global population and the increase in the demand for food in direct proportion to the population make it necessary to protect the soils. There are many reasons for soil degradation, but the most important of these problems and the one that requires urgent solution is erosion. Erosion is a soil loss problem that makes recovery of soils almost impossible (Lal, 2001). Many studies that determine the erosion of soils have emphasized the importance of protecting soils against erosion. Kanar and Dengiz (2015), in their study on the soils of the Menderes basin, determined the relationship between land use and land cover with the erosion sensitivity index and stated that the soils in the Menderes basin are highly sensitive to erosion and that the necessary precautions should be taken against erosion. In Çorum Alaca Basin, located in the Central Kızılırmak Section of the Central Anatolia Region, 42.2% of the soils are medium degree and 57.8% are less erodible (İmamoğlu et al., 2016).

Within the scope of Suruc plain located in the Southeastern Anatolia project, 94,814 ha of agricultural land is planned for irrrigation. . In Suruc plain, agricultural income will increase along with agricultural production compared to dry agriculture by transition to irrigated agriculture such as Harran plain. Lack of planning before seeding and planting in agricultural area and the lack of studies determining the comprehensive soil characteristics of the Suruc plain may cause many unsuitable applications in the plain. Determining the ideal use of agricultural land makes a great contribution to the sustainable use of soil resources for long periods and the amount of product taken from the unit area. Especially if these researches are carried out in company with developing technology, they will cover reliable and accurate information and will be the source of the projects that are planned to be implemented in these areas (Tokmakçı, 2018).

The aim of this study is to determine the erodibility susceptibility of the soils of the Suruc plain and to predict the potential soil loss, and to reveal their relationsheps with some soil properties.

Materials and Methods

Geographical Location

Suruc plain is located between 38 ° 05 ‘and 38 ° 45’ east longitudes 37° 05’ and 37° 45› north latitudes. Suruc district is in the southwest of Sanliurfa province and about 43 km from the city center. The Suruc plain is a big and fertile land that located at the upper mesopotaimia and near Euphrate rivers. The plain is topographycaly flat, 493 m see level and sorrounded bay small highlands (Figure 1).

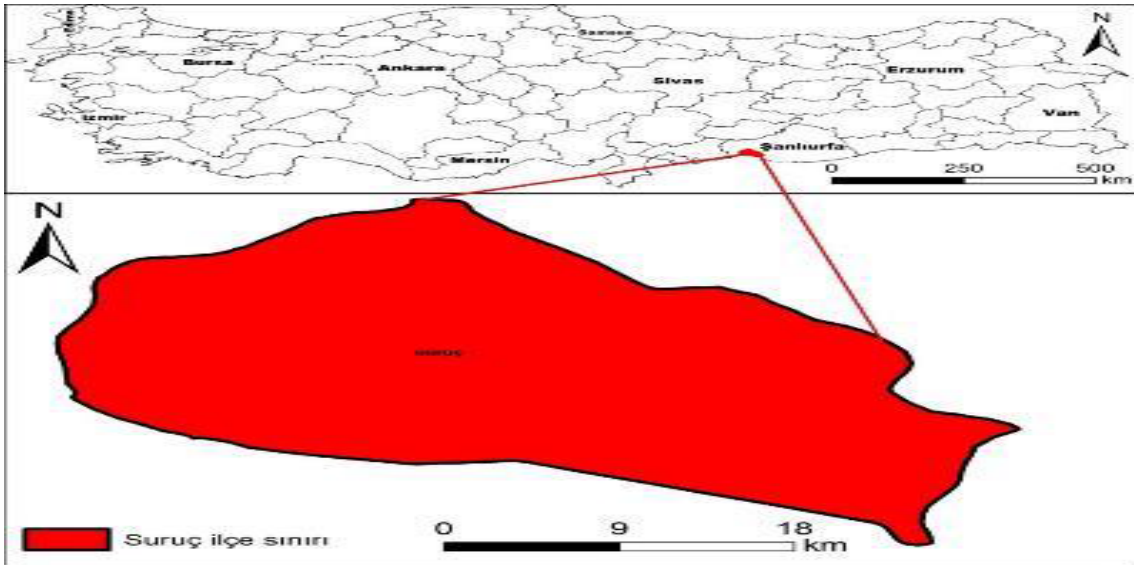

Figure 1. Workspace map

Climate

Suruc plain is characterized as a mediterranean climate whose terrestrial characteristics have partially changed, and the mediterranean climate features dominate, with hot and dry summers and warm and rainy winters. The annual average rainfall of 300-350 mm is not sufficient for the yield of the crop each year.The annual average temperature is 18.4 °C and annual average relative humidity is 54%. According to the American Soil Taxonomy, the moisture regime of the region is Xeric and the temperature regime is Thermic (Süpürkeci, 2014).

Methods

In this study, soil sampling was collected at 50 different points (0-30) in Suruc plain. Soil samples taken from the study area prepared for analysis after passing through a 2 mm sieve. Texture analysis was determined with Bouyoucos Hydrometer by taking 50 g from the oven dry weight soil, adding 10 ml of 10% sodium hexametaphosphate $(NaPO3)_6$ solution and 150 ml of pure water on them, mixing with a drumstick (Bouyoucous, 1951). Hydraulic conductivity was determined by saturating intact soil samples with water for 16 hours (Özdemir, 1998). pH and EC analysis were determined by taking 100 g from the soil with air dry weight and the soil being saturated with graduated burette (Richards, 1954). The total CaCO3 amount was measured with a Schiebler calcimeter by taking 0.5 g of soil sample, adding 5 ml of 10% HCI (Gülçur, 1974). The

organic matter was sieved through a 0.5 mm sieve, 0.5 g of soil sample was placed in a 500 ml flask and 10 ml of 1 N potassium dichromate ($K_2Cr_2O_7$) solution was added on it and determined by wet burning method (Nelson and Sommers, 1996).

The erodiblity (USLE-K) factor of soils: K factor, The data to be obtained from the analysis of soil samples taken from the Suruc plain is determined using the following equation.

USLE-K = ((2.17 x 10-4) x (M1.14) x (12-a) + 3.25 x (b - 2) + 2.5 x (c - 3)) x d

According to the results obtained from the equation (Table 1), the erosion levels of the soils are determined.

Table 1. USLE- K class values.

Category	Mean	Ration
1	Very little Erodiblity	0.00-0.05
2	Little Erodiblity	0.05-0.10
3	Medium Erodiblity	0.10-0.20
4	Surplus Erodiblity	0.20-0.40
5	Excessive Erodiblity	0.40-0.60

Results and Discussion

Sand, silt, clay, organic matter, lime, EC, pH, hydraulic conductivity and erosion (USLE-K) parameters of the soils were examined for 50 soil Samples and statisctical Analysis were applied (Table 1). The statistical analyses resuls showed that the coefficient of variation values (CV), which are considered to be indicators of variability, are classified as 15% small, 16-30% medium, and ≥30% high variables (Wilding et al., 1994).

Table 2. Descriptive statistics for some physical and chemical properties of the study area (0-30 cm) soils

Parametres	n	Minumum	Maximum	Mean	Standard Error	Variance	Coefficient of variation
Sand (%)	50	18.72	56.72	34.24	9.36	87.60	27.34
Silt (%)	50	14.56	32.56	21.80	4.50	20.23	20.64
Clay (%)	50	28.72	60.72	43.96	8.47	71.82	19.27
O.M (%)	50	0.79	3.17	2.10	0.57	0.33	27.14
Lime (%)	50	7.97	59.38	33.86	12.55	157.47	37.06
EC (dS m^{-1})	50	0.01	1.11	0.54	0.21	0.04	38.89
pH	50	6.90	7.80	7.34	0.18	0.03	2.45
H.C (cm h^{-1})	50	0.28	9.862,00	200.49	1.394,23	1.943.882,83	695.41
USLE-K	50	0.01	12.00	0.37	1.68	2.82	454.05

O.M: organic matter, H.C: hydraulic conductivity

When the average values of the soils were examined, it was determined that the soils were clay textured in terms of sand, clay and silt ratios and the organic matter level was 2.10%. Soil pH is 7.34 and EC values is characterized as none saline soils. It has been determined that the hydraulic conductivity rate in Suruc plain is 200.49 cm h^{-1}, and the erosion conditions of the soils are very erodable. The parameters varying in terms of soil properties examined in the soils of Suruc plain, the lowest variation (CV = 2.45) is the pH, the other parameters are respectively the clay ratio (CV = 19.27), the silt ratio (CV = 20.64) moderate, organic matter (CV). = 27.14), and the sand content (CV = 27.34) moderately varied. Highly variable soil parameters, respectively; lime (CV = 37.06), EC (CV = 38.89) and the highest variability was determined to be the hydraulic conductivity of soils (CV = 695.41) (Table 1).

A study conducted by Ozturkmen et al. (2020), reported that the hydraulic conductivity rate of soils varies widely is due to the different land use in the Suruc plain. In a study examining the effect of different land use on hydraulic conductivity, they reported that the highest infiltration rate was determined in orchards and the lowest in barley cultivated agricultural lands, and that the soil parameter most affected by different land use was hydraulic conductivity (Ozturkmen and Ramazanoglu, 2020a; Ozturkmen et al., 2020b).

According to the result of the Pearson Correlation (r = 0.99 **) of the soil erosion (USLE-K) parameter, it was determined that there is a very important relationship at the P <0.01 level. The hydraulic conductivity of soils has a direct effect on the soil erosion process (Biddoccu et al., 2017). Since the hydraulic conductivity of soils has an important effect on the soil erosion process, changing the soil cultivation methods will be a protective soil conservation method to apply less tillage techniques to make soils more resistant to erosion (Blanco et al., 2017). Along with the decrease in the rate of hydraulic

conductivity of soils, it causes surface runoff, flood and erosion (Owuor et al., 2018). When the degree of susceptibility of the study area soils to erosion is examined (Table 4), it was determined that the soils are 10% very little, 26% low, 56% medium and 8% strongly erodable soils (Figure 2).

Table 3. Correlation table between soil parameters

Pearson Correlation	**Sand (%)**	**Silt (%)**	**Clay (%)**	**O.M (%)**	**Lime (%)**	**EC (dS m^{-1})**	**pH**	**H.C**	**USLE-K**
Sand (%)	1								
Silt (%)	-0.428**	1							
Clay (%)	-0.877**	-0.058	1						
O.M (%)	0.071	0.103	-0.133	1					
Lime (%)	0.554**	0.196	-0.508**	-0.161	1				
EC (dS m^{-1})	-0.113	0.247	-0.007	0.178	-0.102	1			
pH	-0.065	0.051	0.045	0.015	0.040	0.066	1		
H.C (cm h^{-1})	-0.085	0.024	0.081	0.038	-0.122	0.048	0.288*	1	
USLE-K	-0.072	0.041	0.058	0.032	-0.110	0.052	0.288*	0.999**	1

** *P<0.01;* * *P<0.05, O.M:* organic matter, H.C: hydraulic conductivity

Table 4. Coordinates of the study area soil samples and erosion sensitivity values

Sample No	**Coordinate (37 Zone, UTM)**		**USLE-K**	**Erodiblity Degree of Soils**
	X	**Y**		
1	444541	4089217	0.09	2
2	444541	4089216	0.16	3
3	444494	4089229	0.09	2
4	443478	4088787	0.19	3
5	443463	4088778	0.10	2
6	443448	4088749	0.19	3
7	443476	4088831	0.07	2
8	443467	4088833	0.33	4
9	443454	4088835	0.21	4
10	442880	4088154	0.08	2
11	442398	4088340	0.19	3
12	442446	4088338	0.09	2
13	442369	4088310	0.04	1
14	442343	4088402	0.15	3
15	442355	4088423	0.21	4
16	442357	4088449	0.19	3
17	442319	4088460	0.17	3
18	442108	4088299	0.15	3
19	442098	4088293	0.19	3
20	4420849	4088276	0.16	3
21	442140	4088229	0.07	2
22	442133	4088222	0.22	4
23	442121	4088218	0.20	3
24	442145	4088203	0.14	3

25	442154	4088187	0.07	2
26	442166	4088172	0.16	3
27	440947	4087547	0.12	3
28	440934	4087559	0.11	3
29	440921	4087557	0.12	3
30	440971	4087512	0.13	3
31	440983	4087496	0.04	1
32	440958	4087486	0.06	2
33	443779	4086858	0.10	2
34	437770	4086832	0.13	3
35	437762	4086816	0.13	3
36	437740	4086814	0.10	2
37	438064	4086961	0.11	3
38	438064	4086961	0.12	3
39	438061	4086972	0.15	3
40	438031	4086968	0.14	3
41	438009	4087007	0.12	3
42	438027	4087078	0.04	1
43	440278	4086664	0.13	3
44	440281	4086658	0.09	2
45	440298	4086654	0.15	3
46	445058	4089820	0.15	3
47	4450580	4089830	0.15	3
48	445075	4089833	0.10	1
49	449492	4097155	0.05	1
50	449501	4097161	0.01	1

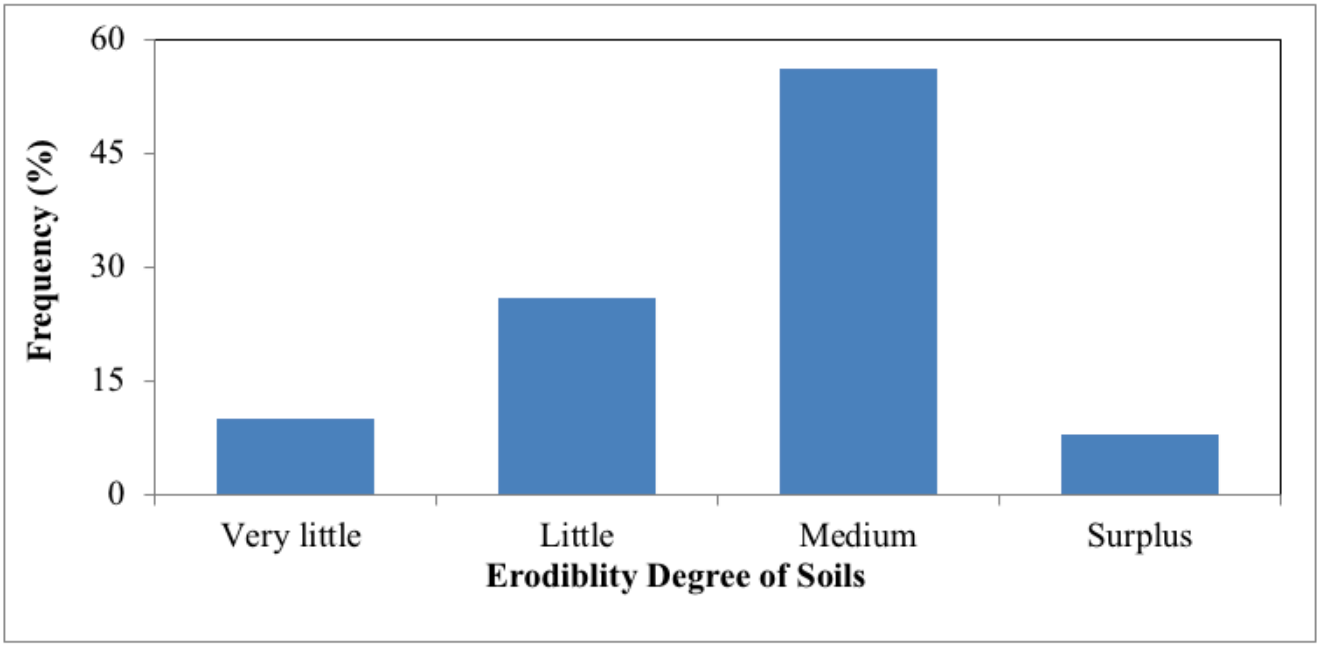

Figure 2.Frequency table for the USLE-K factor of Suruc plain soils

Conclusion

As a result of the study conducted on Suruc's plain soils, it was determined that the soil reaction and lime content was high and level of organic matter was low. It has been observed that there is no problem in terms of salinity in the plain and the soils are generally clay textured soils. When the abrasion resistance of soils is examined, it was determined that the Suruc plain soils are highly susceptible to erosion. Soil protection measures should be taken against erosion in the Suruc plain to be irrigated. In vegetative production, it is necessary to change the existing irrigation systems (surface irrigation) before the Suruc plain is opened to irrigation and to select the appropriate irrigation systems in the plain. It is necessary to improve the infiltration rates of soils in order to determine the infiltration rates of soils and to prevent soil loss by runoff in soils with poor infiltration rates. After the water introduced to the plain, sprinkler or drip irrigation systems should be used instead of traditional irrigation systems. With this study, after many years, the changes and deterioration that may occur in the soil due to irrigation in the Suruc plain will be determined more quickly and will provide background information for future studies.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Research Article

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Antimicrobial Activity of Olive Leaf Extract on Selected Foodborne Pathogens and its Effect on Thermal Resistance of *Listeria monocytogenes* in Sous Vide Ground Beef

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Abstract

Antimicrobial activity of a commercial olive leaf extract (OLE) against *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Escherichia coli* Biotype I, *Salmonella* Enteritidis and *Salmonella* Typhimurium was tested by disc diffusion assay. The Gram negative bacteria tested in this study were more sensitive to OLE than Gram negatives. The highest antimicrobial activity was on *L. monocytogenes* which yielded the largest inhibition zone. Effect of OLE on thermal resistance of *L. monocytogenes* was tested both in Tryptic Soy Broth supplemented with 0.6% yeast extract (TSBYE) and sous vide packed ground beef. OLE added TSBYE (0, 0.5, 1%) tubes and ground beef (0, 1%) samples were inoculated with *L. monocytogenes* (7-8 log cfu/ml-g) and heated at 55, 60 and 65°C up to 30, 20 and 7.5 minutes, respectively. Total reductions in TSBYE tubes added with 0.5 and 1% OLE were slightly higher than control tubes (0% OLE) for all temperatures. Counts of *L. monocytogenes* in sous vide packed ground beef samples added with 1% OLE and then cooked at 55°C (30 min), 60°C (20 min) and 65°C (7.5 min) were 0.31, 1.04 and 0.73 log cfu/g lower than those control samples, respectively. The results indicate that OLE included in formulation may be an additional hurdle to control *L. monocytogenes* in heat processed ground beef.

Keywords: Olive leaf extract, Sous vide, Antimicrobial activity, *Listeria monocytogenes*, Ground beef

Introduction

In recent years, the plant extracts those rich in phenolic compounds have gained increasing attention due to their GRAS (Generally Recognized as Safe) status. Accordingly, they have been preferred as food additives for their antimicrobial and antioxidant functions (Perumella and Hettiarachy, 2011). The olive tree (*Olea europaea* L.) has been cultured for ages in Mediterranean region for not only its fruits but also its oil. Olive leaf extract (OLE) is a byproduct used mainly for medicinal purposes (Erdohan and Turhan, 2011). The content of phenolic compounds in olive leaf is as high as in olive fruit and derived products (Rahmanian et al., 2015). Olive leaf contains fourteen different compounds and among these compounds concentrations of oleuropein, hydroxytyrosol, luteolin-7-glucoside, epigenin-7-glucoside and verbascido are higher than the others (Benaventa-Garcia et al., 2000; Hayes et al., 2011). These bioactive compounds have biological activities including antimicrobial, antioxidant and antiproliferative (Rahmanian et al., 2015). The antimicrobial activity of OLE against foodborne pathogens has been shown by several studies (Pereira et al., 2007; Markin et al., 2003; Sudjana et al., 2009; Aliabadi et al., 2012; Gökmen et al., 2014; Hussain et al., 2014). Gökmen et al. (2014) determined antimicrobial activity of a commercial OLE against *L. monocytogenes* with >32 mg/ml MIC (Minimum Inhibitory Concentration) value. Liu et al. (2017) reported that olive leaf extract reduced cell motility in *L. monocytogenes*.

Sous vide is a cooking method in which food is

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subjected to heat process in vacuum sealed pouches. *Listeria monocytogenes*, a facultatively anaerobic bacterium, can be assumed a risk for sous vide cooked meats considering its heat resistance and ability to grow at refrigerated temperatures (Baldwin, 2012). Natural antimicrobials may contribute to reduce thermal resistance of *L. monocytogenes*. Juneja et al. (2013) has mentioned that the commercial apple powder rich in phenolic compounds added into ground beef reduced thermal resistance of *L. monocytogenes*. Therefore, the plant extracts rich in phenolic compounds may help to assure microbial safety by reducing thermal resistance of foodborne pathogens, when food is undercooked unintentionally or subjected to mild heat treatment intentionally to keep its fresh characteristics.

The aims of this study were to investigate the antimicrobial activity of OLE on selected foodborne pathogens, and to evaluate if the combination of OLE with mild heat treatment could eliminate *L. monocytogenes* in TSBYE and sous vide cooked ground beef.

Materials and Methods

Materials

Olive leaf extract (OLE) in liquid form was obtained from Tariş Zeytin Inc. (İzmir, Turkey). *Listeria monocytogenes* ATCC 7644, *Bacillus cereus*, *Escherichia coli* O157:H7, *Escherichia coli*, *Salmonella* Enteritidis ATCC 13076, *Salmonella* Typhimurium and *Staphylococcus aureus* were from culture collection of Engineering Department of Engineering Faculty, Sakarya University. Ground beef (85% lean) was purchased from a local market, brought to laboratory in cooled conditions and frozen. Frozen ground beef was thawed in refrigerator (4±1°C) overnight before experiments.

Antimicrobial activity assay

Disc diffusion method was used to determine antimicrobial activity of OLE against selected bacterial cultures. *Listeria monocytogenes* ATCC 7644, *Bacillus cereus*, *Escherichia coli* O157:H7, *Escherichia coli*, *Salmonella* Enteritidis ATCC 13076, *Salmonella* Typhimurium and *Staphylococcus aureus* were activated twice in Tryptic Soy Broth supplemented with 0.6% yeast extract (TSBYE, Merck, Darmstadt, Germany). *L. monocytogenes* culture was incubated at 30°C and the other cultures at 37°C for 24 h. From each bacterial culture, a 50 ml-portion was spread on Tryptic Soy Agar supplemented with 0.6% yeast extract (TSAYE, Merck, Darmstadt, Germany). OLE was prepared at different concentrations (5, 10, 20, 30, 40, and 50%; v/v) using distilled water or 1% dimethyl sulfoxide (DMSO). The sterile paper discs (6 mm in diameter) were placed on culture inoculated TSAYE and then discs were impregnated with 50 ml OLE. Same amounts of distilled water or DMSO were transferred to control discs. Plates were incubated at 37°C for 18-24 h and then the inhibition zones larger than 7 mm were measured. Tests were repeated twice and mean values were presented.

Preparation of *Listeria monocytogenes* inoculum

L. monocytogenes stock culture was activated twice in TSBYE by incubating at 30°C for 18-24 h. Active culture was inoculated into TSBYE and incubated for 18-24 h at 30°C. Following incubation, TSBYE culture was centrifuged at 4000 rpm at 4°C for 10 minutes. After discarding supernatant, the pellet was washed twice by adding sterile peptone water (PW; 0.1% peptone) and centrifuging at 4000 rpm for 10 min at 4°C. To finish the supernatant was removed and the pellet was completed to the original volume by sterile PW.

Effect of olive leaf extract on heat resistance of *L. monocytogenes* in TSBYE

To determine the effect of OLE on thermal resistance of *L. monocytogenes* in vitro conditions, one ml *L. monocytogenes* inoculum was transferred into 9 ml TSBYE added with olive leaf extract (0, 0.5 and 1%) (Skandamis et al., 2008). The tubes were placed into a water bath adjusted to 55, 60 and 65°C. Water level was 4 cm higher than then the liquid media in the tubes. The tubes were shaken every 3-5 min during heating. Three tubes were removed at each sampling time and cooled rapidly by immersing in ice slurry.

Inoculation and cooking of ground beef samples

The frozen ground beef was thawed in a refrigerator overnight and then divided into two batches. One batch was added with 1% olive leaf extract and the other with same amount of sterile distilled water. Each batch was mixed by hand for three minutes. Then, each batch was inoculated with *L. monocytogenes* at the level of 7-8 log cfu/g and mixed well for even distribution of the pathogen. Ground beef was shaped using sterile glass petri plates (6 cm in diameter and 1 cm in thickness) and each portion was 25-30 g. Samples were placed into bags (EVP-SV1520-100, 15×20 cm, Elektrola, ÖRKA, Istanbul, Turkey) and the bags were evacuated with 99% vacuum (CromPack Vacuum Systems VM48, İstanbul, Turkey). Samples were heat treated using a temperature controlled sous vide cooker with a circulator (PolyScience, CRC-AC2E, ÖRKA, Istanbul, Turkey) adjusted to 55, 60 and 65°C. Inner temperatures of samples were monitored by a thermocouple inserted into an uninoculated ground beef sample. The total cooking times were 30, 20 and 7.5 min at 55, 60 and 65°C, respectively. At each sampling time two samples were removed and cooled to 4°C by immersing in ice slurry.

Enumeration of *L. monocytogenes*

Serial dilutions from the cooled TSBYE tubes were prepared using MRD (Maximum Recovery Diluent; 1 g/l peptone, 8.5 g/l NaCl) and 0.1 ml portions from appropriate dilutions were spread on Tryptic Soy Agar supplemented with 0.6% yeast extract (TSAYE, Merck, Darmstadt, Germany) and plates were incubated at 30°C for 24-48 h. After incubation colonies were counted manually and the results were expressed as log cfu/ml of TSBYE.

A ten gram ground beef sample was transferred to stomacher bag added with 90 ml MRD and homogenized for 2 min. Serial dilutions were prepared using MRD and 0.1 ml portions from dilutions were spread plated on TSAYE. Plates were kept at 25°C for 3 hours. Then, 10 ml PALCAM Agar (Merck, Darmstadt, Germany) added with selective supplement at 45°C was poured on TSAYE (Miller et al., 2010). Plates were incubated at 30°C for 48 h. After incubation the typical grey-green colonies with black zone were counted as *L. monocytogenes* and the results were expressed as log cfu per gram of ground beef (log cfu/g).

Water activity measurement

Water activity values of ground beef samples were determined by Aqualab Water Activity Meter (Model Series 3, Decagon Devices, Pullman, WA).

Results and Discussion

Antimicrobial activity of OLE

The disc diffusion assay results were presented in Table 1. *L. monocytogenes* and *S. aureus* were more sensitive against OLE. In other words, OLE prepared DMSO or distilled water yielded larger inhibition zones against these two bacteria than the other bacteria. The largest zone diameters were 13.67 and 14 mm obtained by 50% OLE (DMSO) for *S. aureus* and *L. monocytogenes*, respectively. Twenty percent of OLE prepared with DMSO did not yield any inhibition zones for all bacterial cultures, while that prepared with distilled water yielded inhibition zone only against *L. monocytogenes*. On the other hand, 5 and 10% concentrations of OLE whether prepared with DMSO or distilled water did not show antimicrobial activity on any of the bacterial cultures tested in current study.

Table 1. Antimicrobial activity of OLE at different concentrations (Inhibition zones in mm)

Bacterial cultures	OLE concentrations (v/v)							
	DMSO				Distilled water			
	20%	30%	40%	50%	20%	30%	40%	50%
E. coli O157:H7	-*	-	7.75	9.00	-	7.50	7.50	7.67
E. coli	-	-	8.00	8.50	-	7.33	7.33	7.33
S. Enteritidis	-	8.00	8.50	9.33	-	7.00	7.33	7.50
S. Typhimurium	-	-	7.50	8.00	-	7.00	7.50	8.00
B. cereus	-	7.00	8.50	9.00	-	-	7.75	8.00
S. aureus	-	10.25	12.67	13.67	-	8.75	10.50	11.50
L. monocytogenes	-	8.17	10.67	14.00	7.83	10.33	11.83	12.67

* No inhibition zone

According to the antimicrobial activity assay results *L. monocytogenes* and *S. aureus* were determined as more susceptible than the other bacteria. The previous studies on antimicrobial activity of OLE on different bacteria revealed that OLE is effective on both Gram-positive and Gram-negative bacteria including those tested in the current study (Pereira et al., 2007; Markin et al., 2003; Sudjana et al., 2009; Aliabadi et al., 2012; Gökmen et al., 2014; Hussain et al., 2014). However susceptibility of tested microorganisms shows variation. Markin et al. (2003) reported that *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most susceptible against water extract of olive leaf while *B. cereus* was the least susceptible. According to Pereira et al. (2007) *B. cereus* was more susceptible with the extract concentration of 0.63 mg/ml that inhibits 25% of microbial growth (IC_{25}) than the other bacteria including *E. coli* and *S. aureus*. Sudjana et al. (2009) determined that commercial OLE was the most active against *Campylobacter jejuni, Helicobacter pylori* and *S. aureus* (MIC value 0.31-0.78% v/v), while the extract showed little activity against other microorganisms including *L. monocytogenes, B. cereus, E. coli* and *Salmonella.* In a study by Aliabadi et al. (2012) the aqueous extract of olive leaf (15-50 mg/ml) was effective on *S. aureus*, *S. typhimurium, E. coli, K. pneumoniae* and *B. cereus* while *S. typhimurium* was the most susceptible. These variations may arise from several reasons such as composition of OLE, extraction method, the solvent type used for extraction as well as strain differences and test conditions. One of the most possible reasons seems to be OLE composition. Lee and Lee (2010) evaluated individual and combined activities of phenolic compounds found in olive leaf and determined that caffeic acid was effective on *B. cereus*, *E. coli* and *Salmonella* Enteritidis, while oleuropein inhibited only *S.* Enteritidis. Therefore, the differences in OLE compositions may result in variations of antimicrobial activity test results. In current study the most susceptible bacterial cultures were *L. monocytogenes* and *S. aureus* which are Gram-positive. Sudjana et al. (2009) suggested that one or more components within OLE may specifically be active against the Gram-positive cell wall.

Reduced heat resistance of *L. monocytogenes* by OLE

Use of mild processes in food preservation may help to produce natural healthy foods. In other words the low doses of one or more treatments can keep the sensory characteristics and nutritional value of the products. However, it is vital to inhibit the pathogenic bacteria for ensuring microbial safety of mildly processed foods.

The reductions of *L. monocytogenes* counts in TSBYE added with 0, 0.5 and 1% OLE were shown in Figure 1. The total reduction in *L. monocytogenes* counts in TSBYE after heating at 55°C for 30 min was 0.64 and 0.84 log cycle for 0.5 and 1% OLE added tubes, while it was 0.39 log cycle in control tubes (0% OLE). Heating at 60°C for 20 min reduced the number of pathogen by 3.63, 4.03 and 3.83 log cfu/ml in TSBYE tubes added with 0, 0.5 and 1% OLE, respectively. The total reductions in 0.5 or 1 % OLE added TSBYE tubes were approximately 0.90 log cycle higher than control tubes after 7.5 min heating at 65°C.

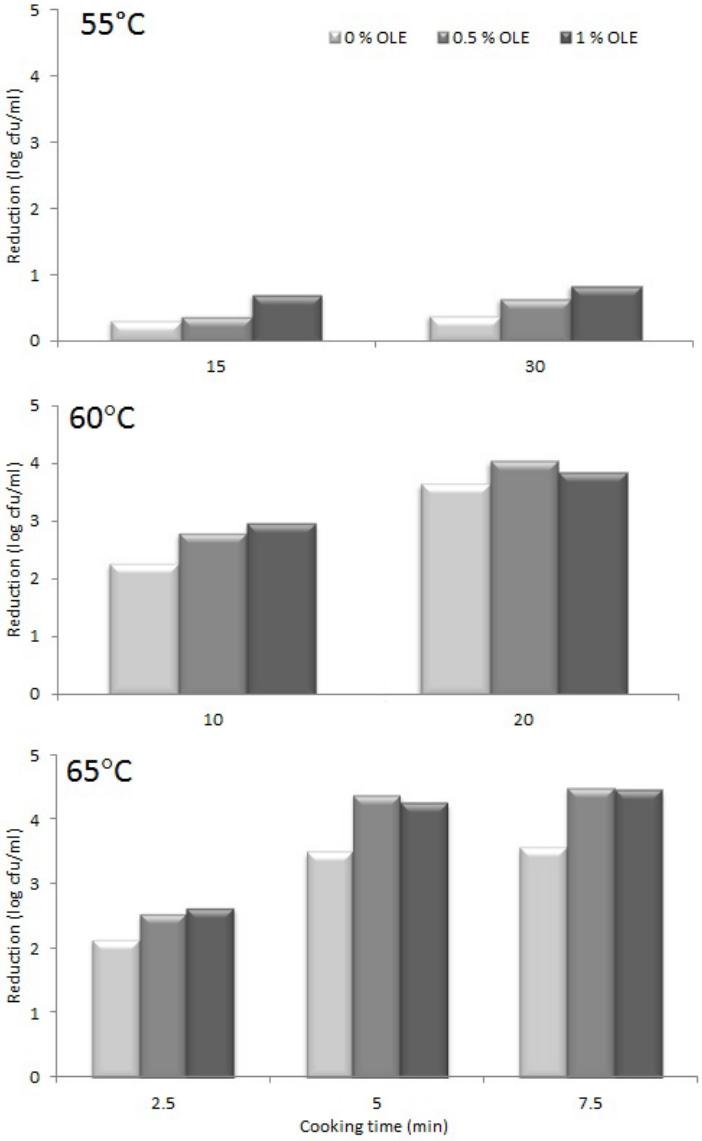

Figure 1. Reduction of *Listeria monocytogenes* in TSBYE with or without OLE at 55, 60 and 65°C (log cfu/ml)

Water activity (a_w) affects thermal resistances of bacteria; accordingly increasing a_w may result in increased thermal sensitivity (Syamaladevi et al., 2016). The a_w values of control ground beef samples (0% OLE) were 0.974 ± 0.003, while that was 0.973 ± 0.002 in 1% OLE added ground beef samples. Therefore, 1% OLE addition did not affect a_w value of ground beef.

The inner temperatures of ground beef samples during sous vide cooking increased gradually and slightly lower than the target temperature (Table 2). Figure 2 shows the reductions in *L. monocytogenes* inoculated in ground beef added with 0 or 1% OLE. When 0 and 1% OLE added ground beef samples were heated at 55°C for 30 min, the counts of *L. monocytogenes* reduced by 0.96 and 1.27 log cfu/g, respectively. Supplementation with 1% OLE resulted in an additional 1.04 log reduction following heat treatment at 60°C for 20 min when compared to non-treated control samples. On the other hand, *L. monocytogenes* counts were 0.73 log cfu/g lower in 1% OLE added ground beef than control samples heated at 65°C for 7.5 min.

Reduction in *L. monocytogenes* counts heated at 55°C were similar both in TSBYE and ground beef. In contrast, when the heating temperature was 60 or 65°C, less reduction was observed in ground beef samples than in TSBYE. These differences may arise from the lower thermal conductivity of ground beef than liquid growth medium. It is well known that composition of the heating medium may affect the heat resistance of *L. monocytogenes* (Doyle et al., 2001).

Table 2. Inner temperatures of ground beef samples during sous vide cooking at 55, 60 and 65°C

Water temperature (°C)	Time (minute)	Inner temperature (°C)
55	0	20.0
	15	51.7
	30	53.7
60	0	26.1
	10	52.4
	20	57.2
65	0	28.3
	2.5	49.9
	5	55.4
	7.5	58.8

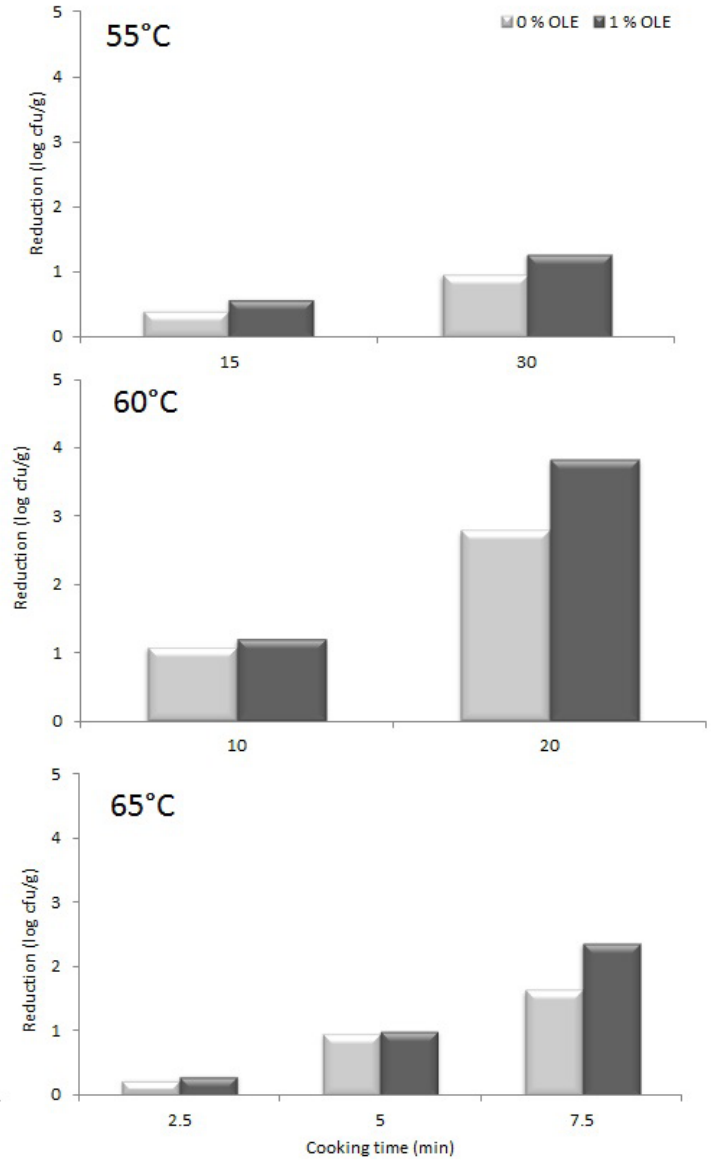

Figure 2. Reduction of *Listeria monocytogenes* in ground beef with or without OLE at 55, 60 and 65°C (log cfu/g)

These results demonstrated that OLE could contribute the inactivation of *L. monocytogenes* depending on the temperature and time. As far as we have known that this is the first report on effect of OLE on thermal resistance of *L. monocytogenes*. The previous studies have shown that OLE may retard growth of spoilage flora and then extend shelf life of animal based food products. Ahmed et al. (2014) found counts of aerobic bacteria and total coliforms were lower at least one log when raw peeled undeviened shrimps than those non-treated samples. Baker (2014) reported lower coliform, psychrophilic bacteria and aerobic plate count in 1-3% OLE added lamb patties than control samples during storage at 4°C for 12 days. Recently, Liu et al. (2017) have mentioned that OLE, at a concentration of 62.5 mg/ml, almost completely inhibited the growth of *L. monocytogenes* and reduced its cell motility demonstrated by the absence of flagella as shown by scanning electron microscope. OLE is rich in phenolic compounds (Pereira et al., 2007). It is well known that phenolic compounds damage the structure of cell membrane proteins, thus change the semipermeable nature of cytoplasmic membrane (Ultee et al., 1999). Juneja et al. (2013) reported that *L. monocytogenes* was more sensitive to lethal effect of heat when ground beef was supplemented with apple polyphenols. Similarly, Char et al. (2010) have reported that vanillin and citral addition to orange juice shortened the inactivation time for *Listeria inocua* (surrogate for *L. monocytogenes)* at 52 and 57°C.

Conclusion

The results of current study revealed OLE has antimicrobial activity on foodborne pathogens, while the Gram positive bacteria were more sensitive than Gram negatives. The combination of stress factors, i.e. OLE addition and thermal treatment, increased inactivation of *L. monocytogenes* reducing the time to destroy the pathogens at temperatures from 55 to 65°C. Although further studies are needed to understand the thermal death kinetics of *L. monocytogenes* in presence of OLE, it is obvious that OLE addition may help to control *L. monocytogenes* in heat processed meat products.

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

Serap Coşansu designed the study and Özlem Kıymetli collected the data. Serap Coşansu wrote the article. All the authors read and approved the final manuscript.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Effects of capsanthin on surface hydrophobicity and auto-aggregation properties of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*

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Abstract

Paprika is a one-year culture plant that grows in temperate climates and derives its color from the carotenoid compounds. The basic red color in paprika originates from capsanthin and capsorubin. People must have a healthy gastrointestinal system to maintain a healthy life. Lactic acid bacteria, which constitute the most important group of probiotic microorganisms, are natural members of a healthy intestinal microflora. Main lactic acid bacteria are *Lactobacillus acidophilus* and *Lactobacillus rhamnosus,* which can inhibit pathogenic microorganisms, strengthen immune system, and improve the microbial balance of the gastrointestinal tract. Such bacteria can be modulated by diet constituents, thusw the present study aims to investigate the effects of capsanthin on probiotic bacteria *Lactobacillus acidophilus* LA-5 and *Lactobacillus rhamnosus* GG. For this, different concentrations of capsanthin were added to growth media of probiotic bacteria, and their effects on bacterial growth kinetics, bacterial surface hydrophobicity (Microbial Adhesion to Solvents - MATS Test) and bacterial auto-aggregation were examined. According to the results, capsanthin did not show any negative effects on the growth, while decreased the hydrophobicity of *Lactobacillus rhamnosus* GG dose-dependent manner but increasing the hydrophobicity of *Lactobacillus acidophilus* LA-5. In auto-agregation, changes were observed depending on the dose and time. This study shows carotenoids taken together with the diet can affect beneficial bacteria.

Keywords: Auto-aggregation, Capsanthin, Hydrophobicity, Paprika, Probiotics

Introduction

The intestinal microbiota begins to resemble the gastrointestinal tract microbiota of a young person after the first year of life and is considered to have reached the adult microbiota composition for an average of 3 years. This bacterial group, which is synbiotic with the human body, is defined as a microbiota with the "Human Microbiome Project". Probiotics are living microorganisms that show beneficial effects on a person's health and physiology when taken in sufficient quantities, and the most important advantage of probiotics in microbiota is their effect in maintaining the proper balance between the pathogens and bacteria required in the organism (İsmailoğlu and Öngün Yılmaz, 2019).

Probiotics are microorganisms that have positive effects on human health when taken into the body in the required amounts (Hill et al., 2014). Probiotic microorganisms have beneficial effects such as vitamin production, reducing lactose tolerance and calcium absorption, by strengthening the immune system, it has benefits such as reducing the risk of intestinal cancer, regulating the digestive system, inhibiting tumor formation, inhibiting diarrhea formation (Gill and Prasad, 2008). Probiotics are also described as health-promoting bacteria.

Lactic acid bacteria (LAB), which constitute the most important group of probiotic bacteria, are gram-positive, catalase-negative, non-spore, cocci or cocobacilli, aerotolerant microorganisms (Vandenbergh, 1993). *Lactobacillus* species

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such as *Lactobacillus casei*, *L. paracei*, *L. rhamnosus,* and *L. plantarum* are among the most widely used probiotic bacteria used in the production of many dairy and non-fermented food products (O'Toole et al., 2017). *Lactobacillus acidophilus* produce lactic acid, hydrogen peroxide and various bacteriosins (acidolin, acidophilin, lactosidine), which can have antibacterial effects against intestinal pathogens (Sanders and Klaenhammer, 2001). Lactic acid lowers the pH of the environment and creates an unsuitable condition for other bacteria, while hydrogen peroxide plays a role in the antagonistic effect against intestinal pathogens (Sezen, 2013). The use of foods for increasing nutritional value, extending shelf life and controlling intestinal infections has made lactic acid bacteria important in recent years (Soomro et al., 2002).

The importance of nutrition on human health has been understood and accordingly, the concentration on natural antioxidant consumption has increased in developed countries. The main color substance of plants that have therapeutic effect against oxidative pressures, diabetes, Alzheimer, neurological diseases and some types of cancer in the last years as well as attracting the attention of many researchers is the carotenoid pigment (Kadakal et al., 2001). Especially, the relationship between cancer and carotenoid is a factor preventing tumor formation (Dai and Mumper, 2010). The antioxidant and anti-inflammatory effect of carotenods is of great importance in terms of cancer diagnosis and treatment methods (Dai and Mumper, 2010). Pigmentation-providing carotenoids cannot be synthesized by animal tissues, and therefore this molecule is derived from food (Fernández-García et al., 2012). The identified carotenoid substance is over 600 and it has potential health benefits as well as its coloring feature (Fernández-García et al., 2012).

Capsanthin is one of carotenoids from red pepper (Figure 1). 70% of carotenoids in all intense varieties of red intense constitute of capsanthin and capsorubin, while green stuffed peppers do not contain them. The ratio of the capsule in different varieties of red-colored species is between 34-60%. More than 60% of the color is red, and 53.5% of this is due to the capsanthin (Kadakal et al., 2001). The coloring agents in red pepper are rich in provitamin A and C, B1, B2, E vitamins (Demiray and Tülek, 2012).

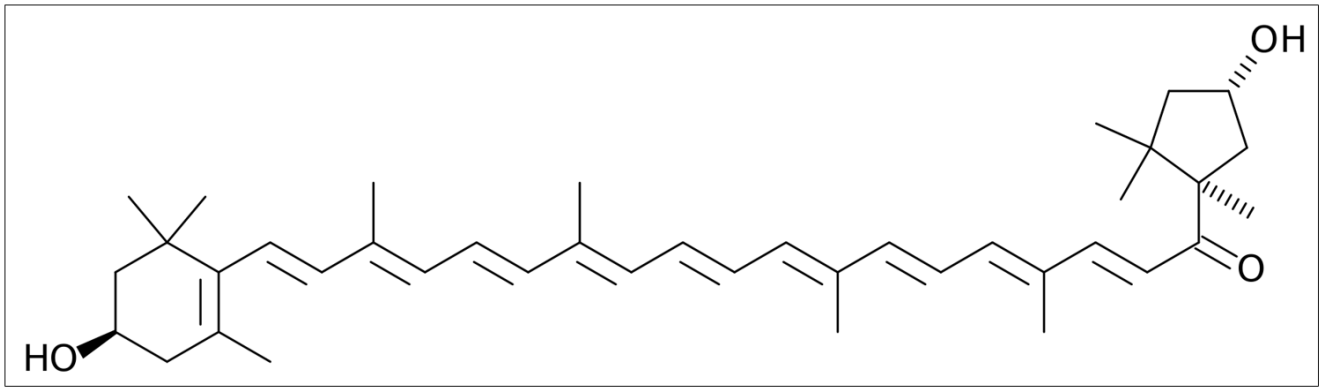

Figure 1. Chemical structure of capsanthin.

Previous researches have found that capsanthin is well absorbed by humans, and has antitumor and anticancer effects, as well this compound plays an important role in the prevention of many diseases (Akdoğan et al., 2008). Capsanthin, which has a beneficial effect, was used in this project and we hypothesized that using it together with probiotic bacteria can have a positive effect on health and prevent gastrointestinal diseases.

The aim of the present study is to determine the effects of capsanthin on probiotic properties, surface hydrophobicity and auto-aggregation of lactic acid bacteria, *Lactobacillus acidophilus* LA-5 and *Lactobacillus rhamnosus* GG.

Materials And Methods

Growth of probiotic bacteria in the presence of capsanthin

The lactic acid bacteria, *Lactobacillus acidophilus* LA-5 and *Lactobacillus rhamnosus* GG, which are kind gift of Chr. Hansen, Turkey, were grown in de Man, Rogosa, and Sharpe (MRS) medium at 37°C in an aerobic environment without shaking (Celebioglu et al., 2018).

Different cultures from the same bacterial stock were grown at different concentrations of the capsanthin (0, 25, 50, 100, 250, and 500 µg mL^{-1}). The control groups were divided into two, Control A and Control B, which have without or with ethanol, respectively. Freeze-dried bacteria were suspended in MRS medium and added to the groups. Capsanthin was added to the treatment groups as 25, 50, 100, 250 and 500 µg mL^{-1}. Bacterial growth was measured by allowing the bacteria to grow at 37°C and their densities were recorded every 4 hours for up to 24 hours as McFarland values (Celebi et al., 2020).

Bacterial Hydrophobicity Test (Microbial Adhesion to Solvents – MATS Test)

Bacterial surface hydrophobicity was measured by microbial adhesion to solvents (MATS) test (Kos et al., 2003; Köroğlu et al., 2019). Bacteria (control and treatment groups) were harvested during the stationary phase (3200xg, 15 minutes), washed with Phosphate-buffered saline (PBS) and resuspended in 0.1 M KNO_3 (pH 6.2). OD_{600} of the suspensions were set to 0.5. One mL of xylene (apolar solvent) was added to 3 mL of bacterial suspension and incubated for 10 minutes

at room temperature. The biphasic system was vortexed for 2 minutes, the aqueous phase was separated and incubated for an additional 20 minutes at room temperature. Bacterial adhesion test to solvents was calculated by measuring absorbance values at 600 nm according to the equation (1).

$$\% \textit{ Adhesion} = (1 - A1\ A0^{-1}) \times 100 \qquad (1)$$

where, A1 is the absorbance value measured after incubation, and A0 is the value measured before incubation.

Bacterial Auto-Aggregation

Bacterial cells were harvested during the stationary phase (3200xg, 15 minutes), washed and resuspended with PBS to OD_{600} 0.5. Then, 4 mL of bacterial suspensions in test tubes were vortexed for 10 seconds and incubated for 4 hours at room temperature.

Absorbances were measured every hour at the wavelength of 600 nm by adding 0.1 mL of suspension and 0.9 mL of PBS to the tube (Kos et al., 2003; Köroğlu et al., 2019). The results were calculated according to the equation (2).

$$\% \textit{ Auto-Aggregation} = (1 - At\ A0^{-1}) \times 100 \qquad (2)$$

where, At is the absorbance value measured after incubation, and A0 is the absorbance value measured at 0^{th} hour.

Statistical Analysis

All experiments were performed with at least 3 replicates. The results were represented with the mean ± standard deviation and the comparison between the control group and treatment groups was performed with Student's *t*-test. Statistical values of $p<0.05$ were considered as significant.

Results And Discussion

Bacterial Growth Kinetics

In this study, capsanthin was used at concentrations of 0, 10, 25, 50, and 100 µg mL^{-1}. As seen in Figure 2, capsanthin did not show any negative effects on the growth of probiotic bacteria. This shows that capsanthin does not have inhibitory effects against probiotic bacteria.

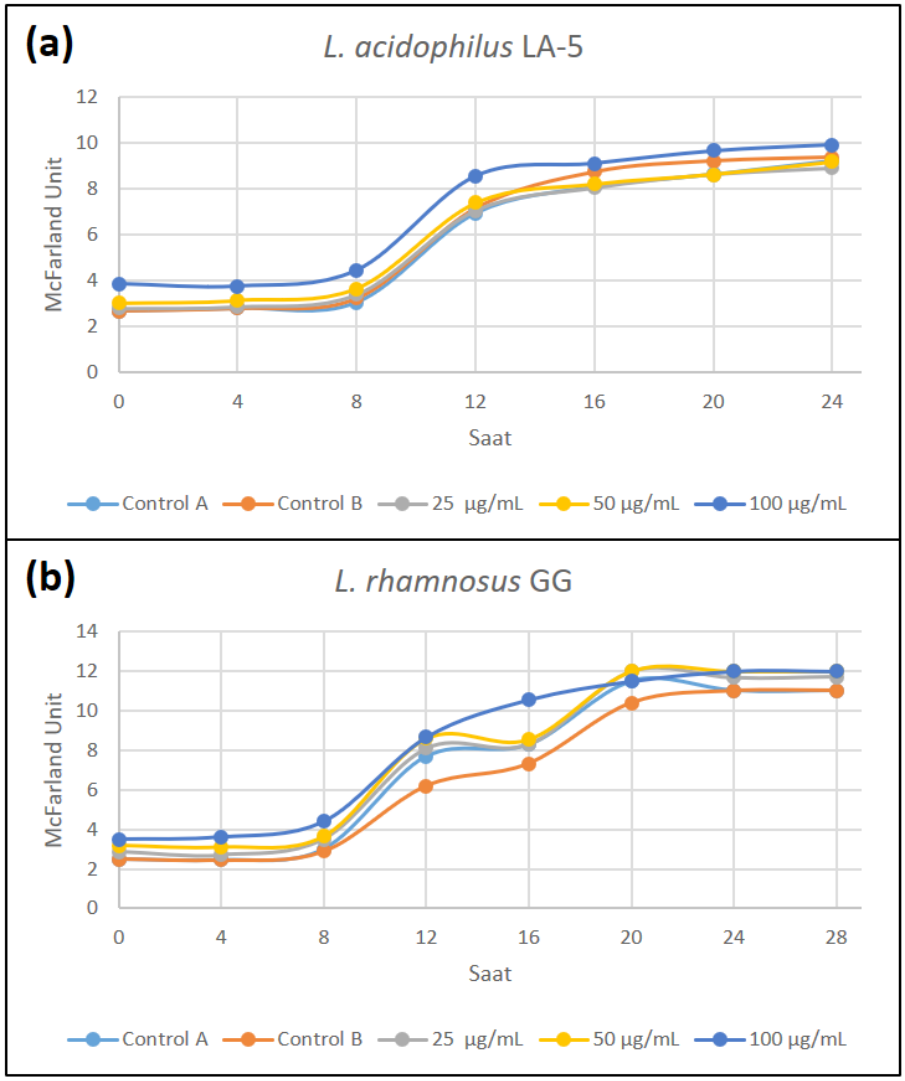

Figure 2. Growth kinetics of probiotic bacteria. **(a)** Growth kinetics of *L. acidophilus* LA-5, grown in the presence of 0, 25, 50 and 100 µg mL^{-1}capsanthin. **(b)** Growth kinetics of *L. rhamnosus* GG, grown at 0, 25, 50 and 100 µg mL^{-1}capsanthin.

Bacterial Surface Hydrophobicity

Bacterial surface hydrophobicity is an important feature for keeping bacteria in the gastrointestinal tract (Vadillo-Rodríguez et al., 2005; van Loosdrecht et al., 1987). This feature is one of the factors that can enable probiotic bacteria to better adhere to the mucosa (Krasowska and Sigler, 2014). In the present study, the Control A group does not contain ethanol, while the Control B group contains ethanol as ethanol was used to dissolve capsanthin. As a result of the MATS test, the hydrophobicity of *L. acidophilus* LA-5 was significantly ($p<0.05$) increased by 500 µg $mL^{-1}$of capsanthin, as compared to the control groups (Figure 3a). Depending on the dose, the capsanthin can increase the surface hydrophobicity of *L. acidophilus* LA-5 bacteria, which can better adhere to these mucous membranes in the gastrointestinal tract. In addition, increased cell surface hydrophobicity may allow *L. acidophilus* LA-5 bacteria to better colonize the intestinal tract to demonstrate their probiotic activity. Therefore, as the surface hydrophobicity increases, it also increases the possibility of *L. acidophilus* LA-5, a beneficial bacterium, to be attached to the mucosa.

The hydrophobicity of *L. rhamnosus* GG showed a dose-dependent decrease in the concentrations of 25, 50, 100, 250 and 500 µg mL^{-1} of capsanthin compared to the two control groups (Figure 3b). Surface hydrophobicity of *L. rhamnosus* GG decreased depending on the dose. This means that no change has been made and was unable to change the adhesion of the *L. rhamnosus* GG bacteria treated with the capsanthin to the mucus layer and major components.

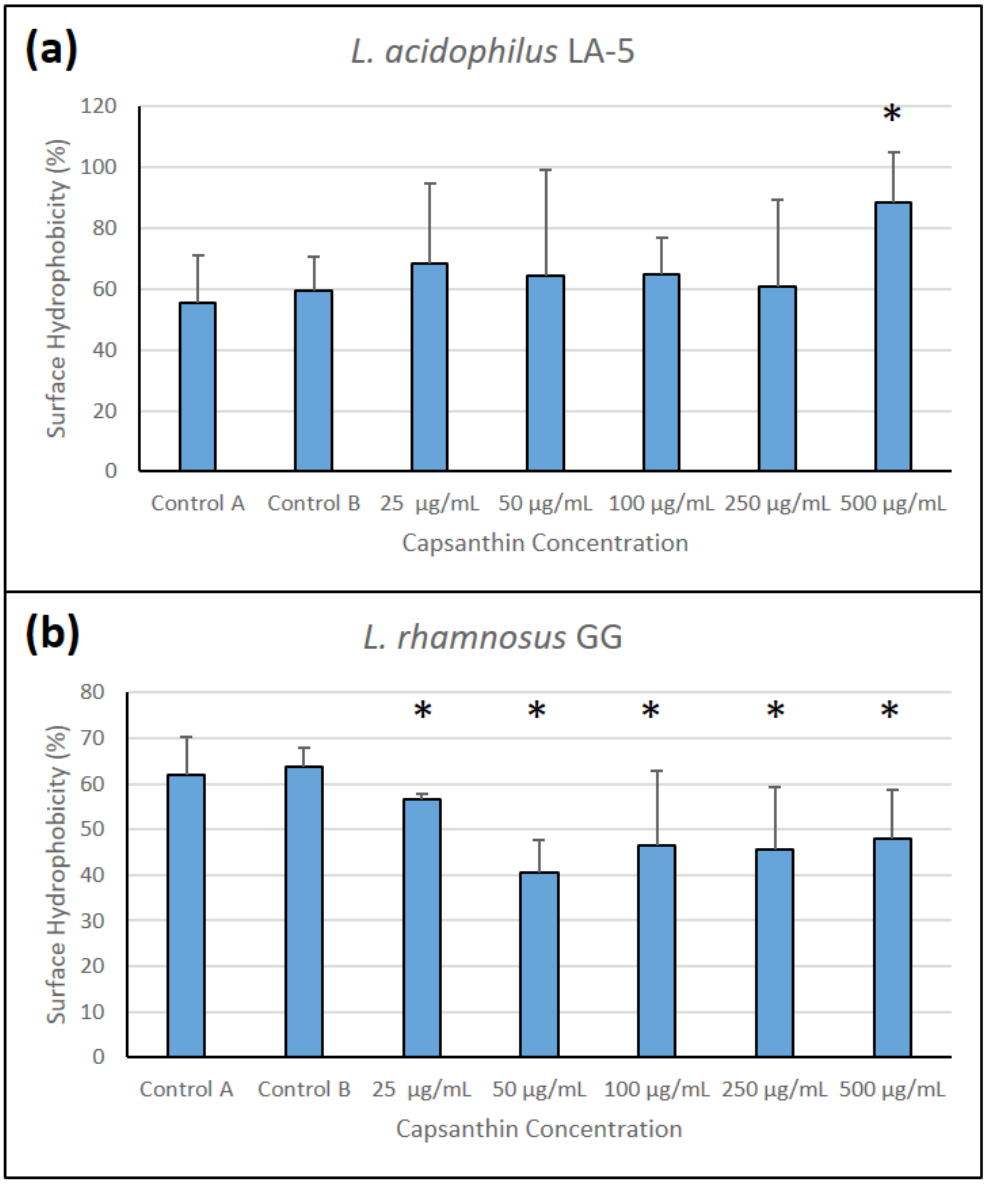

Figure 3. Surface hydrophobicity of probiotic bacteria. **(a)** MATS test result of *L. acidophilus* LA-5 treated at 25, 50, 100, 250 and 500 µg mL^{-1} concentrations. **(b)** MATS test result of *L. rhamnosus* GG treated at 25, 50, 100, 250 and 500 µg mL^{-1} concentrations. Asterisks (*) indicate that the difference is statistically significant ($p<0.05$) as compared to Control B (containing ethanol).

With the use of capsanthin, according to the MATS test result, colonization of *Lactobacillus acihophilus* LA-5 bacteria can lead to better adhesion to the intestinal mucosal structure, which plays important roles in the probiotic properties.

The use of capsanthin with other bacterial strain *Lactobacillus rhamnosus* GG did not cause any change in surface hydrophobicity, and this could not affect the adherence of beneficial microorganisms to the intestinal mucosal structure. However, this effect of capsanthin on *Lactobacillus rhamnosus* GG can be investigated by further studies for the intestinal mucosal system. With different studies, the effect of capsanthin *on Lactobacillus rhamnosus* GG at different doses and time can be examined.

Bacterial auto-aggregation

Bacterial auto-aggregation is defined as clustering of bacteria by sticking together. In addition, high aggregation can cause better adhesion, however, other factors involved in adhesion to the mucosa are proteins in the surface layer of bacteria, particularly S-layer proteins, extracellular polysaccharides, and lipoteichoic acid (Alp and Kuleaşan, 2019). Bacterial aggregation could be important for the binding of probiotic bacteria to the intestinal mucosa (Kos et al., 2003). High aggregation status can be a better adhesion indicator (Del Re B et al., 2000).

Table.1. Auto-aggregation of *Lactobacillus acidophilus* LA-5 and *Lactobacillus rhamnosus* GG. The results are represented as mean value of three independent experiments with standard deviations.

	1st hour	2nd hour	3rd hour	4th hour
L. acidophilus LA-5				
Control A	47.5 ± 6.9	76.5 ± 14.3	N.D.	N.D.
Control B	44.3 ±19.0	93.2 ± 5.4	N.D.	N.D.
25 µg mL^{-1}	56.0 ± 13.6	93.8 ± 1.6	N.D.	N.D.
50 µg mL^{-1}	57.5 ± 27.3	92.9 ± 5.5	N.D.	N.D.
100 µg mL^{-1}	54.7 ± 20.2	91.9 ± 4.4	N.D.	N.D.
250 µg mL^{-1}	45.6 ± 23.9	87.9 ± 7.8	N.D.	N.D.
500 µg mL^{-1}	36.5 ± 27.0	78.8 ± 3.1	N.D.	N.D.
L. rhamnosus GG				
Control A	N.D.	4.9 ± 0.4	46.1 ± 35.6	21.9 ± 10.9
Control B	N.D.	22.3 ± 7.2	50.7 ± 20.6	44.5 ± 8.7
25 µg mL^{-1}	N.D.	12.8 ± 5.0	13.2 ± 11.0	24.2 ± 9.8
50 µg mL^{-1}	N.D.	14.9 ± 3.4	24.0 ± 13.8	27.5 ± 10.6
100 µg mL^{-1}	N.D.	26.5 ±1.1	34.9 ± 15.2	22.9 ± 7.9
250 µg mL^{-1}	N.D.	16.9 ± 9.6	26.7 ± 9.8	20.9 ± 6.7
500 µg mL^{-1}	N.D.	22.9 ± 6.4	25.3 ± 14.0	46.7 ± 9.3

N.D.: Not determined

Bacterial auto-aggregation test results of *L. acidophilus* LA-5 and *L. rhamnosus* GG are given in Table 1. The results showed that bacterial auto-aggregation of *L. acidophilus* LA-5 showed in 25, 50 and 100 µg mL^{-1} capsanthin concentrations are higher than Control A group at 1st hour. In the 2nd hour, concentrations of 25, 50, 100 and 250 µg mL^{-1} of capsanthin concentrations increased the auto-aggregation compared to the Control groups. Changes in auto-aggregation of *L. rhamnosus* GG bacteria depending on time and dose were observed.

The results showed that the auto-aggregation values were increased in 3 different concentrations in growth of *L. acidophilus* LA-5. However, high concentrations of capsanthin showed a reduction in auto-aggregation of *Lactobacillus acidophilus* LA-5. These bacteria, treated with capsanthin, can aggregate and this can provide better binding to the intestinal mucosa. *Lactobacillus acidophilus* LA-5, which adhere better to the intestinal mucosal structure, can have a health-promoting effect when used with capsanthin. On the other hand, when capsanthin was added to the growth of *Lactobacillus rhamnosus* GG, the other probiotic bacterial strain, auto-aggregation values were not significantly altered. However, detailed research can be performed on the adhesion of *Lactobacillus rhamnosus* GG bacteria by treatment with capsanthin.

Adhesion seems very crucial for especially probiotic bacteria, as high attachment of the bacteria to the mucosa could mean higher biological activities, such as higher colonization, inhibition of pathogen binding, and better modulation of immune system (Alp and Kuleaşan, 2019; Bermúdez-Brito et al., 2012). Even though S-layer proteins are very important in adhesion of probiotic bacteria, non-selective interactions on the surface such as hydrophobicity and also auto-aggregation could play roles in adhesion (Alp et al., 2020; Kos et al., 2003). Thus, in the present study, we showed that these properties of the probiotic bacteria could be modulated by a dietary carotenoid, capsanthin.

Conclusion

The present study investigates the effects of capsanthin, one of the major carotenoids present in paprika, on some probiotic properties of lactic acid bacteria *lactobacillus acidophilus*

LA-5 and *lactobacillus rhamnosus* GG. The results indicate that this carotenoid may alter the properties of probiotic bacteria so that they can prolong their resistance in the gastrointestinal system. Further studies can give insight into more details how capsanthin can alter the adhesion properties of probiotic bacteria to the mucus, as well as to the intestinal cells; furthermore, how capsanthin can be metabolized by these bacteria.

Capsanthin, a natural antioxidant compound found in red pepper, will shed light on many research and studies as a result of this project. Today, due to the spread of diseases, healthy nutrition and strengthening the immune system have gained importance. Thus, the red pepper component capsanthin, which is taken in the diet, can be combined with beneficial microorganisms in the intestines and have more effects on the immune system. It can inhibit the growth of pathogenic microorganisms by strengthening the gastrointestinal system. Capsanthin and probiotic bacterial strains together can exert more beneficial effects on the host. This study can assist in more extensive studies to investigate the health effects of taking probiotic bacteria with carotenoid compounds.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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The Author name's in the page header (Aylin Yilmaz Cetinkaya and Selcuk Yurtsever) has been changed from:
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Effects of shoot tip colchicine applications on some grape cultivars

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Abstract

Polyploidization can provide changes in vital features such as growth, development, environmental stress tolerance in plants. Colchicine is one of the most commonly used chemicals as a polyploidization agent. In this study, 2-year-old 'Ekşi Kara', 'Gök Üzüm' and 'Trakya İlkeren' (2x, *Vitis vinifera* L.) saplings grown on their own roots were used. When the enforced shoots reached about 15 cm length, colchicine applied (0, 2.5 g L^{-1}, 5 g L^{-1}, 7.5 g L^{-1}) 24 and 48 hours to the lateral shoot tips. The effects of treatments were evaluated by shoot tip viability, stoma size and density, chloroplast counts, and flow cytometry (FC) analysis, and 'Kyoho' (4x) were used as the control. The maximum stomatal variations were determined in Ekşi Kara cultivar at 2.5 g L^{-1} 24-h application. Based on morphological differences, FC analysis was performed only in 'Ekşi Kara' but there was no genomic duplication. Since the morphological differences were not sufficient in the diagnosis of polyploid in grape cultivars, FC analysis should be performed to achieve confirmed results.

Keywords: Grapevine, Cultivar development, Breeding, Chemical mutagen, Autotetraploidy

Introduction

Grape (*Vitis vinifera* L.) is one of the most important fruit species grown globally as producing table grape, wine, raisin and fruit juice. Approximately 36% of the world production, and 56.1% in Turkey's grape production is used as table grape (OIV, 2019). New grape cultivars are needed to ensure high adaptation to changing environmental conditions for sustainable viticulture and to meet market demands.

Polyploidization, is an important tool employed to create new genetic resources in many plant species, to shorten the time needed for breeding and to obtain properties that cannot be achieved through hybridization (Yue et al., 2017). Mutation, in plants can be stimulated with many chemicals and physical mutagens, colchicine is the most commonly used chemical mutagen for this purpose. This antimitotic agent promotes polyploidy in the cells by blocking the mitosis in the metaphase stage (Planchais et al., 2000). In previous studies reported that there is increasing fruit quality and developing the stress tolerance in polyploid grapes (Notsuka et al., 2000; Park et al., 2004; Yamada and Sato, 2016). This method has been used in grape breeding since 1937 and interest has been increasing in recent years (Olmo, 1937). Polyploid grape cultivars are used commercially and cv. Kyoho (4x) constitutes 44% of the total vineyard area in China, which ranks the first in the world grape production (Olmo, 1937).

'Ekşi Kara' and 'Gök Üzüm' are ancient and autochthonous grape cultivars grown extensively in Central Taurus Region (Kara et al., 2017a). In order to meet the pollen needs of 'Ekşi Kara' (Kara et al., 2017b), and to increase its marketability without loss of adaptation ability to the area where traditional cultivar is grown, its fruit quality characteristics must be improved (Kara et al., 2017b). Previous studies conducted on chromosome doubling differ in terms of application doses, durations, tissue types, and application methods for polyploidy

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stimulation (Değirmenci-Karataş et al., 2010; Dhooghe et al., 2011; Kuliev, 2011; Ma et al., 2014; Kara et al., 2018). Studies are needed to determine the proper colchicine application methods with different cultivars, and to obtain cultivars with increased ploidy levels.

In this study, the effects of different doses and durations of colchicine applications on shoot tips were tested in order to provide genomic duplication in 'Ekşi Kara', 'Gök Üzüm' and 'Trakya İlkeren' grape cultivars *in vivo* conditions.

Materials and Methods

Plant Material

In this study, Ekşi Kara and Gök Üzüm autochthonous cultivars which were obtained from Selçuk University Faculty of Agriculture Department of Horticulture, and cv. Trakya İlkeren obtained from Tekirdağ Viticulture Research Institute, were used. 'Ekşi Kara' and 'Gök Üzüm' are well-adapted and the most intensely grown cultivars in Konya-Karaman provinces and Central Taurus Region. Since 'Ekşi Kara' has functional female flowers, 'Gök Üzüm' is used as a pollinator. Both cultivars are used as table and as raisin locally (Kara et al., 2017a). 'Trakya İlkeren' is preferred in early ecology in terms of its early yield, it can also be successful in short-vegetation areas (Köse and Ateş, 2017; Gülcü et al., 2020). Plants obtained from cuttings were used in study. Thermotherapy was applied for 30 minutes to the cuttings that would be used in the study at 50°C before rooting (Waite and Morton, 2007). After thermotherapy, cuttings were rooted in the mixture (peat: perlite 2:1 v:v) in the greenhouse. Rooting plants were planted in pots that containing a 2: 1 peat-perlite mixture. The chloroplast counts in stoma guard cells of plants were compared to 'Kyoho' (4x) (Yamada and Sato, 2016).

Chemical Mutagen Colchicine Applications

Colchicine (Sigma-Aldrich) applications were made to stimulate the polyploidy, which is effective even in low doses (0.5 mM< dose) in plants (Allum et al., 2007). In the present study, 1% dimethyl sulfoxide (DMSO) dose was used as the solvent for colchicine (Yang et al., 2006). 2-year-old potted plants grown on their own roots in greenhouse conditions, pinching was applied in early active growth period, when the shoots reached at 15 cm length. Lateral shoots tips were exposed to colchicine, 3 different doses (2.5 g L^{-1}, 5 g L^{-1}, 7.5 g L^{-1}) and 2 different time which 24 [3 times in 24 hours, (morning, noon and evening)]-48 hours [3 times in every 24 hours, (morning, noon and evening)]. After 24 hours from the first application, shoot tips were washed with sterile water. Control plants underwent washing only with a sterile water.

Determination of plant growth and ploidy level after colchicine applications

Survival rates of shoot tips (%)

Two weeks after the applications, the number of surviving shoot tips was determined by proportioning the number of alive shoot tips to all shoots of treated plants (%) (Kara et al., 2018).

Stoma Length (μm), Stoma Width (μm) and Stoma Density (stoma mm^{-2}) Observations

Two mounts after the treatments, the leaf epidermal traces of the plants that underwent the application were examined at the abaxial side of the fourth leaf from the end on the developing shoots after the application was carried out. The lower epidermis was removed by pasting with transparent nail polish from three different areas, and placed on the slide to determine the width and length of the stoma with a ×400 microscope (Moghbel et al., 2015).

Chloroplast Count (pcs $stoma^{-1}$)

Two mounts after the treatments, the changes in chloroplast counts were examined in stoma guard cells in all treated plants survived shoots. In the leaves that were taken for the stoma sample, the colour of the leaf sections was decolorized with Carnoy's Solution (3-part ethyl alcohol: 1-part glacial acetic acid v/v). The leaf sections that were taken out of the solution were kept in sterile water for 2-5 minutes, and then stained with 1% I-KI for 30 seconds. A total of 30 stoma chloroplast counts were performed in each sample. Chloroplast numbers were detected with ×400 microscope (Yuan et al., 2009), and were compared to diploid parents and tetraploid 'Kyoho'.

Flow Cytometry (FC) Analysis

Fresh leaf samples (3-4 weeks) were taken to a petri dish of 0.5 cm^2 for each application, 500 µL isolation buffer (Partec-Nuclei Buffer Extraction) was added, and the leaf texture was divided into small pieces with razor blades. The samples in the petri dish were shaken for 10-15 seconds, filtered with Partec-CellTrics 30 µm- green filter into the tube (Partec-Sample Tubes, 3.5 ml, 55x12 mm). A total of 1600 µL staining solution [Partec-DAPI (4.6 diamidino-2-phenylinole) Staining Buffer] was added to the tubes and was kept for 5 minutes in a medium isolated from light. Then the samples were analysed with the FC device. Samples were compared based on peak channels formed by diploid parents and tetraploid (4x) control in the FC device (Pazuki et al., 2018).

Statistical Analysis

The experiment was conducted in completely randomized design, with 3 repetitions, and with 10 shoot tips per repeat. The effects of the applications dose and duration interaction were compared in the JMP 13.0 Statistical Program with the Tukey test at $p<0.05$ significance level (Yue et al., 2017).

Results and Discussion

Survival Rates of Shoot Tips (%)

The shoot tip viability rates were varied according to the interaction of the cultivar, dose and duration. The colchicine doses and application times tested in this study affected the survival rates of the shoot tips in varying degrees according to the cultivars. The minimum shoot viability rates in 'Ekşi Kara' (83.67%) as a result of the toxic effect was recorded in 2.5 g L^{-1} 24-h, while in the control all of them were alive. The lowest shoot tip viability rates in 'Trakya İlkeren' and 'Gök Üzüm' were detected in 2.5 g L^{-1} 48-h (86.22%) and 2.5 g L^{-1} 24-h (84.89%) applications, respectively. It was observed that in 'Gök Üzüm' shoot tip viability rates were higher than other cultivars (Table 1).

Since the microtubules are in different tubule compounds in explant sources like shoot tips, the sensitivity levels of explant sources to chemical mutagens might vary. Sekiguchi et al. (1971) indicated that shoots did not grow as a result of the

Table 1. Effects of applications on survival rates of shoot tips (%)*

Time		Ekşi Kara	Gök Üzüm	Trakya İlkeren
24 h.	Control	100.00±0.00	100.00±0.00	100.00±0.00
	2.5 g L^{-1}	83.67±1.53	84.89±2.70	87.62±2.27
	5 g L^{-1}	87.72±1.55	95.03±2.27	88.48±1.34
	7.5 g L^{-1}	84.94±2.84	93.86±1.97	86.27±2.23
48 h.	2.5 g L^{-1}	84.45±0.57	92.88±2.83	86.22±3.36
	5 g L^{-1}	83.69±3.49	90.40±1.20	89.15±1.82
	7.5 g L^{-1}	86.00±3.61	92.85±1.90	89.84±2.64

*Colchicine applications and time interactions are non-significant at $p<0.05$

damage to the shoot tip area due to applications with mutagenic effects in some species. Also, it was reported that the shoot tips dyed at varying rates in antimitotic applications made to different types of tissues in rootstocks and grape cultivars due to the toxic effect of the chemical used (He et al., 2016; Kara et al., 2018). The findings from the present study are similar to these results.

Stoma Length (*µm*), Stoma Width (*µm*) and Stoma Density (stoma mm^{-2}) Results

The effects of the applications varied according to the cultivars, and the increases in stoma lengths were determined. Applications of 2.5 g L^{-1} and 7.5 g L^{-1} doses in 'Ekşi Kara' for 24 and 48 h caused elongation in stoma length compared to the controls (19.73 µm), while 5 g L^{-1} application caused decreases (19.10 µm). Similarly, in 'Trakya İlkeren' the 2.5 g L^{-1} and 7.5 g L^{-1} for 24-h and 48-h applications increased the stoma length. The longest stoma was recorded in the 2.5 g L^{-1} 24-h (26.23 µm) application in 'Gök Üzüm'. In the 'Trakya İlkeren' stoma length was increased in the 7.5 g L^{-1} 24-h (24.17 µm) application.

The stoma widths significantly ($p<0.05$) affected by colchicine applications, varied according to the cultivars. The 7.5 g L^{-1} colchicine for 48-h application in 'Ekşi Kara' and 'Trakya İlkeren' increased stoma width as 17.16 µm, 16.56 µm, respectively. In 'Gök Üzüm', the highest width was achieved in the 2.5 g L^{-1} 24-h (17.61 µm) (Figure 2).

The effects of colchicine dose and time of applications combinations in all grape genotypes in terms of stoma count per unit area were statistically important ($p<0.05$). Stoma densities were decreased depending on colchicine applications in all cultivars. The lowest stoma density values were determined in 'Ekşi Kara' the 5 g L^{-1} 24-h (297.10 stoma mm^{-2}), in 'Trakya İlkeren' the 7.5 g L^{-1} 48-h (408.11 stoma mm^{-2}) and in 'Gök Üzüm' with 5 g L^{-1} 48-h (433.91 stoma mm^{-2}) applications (Figure 3).

Stoma data, ensures approximate identification of genome size for the autopolyploid stimulated genotypes (Yang et al., 2006). The increase in cell size causes depending on the response of the species and cultivars, increasing occur in the shoot diameter, pollen, leaf and stoma sizes in tetraploid plants (Motosugi et al., 2002; Sattler et al., 2016). As a result of the increase in stoma size, decreases are detected in stoma count per unit area ($mm^{2)}$ (Ma et al., 2014; Xie et al., 2015). According to the findings in present study, stoma data can be used for pre-evaluation of the ploidy detection, the stoma data obtained outside the full genome folding might vary, and be affected by environmental conditions.

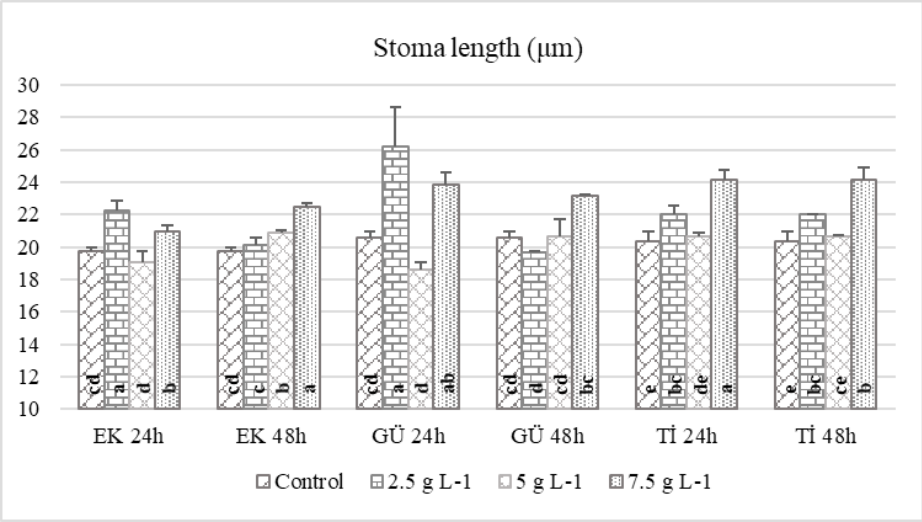

Figure 1. Effects of applications on stomata length (µm) (EK: Ekşi Kara, GÜ: Gök Üzüm, Tİ: Trakya İlkeren)

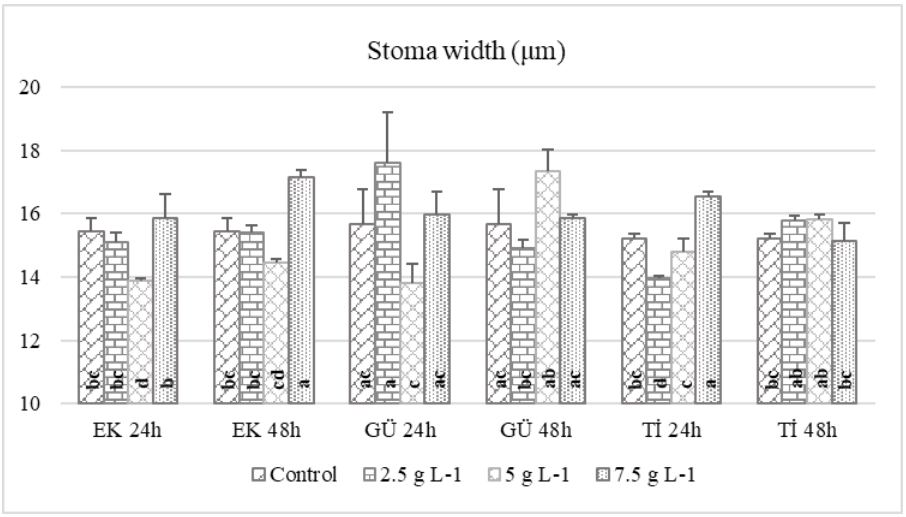

Figure 2. Effects of applications on stoma width (µm) (EK: Ekşi Kara, GÜ: Gök Üzüm, Tİ: Trakya İlkeren)

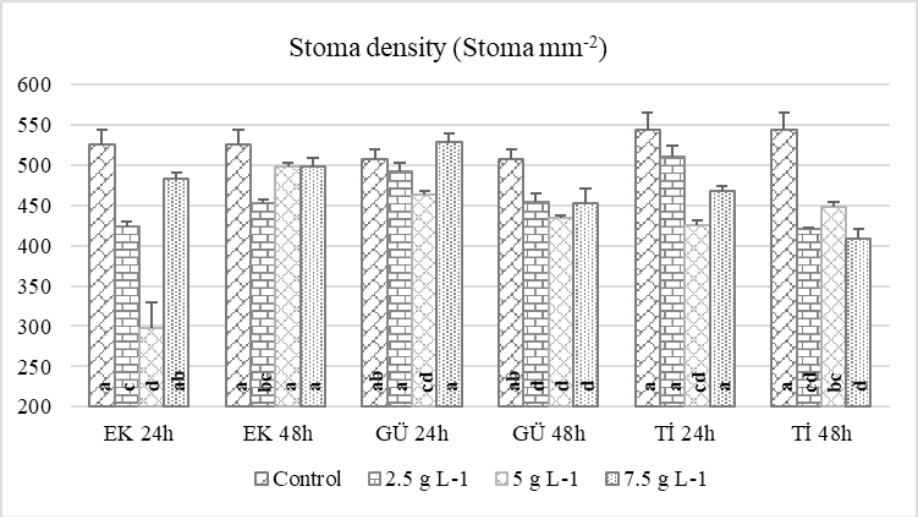

Figure 3. Effects of applications on stomata density (stoma mm^{-2}) (EK: Ekşi Kara, GÜ: Gök Üzüm, Tİ: Trakya İlkeren)

Chloroplast Count (pcs stoma^{-1}) Results

Chloroplast counts of stoma guard cells differed in 'Ekşi Kara' which were colchicine applied, and in control 'Kyoho'. The range of chloroplast count was between 18-28 in mutagen applied grape cultivars, and 38-40 in tetraploid 'Kyoho' (Table 2). The chloroplast numbers of the colchicine applied samples in Trakya İlkeren and 'Gök Üzüm' were similar to those of the controls (18-20); however, that was increased in 'Ekşi Kara' a dose-dependent, and the maximum value was 24.92 in the 2.5 g L^{-1} 24-h application.

The previous studies were reported that there is an association between the chloroplast counts and ploidy levels in stoma guard cells (Chen et al., 2009). Xie et al. (2015) indicated that chloroplast counts were made easier and earlier in stoma guard cells compared to the chromosome counts and FC analysis. In the present study, chloroplast counts increased compared to the original diploids; however, its frequency remained low comparted to the tetraploid control 'Kyoho'.

Flow Cytometry (FC) Analysis Results

Based on the numerical differences occurring in chloroplast counts, FC analysis was performed on plants whose ploidy levels were estimated to be different. As a result of FC analysis, it was determined that the ploidy levels of the samples examined did not change and they maintained their diploid forms (Figure 4).

In previous studies, FC analyses were used to determine the change in the ploidy levels of plants. In our study, FC analysis results were similar to the literature (Yang et al., 2006; Dhooghe et al., 2011; Acanda et al., 2013; Acanda et al., 2015).

Table 2. Effects of applications on chloroplast number (stoma mm^{-2})*

		Ekşi Kara		Gök Üzüm		Trakya İlkeren	
	Number of stoma	Average	Range	Average	Range	Average	Range
Kyoho	30	38.50±0.50 a	38-40	38.33±0.58 a	38-40	38.75±1.09 a	38-40
Control	30	19.73±0.28 d	18-20	20.05±0.23 cd	18-22	20.53±0.42 b	18-22
2.5 g L^{-1} 24h	30	24.92±1.01 b	18-28	19.71±0.25 d	18-22	20.45±0.08 b	18-22
2.5 g L^{-1} 48h	30	20.42±0.05 de	18-22	20.88±0.39 bc	18-22	20.25±0.12 b	18-22
5 g L^{-1} 24h	30	21.34±0.15 d	18-22	21.76±0.48 b	18-22	20.91±0.21 b	18-22
5 g L^{-1} 48h	30	20.26±0.09 de	18-22	20.06±0.26 cd	18-22	20.29±0.15 b	18-22
7.5 g L^{-1} 24h	30	23.11±0.12 c	18-26	20.03±0.20 cd	18-22	20.20±0.00 b	18-22
7.5 g L^{-1} 48h	30	19.58±0.04 d	18-20	20.83±0.15 bc	18-22	19.84±0.01 b	18-22

*Mean separation within columns by Tukey multiple test at, 0.05 level

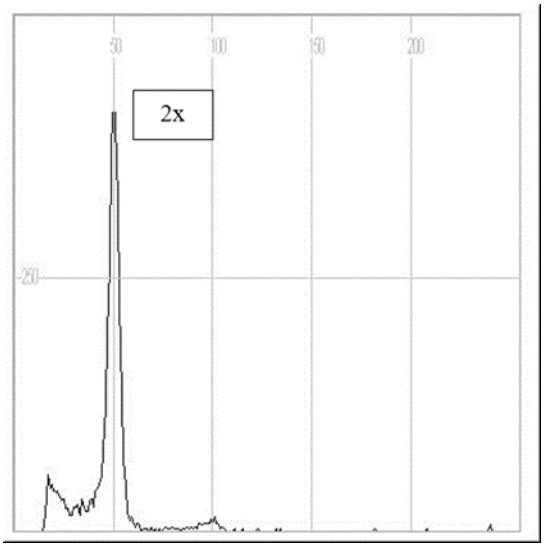

Figure 4. FC analysis result of 'Ekşi Kara' 2.5 g L^{-1} 24-h application (diploid, 2n = 2x)

Conclusion

The polyploidy breeding method is used in the breeding of economically important plants since its provides potentially beneficial results. Although differences were detected in the morphological and stoma sizes of the grape cultivar that underwent colchicine applications, it was determined with the chloroplast counts and FC analyses in stoma guard cells that these changes did not cause differences at the genome level. It was observed in the study that the reactions to *in vivo* applications varied on cultivar basis, and that the colchicine applications had limited effects on the development of grape cultivars that had domestic and regional importance. It is considered that future studies should focus on different cultivars and tissue types, dose and duration combinations.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Not applicable.

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Data availability

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Consent for publication

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