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Research Article
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Evaluation of the insecticidal potential of two medicinal plants and an entomopathogenic fungi against *Tribolium confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae), a pest of stored foods

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Abstract
Stored products are among the most important foods in human nutrition. However; these products are under the pressure of many harmful organisms.
One of these pests is *Tribolium confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae). In this study, effects of two essential oils (*Mentha rotundifolia* and *Satureja calamintha*) and an enthomopathogenic fungi on *Tribolium confusum* has been tried under the laboratory conditions, contact and repellent effects of essential oils and contact effect of dry plant extract were determined.
As a result, essential oil, 3, 5, 10, 15 and 20 µl / ml doses of *Mentha rotundifolia* showed 100% effect after 24 hours, while *Satureja calamintha* reached 100% effect after 96 hours.
15 µl / ml dose of *M. rotundifolia* and 20 µl / ml dose of *S. calamintha* reached 100% detrimental effect after two hours. Powder doses of 0.6, 0.8 and 1.0 g of these plants, which were applied, caused the death of all the male and female individuals after 24 hours. In addition, the effects of $1x10^7$ conidia / mL dose of the entomopathogenic fungus *Beauveria bassiana* after 24, 48 and 72 hours were 87.5%, 97.5% and 100%, respectively. It was seen that both essential oils and *B. bassiana* were successfully in suppressing the depot pest *Tribolium confusum*.

Keywords
Beauveria bassiana, Cereals, Essential oils, *Mentha rotundifolia*, Repellent, *Satureja calamintha*

Introduction
Pests are a serious problem in grain during storage and its derived industry (Pérez et al., 2004).

Even if the problem is a global, it is more important in developing countries and in those of Africa in particular because of the climatic conditions favourable to their development (Stored food could be attacked by insects, fungi and rodents. Insect damage is the mostimportant. Even if the problem arises in such a wayoverall, it is more important in developing countries. Development and in those of Africa in particularbecause of the climatic conditions favorable to their development (Ndomo et al., 2009). Confused flour beetle "*Tribolium confusum*" is one of the most important species on grain stocks. It is found all over the world. The protection of cereals against these stock pests is of great importance for human survival. There are many obstacles to the timing of chemical use, including: increasing resistance in these pests and economic problems.

Following the increasing attention of the national and international scientific community on the risks related to the use of chemical pesticides, it is therefore important to seek new alternative control strategies to

overcome this scourge. Hence the urgent need to search for natural insecticides which, while being equally active, volatile and available. These products have a bio-insecticidal activity and, above all, are not toxic for human. Which has been the subject of various studies (Righi et al., 2017; Kemassi et al., 2019; Benayad et al., 2012).

It is with the aim of making our contribution to this aspect of research concerning the protection of foodstuffs stored by natural means as an alternative to chemicals against this dreadful pest, that we have attempted to test new bioinsecticide molecules through the choice of two medicinal plants and the use of an entomopathogenic fungus *Beauveria bassiana*.

Materials and Methods

Insects and substrate

The strain of *Tribolium confusum* used originates from Mascara (Algeria) and its molecular identification was confirmed in a molecular entomology laboratory in Turkey. Several generations were obtained from mass rearing techniques which were carried out in glass jars filled with wheat flour. The whole is placed in an oven at a temperature of 28°c and a relative humidity of 70%.

Plant materials

The two plants (*Mentha rotundifolia* and *Satureja calamintha*) belonging to Lamiaceae family were harvested in March 2019 from Mascara region (West of Algeria). They are identified by Dr. Righi, a botanist in the Department of Agronomic Sciences of the University of Mascara. The aerial parts (stems, leaves and flowers) of each plant were dried and kept away from light and humidity. Part of the plant material of each of the two species is coarsely finely ground in an electric mill while the other part is used for the extraction of essential oils.

Extraction of essential oils

Essential oils were obtained by the hydro-distillation process using a Clevenger extraction device. The yield was 1.86% for *Mentha rotundifolia* and 1.77% for *Satureja calamintha.*

Bioassays

All bioassays were performed in the Entomology Laboratory of the University of Mascara under controlled conditions (28 °C, 70% RH).

Contact toxicity of essential oils

0.4g mass of flour substrate are put in petri dishes treated with *Mentha rotundifolia* and *Satureja calamintha* separately at different doses (5,10,15 and 20µL/ml) of acetone solution of essential oil and 3µL/ml only for *Mentha rotundifolia*. A batch of 10 adults male and female individuals is introduced into each petri dish. Five replicates were carried out for each dose and each plant. Mortalities in the treated plates (Mo) were expressed according to Abbott's (1925) formula of corrected mortality (Mc) taking into account the natural mortality observed in the control plates (Mt): Mo-Mt / 100-Mt x 1OO.

Repellent effect of essential oils

The repellent effect of the ssential oil towards adults of *Triboliumconfusum* was evaluated using the preferential area on filter paper method described by McDonald et al., (1975). Forexample, the 5cm diameter filter paper discs used for this purpose were cut into two equal parts, each with a surface area. Four doses of acetone solution of essential oil of *Satureja calamintha* and *Mentha rotundifolia* (5, 10 15and 20 µl/ml) and 3µL/ml for *Mentha rotundifolia* were used for this test. Each of these doses was spread evenly over one half of the disc; the other half received only 1ml of acetone. A batch of 10 adult insects, no more than 2 days old, was placed in the center of each disc. Four replicates were made for each dose and each plant. After two hours, the number of insects present on the part of the filter paper treated with essential oil (Nt) and the number present on the untreated part (Nc) were recorded. The percentage repellency (PR) was calculated using the following formula:

PR%= Nc - Nt X 100

The average percentage of repellency for the essential oil was calculated and assigned according to the classification of McDonald et al. to one of the different repellent classes ranging from 0 to V:

Class 0 (PR< 0.1%). Class III (PR= 40.1 - 60%).

Class I (PR= 0.1 - 20%). Class IV (PR= 60,1 -80%).

Class II (PR= 20.1 - 40%). Class V (PR= 80,1 - 100%).

Contact toxicity of test plant powders

10 adult male and 10 females *Tribolium confusum* individuals were introduced in petri dishes each containing 0.4 g of flour mixed with the powder of each plant studied. Three doses (0.6, 0.8 and 1 g) are tested, the experiments were repeated four times for each dose and each plant in the presence of a control.

Fungi and inoculum's preparation

Fungal strains of *Beauveria bassiana*isolated from soils in Isparta Province, Turkey in 2014 were obtained from Plant Protection Department, Agricultural Faculty, Isparta University of Applied Sciences, Turkey. The culture was maintained on a Sabouraud Dextrose Agar (SDA) slant at 4 °C. After were scraped with 10 ml of sterile water that contained 0.05% Tween 80. The spore suspension was adjusted to the desired concentration (10^7 conidia/ml) and counting with Malassez cell.

Application of entomopathogen fungi on insect

The $1x10^7$ conidia/mL of the final concentration conidial suspension of isolate were sprayed two times from 30 cm distance with hand sprayers on the insects placed in the petri plates. Each assay consisted of 4 replicates with 10 insects. Control adult were treated with sterile distilled water with a 0.3% Tween-80. Petri dishes were kept at room temperature of 25 °C. The mortality rates of insects were evaluated on 1^{st}, 2^{nd}, and 4^{th} day after inoculation. Tukey test was applied after one-way ANOVA.

Results

Contact toxicity of essential oils

The variation in the cumulative mortality rate of *T. Confusum* adults as a function of exposure time to essential oils extracted from two plants (*M. rotundifolia* and *Satureja calaminha*) was shown in Figure 1 and 2. Mortality rate in all doses of *M. rotundifolia* reached 100% less than 24 hours (Figure 1). According to figure 2, we noticed that the mortality rate of *S. calamintha* was 88% at the dose of 5 µl on the insects during the first 24 hours. After three days the mortality rate reaches 100%.

The analysis of variance reveals a significant effect with F Cal =3.49 and F Theo =3.15.

Contact toxicity of test plant powders

All tested doses (0.6g, 0.8g and 1g) powdered of *M. retundifolia* and *S. calamintha* killed all males and females of insects within the first 24 hours.

Repellent effect of essential oils

The results of the calculation of the percentage repellent of the two plants on the adults of *Tribolium confusum* by the method of Mc Donald et al., (1975) were shown in Table 1. After an exposure time of two hours, the results showed that the oil of *M. rotundifolia* is very repellent with a percentage of 96.3% compared to that of *S. calamintha* (90.37%) at the low dose of 3µl.

Effect of *Beauveria bassiana* on adults of *Tribolium confusum*

After the first few days of application, we observed the whitish mycelium of *Beauveria bassiana* curling over the entire surface of the adults of *Tribolium confusum*

According to time, an increase in mortality rates of adult *T. confusum* was observed (Table 2, Figure 3). The results reported in Figure 3 showed that *B. bassiana* caused with a mortality rate of 100% contrary to the control. No mortality was observed during the whole four-day exposure period.

Beauveria bassiana was very effective on the pest (Table 2). Numbers of dead insects in the 24th hour after treatment were 8.75 and it was different from control statistically. In the 48th hour, dead insect numbers were 9.75 and then all insects at 72nd hour were dead. Results of 98th and 72nd hours were different from other times statistically.

Discussion

The yields obtained are higher, whatever the species considered (*Mentha rotundifolia* 1.86% and *Satureja calaminha* 1.77%), than the values obtained in the literature (Lebyoud et al., 2015; Ech-Chahad et al., 2013; Bardeau, 2009; Righi et al., 2017; Bounihi, 2016).

These results can be explained by the fact that the values were calculated on the basis of the dry weight of the samples. Environmental, climatic and geographical conditions, the harvest period and the distillation technique influence the yield of essential oil (Lahlou, 2004).

According to Kemassi et al. (2019), mortality is the first criterion for judging the effectiveness of a chemical or biological treatment.

It was reported that the Lamiaceae family is the most effective family on the pests (Regnault-Roger & Hamraoui, 1993). In the present study, the two essential oils (*M. rotundifolia* and *S. calamintha*) demonstrated insecticidal activity on adults of *T. confusum* by contact with.

This is in line with the confirmations of several studies (Benayadet al., 2012; Righi et al., 2010; Butnariu et al., 2012; Righi et al., 2016).

According to Kim et al., (2003), the toxic effects of essential oils depend on the pest, plant material tested and the duration of exposure.

Tests carried out by Yahyaoui (2005), on the effectiveness by inhalation and contact of spearmint essential oils on *Rhyzopertha dominica* and *Tribolium confusum* showed that at a dose of 3.12% the essential oil acts practically in the same way on the two insects with 100% mortality. These results corroborate with those of (Benayad et al., 2012) in Morocco or *Mentha rotundifolia* was found to be too toxic with a mortality rate of 85% in the first day of exposure for 100% in the second day at the dose of 3µL.

At the low dose of 3µL/ml *Mentha rotundifolia* oil causes 100% mortality after 24 hours of exposure which corresponds to work of Righi et al., (2017), revealed that: the essential oil of *M. rotundifolia* is more effective on *R. dominica* with a mortality rate of 100% at a dose of 3 µL (highly significant effect with F Cal = 3.49 and F Théo = 0.74).

The oils of seven plants belonging to the Lamiaceae family were more toxic causing a 100% mortality after 1-4 days of exposure, and at low dose 10^{-2} µL/cm^3 on the *A. obtectusbruche* (Regnault-Roger & Hamroui, 1994).

Similarly, the repellency increases with dose and exposure time.

Ndomo et al., (2009), reported that after 2 hours of exposure, *Callistemon viminalis* leaf oils caused a level of repulsion ranging from 36.6 to 80% against adult *A. obtectus* (Coleoptera: Brachidae), clearly showing that the percentage of repulsion increases with dose.

Other previous studies have also shown that repellency can be used as a control method. For instance, Righi et al., 2016 demonstrated repellent properties of *Mentha rotundifolia, Satureja calamintha* and *Schinus moll* essential oils against *R. dominica.* In addition 100% repellency of *M. longifolia* (L.) Huds essential oil was observed against *Sitophilus zeamais* (Odeyemi et al., 2008). While our own results show that *M. retundifolia* oil with (PR= 96,3% at 3µL/ml and *S. calamintha* with PR=90,37% at 5µL/ml) are very repellent.

Powders from the leaves of aromatic plants have a toxic effect on insects as they can act as a physical barrier Enobakhare (2007).

The Lamiceaes family plants have been considered among the most traditionally used species against bruchids and other stored food beetles.

Our results (consistent with the work of other researchers who have demonstrated the insecticidal effect of certain plant extracts in the form of powders against pests of stored seeds (Righi, 2016; Barbat, et al., 2013).

The results of present study indicate that *Beauveria bassiana* can be used successfuly against *Tribolium confusum*.

This is consistent with the work of other researchers who have found that Beauveria bassiana is a well-known entomopathogen fungi with a broad host range and is regarded as a safe biopesticide and has shown good results against many stored-grain insect species (Lord, 2005; Akbar et al., 2004; Athanassiou & Steenberg 2007, Ak et al., 2019; Sultan et al., 2019).

Akbar *et al.* (2004), in their research demonstrated that adults of *T. castaneum* exhibited very little susceptibility to *B. bassiana*.

Sheeba et al. (2001), applied *B. bassiana* against *S. oryzae* and recorded (86.2%) the mortality rate in adults after day. These results were also in line with Kassay et al. (2011) who tested 11 isolates of *B. bassiana* against adults of *S. zeamais* and *Prostephanus truncatus* (larger grain borer) (Coleoptera: Bostrychidae), and as a result,

determined that *P. truncatus* was more susceptible to the *B. bassiana* than *S. zeamais*.

It was noted by Khashaveh et al. (2011), that the commercial product of *Beauveria bassiana* showed an interesting insecticidal effect against *S. granarius*, *Oryzaephilus surinamensis*, *Tribolium castaneum* with a mortality rate of 88.33, 78.31, and 64.99%.

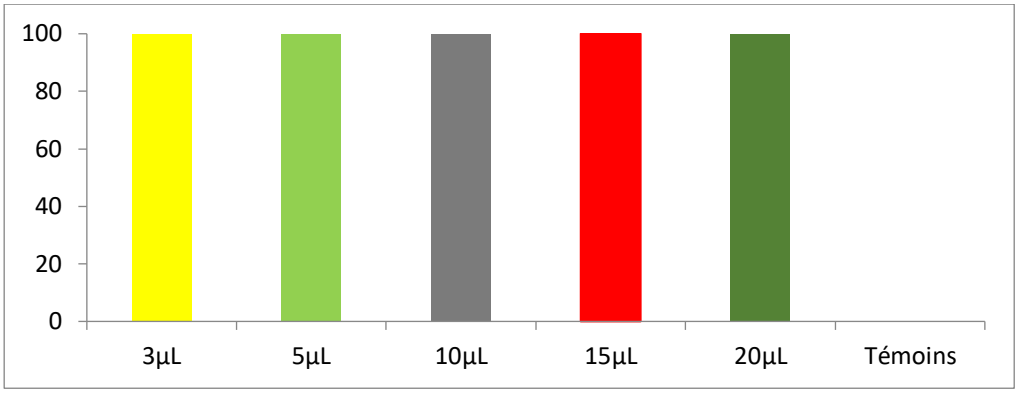

Figure 1. Bio-insecticidal contact effect of *Mentha rotundifolia* against *Tribolium confusum* after 24hours

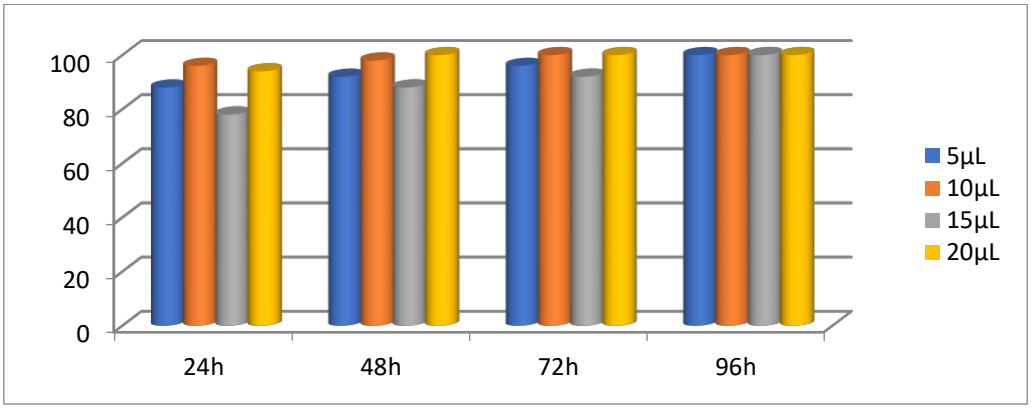

Figure 2. Bio-insecticidal contact effect of *Satureja calamintha* against *Tribolium confusum*

Table 1. Repellent effect of essential oils

Essential oils	3µL	5µL	10µL	15µL	20µL	PR %
S. calamintha	-	76,5%	89%	96%	100%	90 ,37%
M. rotundifolia	88,5%	95%	98%	100%	100%	96 ,3%

Table 2. Dead insect numbers according to time (Mean ±SE)

Time	Dead insect
98h	10.00±0.00* a
72h	10.00±0.00 a
48h	9.75±0.25 ab
24h	8.75±0.47 b
Control	0.00±0.00 c

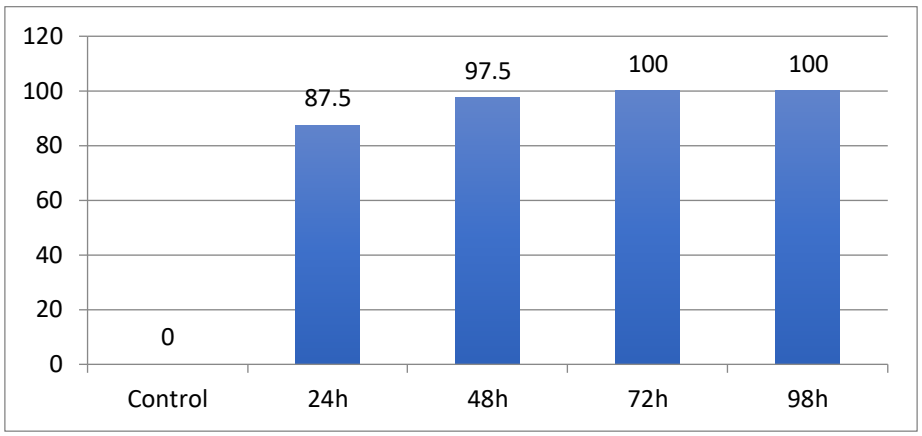

Figure 3. Mortality rates of *Tribolium confusum* treated by *Beauveria bassiana*

Conclusion

This study suggests that *Mentha rotundifolia* and *Satureja calamintha* medicinal plants as well as the entomopathogenic fungus *Beauveria bassiana* revealed that they contain active compounds with a remarkable insecticidal effect may therefore constitute an alternative to chemical control and may be potential protectors against *T. confusum.* and Thus, the study resuls have given the signals that they can be used against other storage pests too.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

All the authors read and approved the final manuscript.

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Influence of different manganese concentrations on eggplant (*Solanum melongena* L.) grown in a hydroponic system

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Abstract
The study aimed to evaluate the effect of three different manganese (Mn) concentrations on the plant growth, leaf chlorophyll, carotenoid content, photosynthetic activity, and root morphological development in eggplant (*Solanum melongena* L. 'Adana cv. Dolmalık' and 'Köksal cv. F1'). Plants were grown continuously in aerated nutrient solution by using a deep-water culture (DWC) technique in a fully automated climate room. A randomized block design with three replications was used. Both excessive (400 µM) and insufficient (0.4 µM) Mn concentrations in the root zone reduced the shoot fresh and dry matter, branch number, leaf area, and leaf chlorophyll content in both examined genotypes in comparison with optimal Mn concentrations (200 µM). Köksal cv. F1 produced higher shoot and root biomasses, root:shoot ratio, total leaf number, leaf total chlorophyll and carotenoid content, total leaf area, and average root diameter at the low concentration of Mn. Conversely, Adana cv. Dolmalık produced significantly higher stem length, shoot and root biomasses, total root length and root volume at high Mn concentrations. Overall, both deficit and excess Mn nutrition could induce disorders in the growth and development of eggplant which may reduce crop yield.

Keywords
Photosynthesis, Mn^{2+}, Deficiency, SPAD, Root morphology

Introduction
Eggplant (*Solanum melongena* L.), also known as the aubergine, guinea squash or brinjal, is an economically important vegetable crop in tropical and subtropical regions of the world. After tomato, watermelon, onion, cabbage, and cucumber, it is the world's sixth most important vegetable. It is widely cultivated in Asia and to a lesser extent in the Mediterranean basin, including Turkey. The approximate total world production for eggplants in 2019 was 52.309.119 metric tons, up by 2.2% from 51.192.811 tons. China was by far the largest producer of eggplants, accounting for over 62% of global production (FAO, 2019). It is highly valued for its taste, nutritional and health benefits (Adamczewska-Sowinska and Kotota, 2010), as it contains 1.4 g protein, 0.30 g fat, 0.30 g minerals, 0.30 g fiber, 4.0 g carbohydrates, 18.0 mg calcium (Ca), 18.0 mg oxalic acid, 47.0 mg phosphorus (P), 2.0 mg potassium (K), 124 I.U vitamin A, 0.11 mg riboflavin and 12.0 mg vitamin C per 100 g of edible portion as well as dietary fiber (Sánchez-Castillo et al., 1999). In addition, Eggplant fruits have shown high hydrophilic oxygen radical absorbance capacity (ORAC) (Cao et al., 1996), which has been correlated to phenolic compounds presence, including delphinidin as a major component in peel (Koponen et al., 2007) and chlorogenic acid in flesh (Whitaker and Stommel, 2003). The young and almost mature fruits are used as vegetable. Since it is able to absorb large amounts of sauces and cooking fats, it is used in preparing very rich dishes, while in Indonesia

and Malaysia they are also eaten raw (Sutarno et al., 1993). Anthocyanins had proven to be abundant in eggplant, Delphinidin glucosides are the type of anthocyanins which contribute to the purple dark color of eggplant (Gürbüz et al., 2018). Eggplant has some properties such as antibacterial, anti-inflammatory, antibacterial, antiallergic and anticarcinogenic which are useful to human health (Martínez-Ispizua et al., 2021).

Manganese (Mn) is an essential micronutrient necessary for plant growth and metabolism. Mn has a profound influence on three physiological processes in plants: (i) it participates in the structure of the water-splitting system of photosystem II (PSII), which provides the necessary electrons for photosynthesis (Graham, 2018). Mild Mn deficiency harms the stability and functionality of PSII, causing reduced photosynthetic CO_2 assimilation (Schmidt et al., 2020), (ii) is required for N metabolism, it functions in nitrate reduction, by acting as an activator for the enzymes nitrite reductase and hydroxylamine reductase, and (iii) it is essential for the biosynthesis of aromatic amino acids (tyrosine) and secondary products like lignin and flavonoids (Buchanan et al., 2000). Mn is a crucial constituent of Mn-superoxide dismutase, a major antioxidant enzyme (Lidon et al., 2004). It also participates in carbohydrate and lipid biosynthesis. Mn also performs as a cofactor of many enzymes, like Mn-catalase, Mn-peroxidases, TCA cycle decarboxylases, RNA polymerases and numerous glycosyltransferases (Lidon et al., 2004). Mn deficiency appears as interveinal chlorosis (yellow leaves with green veins) of the young leaves, sometimes with tan, sunken spots in the chlorotic areas between the veins as well as plants reduced growth and stunted in size (Heine et al., 2011). Despite lack of visual symptoms, latent Mn deficiency in early growth stages can cause a substantial reduction of crop yields (Schmidt et al., 2019). On the other hand, the high concentration of Mn causes brown spots on leaves, followed by chlorosis, necrosis and leaf shedding, reduced growth of plants (Mou et al., 2011); interrupts essential metabolic and reproductive processes, such as absorption, translocation, and utilization of other mineral elements in plants, stimulates the phenolic metabolism, affects the energy metabolism, decreases respiration rates, and causes oxidative stress (Shanahan et al., 2007).

Mn accumulates significantly in shoots by in roots; thus, the shoot is more affected from Mn toxicity than roots (Millaleo et al., 2010). Furthermore, excess Mn is stored in vacuoles and in cell walls in plant tissues. It negatively impacts photosynthesis by reducing biosynthesis of chlorophylls and carotenoids, preventing electron transport activity in light reaction of photosynthesis, modifying the activity of Rubisco, etc. (Millaleo et al., 2010; Parashar et al., 2014). Mn-excess in plants inhibits the functioning of PSII and oxidized form of Mn and different phenolic compounds accumulate in the leaf apoplast (Marschner, 2012). Cell homeostasis gets interrupted in Mn-treated plants. Mn binds to the outer thylakoid membranes of the chloroplasts (Rajpoot et al., 2018). Generally, Mn-tolerant genotypes employ a serial detoxifying strategy to detoxify excess Mn, such as sequestration and translocation of Mn into the vacuole and endoplasmic reticulum, chelation in the cytosol and evoking the induction of antioxidant enzymes (Boojar and Goodarzi, 2008). Phytotoxicity in growth media occurs due to low pH and also high Mn dose. The bark is used as a substitute for peat media and mostly used when peat media is scarce to find. The bark is effective in preventing phytotoxicity (Sabatino, 2020). Growth media with low pH could be adjusted by applying lime (Savvas and Gruda, 2018).

In higher plants, Mn stress caused by deficiency or toxicity experiments, which involve good control over the root environment, is generally carried out in solution culture experiments rather than soils. While solution culture disregards significant impacts of the soil and rhizosphere, it allows control of element content and pH of the nutrient solution, which are crucial factors in controlling microelements uptake and regulating of microelements methods in plant metabolism. Several nutrient solution compositions have traditionally been employed in hydroponic culture (Shenker et al., 2004). Hydroponic screening has been extensively applied to evaluate genotypic variation for Mn tolerance and accumulation ability (Khabaz-Saberi et al.,2010). Genotypic differences in Mn tolerance and accumulation have been studied in different crops such as durum wheat (Khabaz-Saberi et al.,2010), bread wheat (Sadana et al., 2002), barley (Hebbern et al., 2005), rice (Wang et al., 2002), rapeseed (Moroni et al., 2003), and other woody plants (Kitao et al., 2001; Ducĭć et al., 2006; Yao et al., 2012).

However, to date, and our knowledge, Mn tolerance and accumulation inhibiting plant biomass, photosynthesis, accumulation of reactive oxygen species (ROS) and crop yield in eggplant genotypes are poorly understood. Therefore, this study aimed to evaluate the response of two different eggplant genotypes to Mn tolerance and accumulation in a hydroponic system.

Materials and Methods

Plant Material, and Experimental Design

This study was carried out in the summer season of 2018 in the Plant Nutritional Physiology Laboratory of Erciyes University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, in Kayseri, Turkey. Two eggplant genotypes, (‘Adana cv. Dolmalık’ and ‘Köksal cv. F1’) were used as plant materials in this study. Plants were grown continuously in aerated nutrient solution by using a deep water culture (DWC) technique in a fully automated climate room. The eggplant seeds were sown in 77-cell multipots with a mixture of peat (pH: 6.0 – 6.5) and perlite (2v:1v). For the vegetation period, the average day/night temperatures were maintained at 25/22 °C, and the relative humidity was 60-80%. The supplied photon flux in the growth chamber was 350 µmol m^{-2} S^{-1} with a photoperiod of 16/8 h (light/dark). When the seedlings produced 3-4 leaves, they were transplanted to plastic pots for six weeks, after roots were washed. Each vessel was filled with an 8 L modified composition of Hoagland solution. Sufficient dissolved oxygen was supplied into the solution with the aid of an air pump. Due to transplanting small seedlings, the solutions were

entirely changed in the first two weeks and subsequently every 7th day.

Three different (Mn) concentrations: (low Mn: 0.4 µM, optimal: 200 µM, and high: 400 µM) were supplied. $MnSO_4$ was used as Mn source. The nutrient solution had the following composition (µM): $(NH4)_2SO_4$ (500); $Ca(NO_3)_2$ (1500); K_2SO_4 (500); KH_2PO_4 (250); $CaSO_4$ (1000); $MgSO_4$ (325); NaCl (50); H_3BO_3 (8); $ZnSO_4$ (0.4); $CuSO_4$ (0.4); $MoNa_2O_4$ (0.4); Fe-EDDHA (80). A randomized complete block design with three replications was used.

Harvest, Shoot, and Root Dry Weight and Main Stem Length Measurements

For the fresh weight determination, plant organs were fractioned into the leaf, stem and roots and then weighted (g/plant) at the final harvest. Plant dry matter was determined by drying the plant tissues at 70 °C for 72 h in a forced-air oven first and then by weighing them on an electronic digital scale. Total shoot dry matter was determined by adding each aerial vegetative plant parts (leaves + stems) separately. To calculate the shoot/root ratio, the sum of leaf and stem dry weights was divided by the total root dry weight. With the aid of a ruler main stem length (cm) of each plant was measured.

Leaf Area, Total Leaf Number, Branch Number and Photosynthetic Activity Measurements

The leaf-level CO_2 gas exchange (µmol CO_2 m^{-2} s^{-1}) was measured nondestructively by using a portable photosynthesis system device (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). The measurement was performed by using an artificial light source (photosynthetically active radiation (PAR) = 1000 µmol m^{-2} s^{-1}) and artificial CO_2 (400 µmol mol^{-1}) tube of the device. The photosynthesis was measured in the third and fifth weeks of the growth period on the youngest fully expanded leaves, using four replicate leaves per treatment. The total 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 as cm^2. Each fully developed leaf was counted and recorded as leaf number per plant (LN/plant). The branch number (BN) was counted and recorded as BN/plant. SPAD readings were taken with the Minolta SPAD-502 chlorophyll meter for each experimental treatment. During the growth period, two series of SPAD 502 chlorophyll meter readings were performed at the center of the leaves on the fully expanded youngest leaf for each treatment.

Leaf Total Chlorophyll and Carotenoid Content Measurements

A day before harvesting, 100 mg of fresh leaf samples from each replication of the two treatments was taken to measure the leaf total chlorophyll and carotenoid contents using UV–VIS spectroscopy. The samples were put into 15 mL capped containers where 10 mL of 95% (v/v) ethanol was added. Afterward, to allow for the extraction of the leaf pigments, the samples were held overnight in darkness at room temperature. Measurements were done using a spectrometer (UV/VS T80+, PG Instruments Limited, UK) at 470, 648.6, and 664.2 nm wavelengths. Total chlorophyll (a-Total-Chlo) and total carotenoids (b-TC) were estimated from the spectrometric readings using the formulae described by Lichtenthaler (1987):

a) Total-Chlo (mg/g plant sample) = [5.24 WL664.2 − 22.24 WL648.6 × 8.1]/ weight plant sample (g)

b) TC (mg/g plant sample) = [(4.785 WL470 + 3.657 WL664.2) − 12.76 WL648.6 × 8.1]/ weight plant sample (g).

(Note: WL470, WL648.6, and WL664.2 refer to spectrometric readings at wavelengths 470, 648.6, and 664.2 nm, respectively).

Morphological Root Measurements

The plant root morphological parameters such as total root length (m), total root volume (cm^3) and average root diameter (mm) were measured by using a particular image analysis software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc., Québec, QC G1V 1V4, Canada) in combination with recording device of Epson Expression 11000XL scanner (Long Beach, CA, USA).

Statistical Analysis

Statistical analysis of the data was performed using the PROC GLM procedure of the SAS Statistical Software (SAS for Windows 9.1, SAS Institute Inc., Cary, NC, USA). A two-factor analysis of variance was performed to study the effects of genotype or Mn-rates and their interactions on the variables analyzed. The levels of significance are represented at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), or n.s. as not significant (*F*-test and Pearson correlation coefficients). Differences between the treatments were analyzed using Duncan's multiple range test ($p < 0.05$).

Results and Discussions

Changes in Leaf Chlorophyll (a+b) and Carotenoid Content, Photosynthesis and Leaf Chlorophyll Index (SPAD)

The results of the leaf chlorophyll (a+b) content, leaf carotenoid content, photosynthesis and leaf chlorophyll index (SPAD) at the end of the growing cycle of eggplant genotypes grown in different Mn-rates are shown in Figure 1. Leaf chlorophyll (a+b) content, leaf carotenoid content, photosynthesis and leaf chlorophyll index (SPAD) were significantly ($p < 0.001$) affected by genotypes, different Mn rates, and genotype x Mn-rate interaction. However, no differences were observed in carotenoid content by Mn-rate (Figure 1A, 1B, 1C and 1D). Leaf chlorophyll (a+b) content and leaf carotenoid content are the main photosynthetic pigments of higher plants. Low and high Mn-rates decreased the amount of chlorophyll a and b in both eggplant genotypes under hydroponic conditions. Elevated levels of Mn usually have a positive effect on the intensity of photosynthesis and leaf chlorophyll index. Increasing Mn concentration in the nutrient solution increased the intensity of photosynthesis 38.43% and SPAD by almost 3.49% at high Mn-rate than low Mn-rate under hydroponic conditions, though the leaf chlorophyll (a+b) content declined by almost 0.7% and leaf carotenoid content by almost 21.71% at high Mn-rate than low Mn-rate under hydroponic conditions.

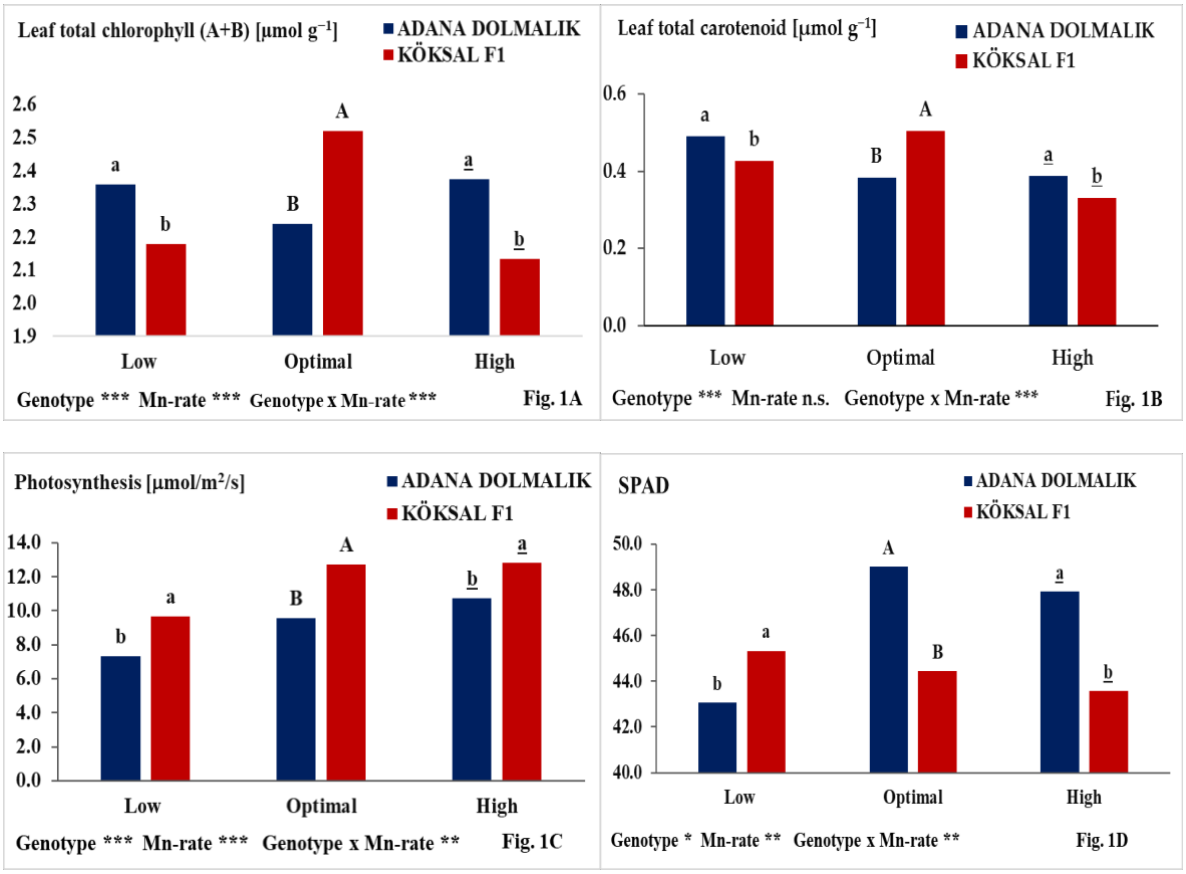

Figure 1. Leaf chlorophyll (a+b) (1A), carotenoid content (1B), photosynthesis (1C), and leaf chlorophyll index (SPAD) (1D) of eggplant genotypes (Adana cv. Dolmalık and Köksal cv. F1) grown under low, upper, and underlined case letters for low, optimal and high Mn supply, respectively) are significantly different between genotypes within columns. Significance of main and interaction effects *F* values: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Like our study Ceballos-Laita et al. (2018) stated that total chlorophyll content in old and young leaves decreased by approximately 50% in plants affected by Mn toxicity in tomato plants. Lidon et al. (2004) did not find any significant changes in the parameters of chlorophyll a fluorescence in plants exposed to increased concentrations of Mn and suggested that the accumulation of photosynthetic electron carriers is a major factor affecting photosynthesis in Mn-excess conditions. In another study, high levels of Mn usually have a positive effect on the chlorophyll content, and this was especially evident in hydroponically grown lettuce (cv. Locarno), in which a significant increase of 24-147% was noted (Przybysz et al., 2017). In contrast to our study, Silber et al. (2009) stated that leaf chlorophyll content was not affected by low Mn concentrations in tomato plants. Chlorophyll a fluorescence measurement of the maximum quantum efficiency of photosystem II (Fv/Fm) has proved useful for characterization of Mn deficiency in barley (Schmidt et al., 2016). This feature reflects the central role of Mn in the water-splitting complex of PSII (Schmidt et al., 2020). In the present work, we observed substantial differences among two eggplant genotypes in PSII quantum efficiency in response to incipient Mn deficiency. Under low and high Mn-rates, the eggplant genotype of Adana cv. Dolmalık produced significantly higher leaf chlorophyll (a+b) content. On the other hand, under optimal Mn-rate Köksal cv. F1 produced significantly higher leaf chlorophyll a and b (Figure 1A). Leaf carotenoid content was decreased under low and high Mn-rates. Under low and high Mn-rates, the higher carotenoid content was observed in Adana cv. Dolmalık, though lower carotenoid content was observed in Köksal cv. F1. Under optimal Mn-rate, significantly higher carotenoid content was produced in Köksal cv. F1 plants (Figure 1B).

Irrespective of the genotype, the addition of Mn increased gas exchange in the examined plants. Regarding the intensity of photosynthesis of the two eggplant genotypes grown in optimal and high Mn-rates, eggplant genotypes produced significantly higher photosynthesis as compared to low Mn-rate. Among the eggplant genotypes, Köksal cv. F1 produced significantly higher photosynthesis under low, optimal and high Mn-rates; on the other hand, Adana cv. Dolmalık produced significantly lower intensity of photosynthesis under low, optimal and high Mn-rates (Figure 1C). The photosynthetic apparatus might also be impaired due to a reduction in the number of chloroplasts and a decrease in the chlorophyll content resulting from excessive Mn accumulation (Demirevska-Kepova et al., 2004), and enhanced production of carotenoids, leading to symptoms similar to those triggered by photo inhibition (Doncheva et al., 2009). Long et al. (2021) documented substantial genotypic differences in maize genotypes in photosynthetic responses to Mn deficiency.

Manganese is taken up by plants as Mn^{2+} cations. Although it is a heavy metal, it may appear in plant tissues in concentrations higher than necessary for the proper functioning of organisms. Manganese is a

nutrient that has many physiological functions, including participation in many enzymes: Mn-catalase, dehydrogenase, decarboxylases, hydroxylases, acid phosphatases, transferases, SOD superoxide. Furthermore, it is present in xylogenesis, flavanols, and PS II complex-protein. Of particular importance is the share of micro-fission reactions of water in the light phase of photosynthesis (Ducic and Polle, 2005; Humphries et al., 2007). Excessive Mn nutrition may interfere with this physiological process and with the nutrient uptake, which could be a reason for worse yielding.

Regarding leaf chlorophyll index (SPAD), plants enhanced SPAD index under optimal and high Mn-rates as compared to low Mn-rate. There were no significant differences stated among eggplant genotypes under low Mn-rate. The eggplant genotype of Adana cv. Dolmalık increased SPAD index under both optimal and high Mn-rates. One of the first signs of the lack of manganese in the growth of plants, chlorophyll formation was collapsed, and the young leaves and branches were not achieving a normal green color. Furthermore, it has been shown by qualitative tests that the starch and sugar content

of the leaves of plants that became chlorotic is much less than the starch and sugar content of similar leaves that were grown during the same time. These facts show that manganese is an important and necessary factor in the synthesis of chlorophyll. Manganese occurs in the largest concentrations in the leaves and the pericarp and germs of seeds of plants (McHargue, 1926). In contrast to our study, Shenker et al. (2004) stated that the extreme Mn levels resulted in a gradual decrease in chlorophyll concentration in 14 days old tomato seedlings.

Biomass Production

Significant differences ($p < 0.001$) were found among genotype, Mn-rate and genotype x Mn-rate interaction in terms of shoot and root fresh matter. Mn is an essential metal (Marschner, 2012). Increasing Mn concentration in the nutrient solution increased the root fresh matter by 34.56% and main stem length %5.40 at high Mn-rate than low Mn-rate. Shoot fresh matter declined by almost 1.29% at a high Mn-rate than low Mn-rate under hydroponic conditions. The significantly higher shoot and root fresh matter were observed at Köksal cv. F1 under low Mn-rate and optimal Mn-rate, while under high Mn-rate significantly lower shoot and root fresh matter were observed at Adana cv. Dolmalık (Table 1). Eggplant "Köksal cv. F1" could be classified as tolerant while "Adana cv. Dolmalık" could be classified as sensitive genotype under Mn deficiency (Table 1). The study of Akinci et al. (2010) claim that in tomato plants root, shoot and leaf growth were generally enhanced at low Mn dose (50 mg L^{-1} Mn) than moderate Mn dose (100 mg L^{-1} Mn) and high Mn dose (200 mg L^{-1} Mn). Similarly, root, shoot and leaf biomass increased in low Mn concentration but decreased with increased Mn from moderate to high Mn concentration. Furthermore, Mou et al. (2011) stated that total plant biomass, shoot biomass and root biomass were significantly decreased under excess Mn concentration which was in line with our studies. With respect to main stem length, there were significant ($p < 0.05$) differences observed at the interaction of genotype x Mn-rate, while there were no significant differences found among genotype and Mn-rate. Increasing Mn concentration in the nutrient solution significantly increased main stem length of the eggplant genotype Adana cv. Dolmalık. However, in eggplant genotype Köksal cv. F1 under optimal Mn-rate recorded higher stem length growth than low and high Mn-rates (Table 1). According to Akinci et al. (2010) shows that increase in Mn-rate of tomato plant significantly decrease its stem length which contrasts with our finding where increasing Mn concentration caused stem length of Adana cv. Dolmalık genotype to decrease.

Table 1. Shoot and root fresh weight and main stem length of eggplant genotypes (Adana cv. Dolmalık and Köksal cv. F1) grown under different concentrations.

Mn rates	Shoot fresh weight (g plant^{-1})		Root fresh weight (g plant^{-1})		Main stem length (cm plant^{-1})	
	Adana D	Köksal F1	Adana D	Köksal F1	Adana D	Köksal F1
Low	117.0 a	115.0 a	19.3 b	33.4 a	16.3 a	15.2 b
Optimal	139.7 A	124.0 B	28.3 B	34.3 A	14.2 B	17.0 A
High	128.0 a	101.0 b	39.2 a	31.7 b	17.8 a	15.4 b
***F*-test**						
Genotype	***		***		ns	
Mn rate	***		***		ns	
Genotype × Mn rate	***		***		*	

[z] Values denoted by different letters (lower, upper and underlined case letters for low, optimal and high Mn supply, respectively) are significantly different between genotypes within columns at $p < 0.05$. Significance of main and interaction effects F values: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***)

There were significant differences ($p < 0.001$) observed among genotype, Mn-rate, and genotype x Mn-rate interaction in terms of shoot and root dry matter and root:shoot ratio, while no significant differences were stated regarding root:shoot ratio at the interaction of genotype x Mn-rate (Table 2).

Increasing Mn concentration from low to high Mn rate in the nutrient solution recorded a percentage increase of 3.72% in shoot dry matter, 32.89% in root dry matter and 34.47% in root: shoot ratio (Table 2). As compared to optimal Mn-rate, a slight reduction in the biomass accumulation in eggplant was preceded by a decrease in the efficiency of the photosynthetic apparatus, which is in line with the findings of Li et al. (2010) and Lee et al. (2011). The significantly higher shoot and root dry matter and root:shoot ratio were found at Köksal cv. F1 under low Mn-rate. Higher toxicity of Mn in aerial parts than in roots is a

characteristic shared by many species Li et al. (2010; Rezai and Farboodnia, 2008). It can result from the fact that the inhibition of Mn uptake or its retention in roots is not a common strategy for maintaining normal growth under Mn excess. Under high Mn-rate significantly higher shoot dry matter was detected at Adana cv. Dolmalık. Under both optimal and high Mn-rates, there were no significant differences stated among genotypes regarding root dry matter. Excess Mn accumulation as compared to optimal Mn level caused to growth and biomass depression for eggplant genotypes. These results are similar to experiments with peas (Rezai and Farboodnia, 2008) and tomato (Akinci et al. 2010) that Mn toxicity limited plant growth and biomass.

High Mn-rate Köksal cv. F1 recorded the highest root:shoot ratio followed by low Mn-rate and medium rate accordingly (Table 2). There is more than one reason for decreased eggplant biomass yield under Mn-stress caused by the accumulation of manganese. Generally, excessive or toxic manganese concentrations influence negatively plant nutrition. The highest Mn-concentrations cause toxic symptoms on plants and probably symptoms with regarding to deficit of other nutrients, occurring for example on leaves (Kleiber, 2014).

Similar results were obtained by Mou et al (2011) in grape cultivars under 15–30 mM Mn-rates. These results suggest that genotypic differences in Mn concentration significantly influenced the fresh and dry biomasses, particularly in eggplant genotype of Köksal cv. F1 plants grown under low Mn-rate. Like our study, Mn concentrations on Mn toxicity were often below 5 mM in previous studies (Sarkar et al., 2004). In our study, plant growth in two eggplant genotypes was stimulated by as low as 0.000025 mM, 0.2 mM Mn, or 0.4 mM Mn. Savvas et al. (2009) and Kleiber (2014) reported that manganese nutrition had a significant influence on tomato yielding, while Chohura et al. (2009) and Kołota et al. (2013) claimed that in research on microelements the form of ions is also important. The optimal content of manganese in a nutrient solution is varied depending on a cultivar (Kleiber, 2014).

Table 2. Shoot and root dry weight and root:shoot ratio of eggplant genotypes (Adana cv. Dolmalık and Köksal cv. F1) grown under different concentrations.

Mn rates	Shoot dry weight (g plant^{-1})		Root dry weight (g plant^{-1})		Root:shoot ratio (g g^{-1})	
	Adana D	Köksal F1	Adana D	Köksal F1	Adana D	Köksal F1
Low	11.2 b	13.0 a	1.70 b	3.37 a	0.15 b	0.26 a
Optimal	15.2 A	14.0 A	2.80 B	3.37 A	0.18 B	0.24 A
High	14.5 a	10.6 b	3.27 a	3.47 a	0.23 b	0.33 a
***F*-test**						
Genotype	***		**		**	
Mn rate	**		***		***	
Genotype × Mn rate	***		**		ns	

[z] Values denoted by different letters (lower, upper and underlined case letters for low, optimal and high Mn supply, respectively) are significantly different between genotypes within columns at $p < 0.05$. Significance of main and interaction effects F values: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Changes in Total Leaf Number, Branch Number and Leaf Area

Total leaf number and total leaf area were significantly affected by genotypes, Mn-rate, and genotypes X Mn-rate interaction. There were significant differences among genotypes with respect to branch number but there were no significant differences found in Mn-rate and genotypes X Mn-rate interaction regarding branch number. Increasing Mn concentration in the nutrient solution increased the total leaf number by almost 25.0%, branch number 76.67% and total leaf area 4.58% at high Mn-rate than low Mn-rate under hydroponic conditions. Decreasing Mn concentration in the nutrient solution significantly increased the total leaf number at the eggplant genotype of Köksal cv. F1. However, no visible foliar Mn deficiency symptoms were observed. Though, there were no significant differences stated among genotypes regarding total leaf number under both optimal and high Mn-rates. In hydroponics, excessive accumulation of this element can be avoided by monitoring Mn levels in water used to prepare the growing medium and correctly adjusting its pH because the availability of Mn increases with a decreasing pH (Ducic and Polle, 2005). Nevertheless, the risk associated with Mn concentration in plant products is often underestimated.

Regarding branch number, there were no significant differences stated among eggplant genotypes under low, optimal and high Mn-rates. In contrast to our study, Maher and Thomson (1991) stated that reduced growth of tomato seedlings was associated with high levels of manganese in the plant. With respect to total leaf area, significantly higher values were noted at the eggplant genotype of Köksal cv. F1 under low, optimal and high Mn-rates (Table 3). Previous investigations have shown that the growth and yield of tomato may be restricted by both Mn deficiency and Mn toxicity (Shenker et al., 2004). In our study, the eggplant plants were more severely affected at the low than high Mn-rates in terms of total leaf number, branch number and total leaf area. In contrast to our study, Akinci et al. (2010) claim that under low Mn-rate (50 mg L^{-1} Mn) and control (0 mg L^{-1} Mn) total leaf area of tomato plants increased significantly as compared to moderate (100 mg L^{-1} Mn) and high Mn-rates (200 mg L^{-1} Mn).

The Effects in the Root Systems

Eggplant plants under low Mn supply produced higher total root length and total root volume than plants under high and optimal Mn supply (Table 4). In the present study, root parameters were variously affected by Mn rates in different cultivars.

Table 3. Total leaf number, branch number and total leaf area of eggplant genotypes ("Adana cv. Dolmalık" and "Köksal cv. F1") grown under different concentrations.

Mn rates	Total leaf number (LN plant^{-1})		Branch number (BN plant^{-1})		Leaf area (cm^2 plant^{-1})	
	Adana D	Köksal F1	Adana D	Köksal F1	Adana D	Köksal F1
Low	25.0 b	40.3 a	5.3 a	4.7 b	2274.1 b	3316.7 a
Optimal	34.7 A	32.3 A	12.3 A	11.3 A	2855.0 B	3424.9 A
High	41.3 a	40.3 a	8.7 a	9.0 a	2593.0 b	3253.7 a
***F*-test**						
Genotype	**		***		***	
Mn rate	**		ns		***	
Genotype × Mn rate	***		ns		***	

[z] Values denoted by different letters (lower, upper and underlined case letters for low, optimal and high Mn supply, respectively) are significantly different between genotypes within columns at $p < 0.05$. Significance of main and interaction effects F values: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Total root length, root volume and average root diameter were significantly ($p < 0.001$) affected by genotype, Mn-rate and the interaction of genotype X Mn-rate. Plants under low Mn supply exhibited usually an improved performance in root growth and root morphological development than plants grown under high and optimal Mn supply. The eggplant genotype of Adana cv. Dolmalık produced significantly higher total root length and root volume than "Köksal cv. F1" under low, optimal and high Mn-rates. Köksal cv. F1 produced significantly higher average root diameter under low, optimal and high Mn-rates as compared to Adana cv. Dolmalık. This reason might be the eggplant genotype of Adana cv. Dolmalık under stressful conditions produces more root surface but lower root diameter. A large root system is considered to be an advantage for nutrient acquisition (Lynch, 2013), especially for elements with low mobility in soils, such as Mn. Deficiency of Mn is known to negatively affect the extension of the main root axis as well as the formation of lateral roots (Broadley et al., 2012). A recent study on Douglas fir showed that root growth relied on hydroponic Mn concentration (Ducic and Polle, 2007).

Bromfield (1978) and Sonneveld and Voogt (1980) also stated a tendency for Mn to be depleted in the root zone of soilless-grown crops and ascribed this phenomenon to immobilization of soluble Mn by oxidizing bacteria, which are common in nutrient solutions. It seems, therefore, that in our experiment, part of the Mn supplied to the plants through the nutrient solution was immobilized by microorganisms. The microbial oxidation of Mn in nutrient solution is beneficial when Mn is supplied at excessively high rates but potentially harmful if Mn is supplied at rates close to the standard requirements for plant uptake.

Similar to our study, Mou et al. (2011) stated that in a grape cultivar of Jinshou and Shuijin, except for the root volume increased by 15 mM Mn treatment, root length and root area were decreased with an increase in Mn concentration. Root diameter was increased with an increase in Mn levels in all grape cultivars except for Shuijin at an extremely high Mn level (60 mM). For root hairs, grape cultivars of Jinshou and Shuijin were much more affected by Mn than grape cultivar of Combier. At the concentration of 30 mM Mn treatment, pronouncedly decreased root hair elongation and hair density in grape cultivars of Jinshou and Shuijin was found although it had little effect in cultivar Combier; while under 60 mM Mn treatment, root hairs were torn down, and little hair remained in root surface in all cultivars. In contrast to our study, they stated that in grape cultivar of Combier root area and root volume increased with the increasing of Mn levels (ranged from 0 to 30 mM), but all these three root parameters were decreased under higher Mn^{2+} levels (45–60 mM).

Table 4. Total root length, root volume and average root diameter of eggplant genotypes (Adana cv. Dolmalık and Köksal cv. F1) grown under different concentrations.

Mn rates	Total root length (m plant^{-1})		Total root volume (cm^3 plant^{-1})		Av. root diameter (mm)	
	Adana D	Köksal F1	Adana D	Köksal F1	Adana D	Köksal F1
Low	43.95 a	24.42 b	2.78 a	1.91 b	0.29 b	0.38 a
Optimal	35.87 A	16.73 B	2.62 A	2.00 B	0.31 B	0.41 A
High	35.71 a	24.74 b	2.39 a	1.94 b	0.29 b	0.36 a
***F*-test**						
Genotype	***		***		***	
Mn rate	***		***		***	
Genotype × Mn rate	***		***		**	

[z] Values denoted by different letters (lower, upper, and underlined case letters for low, optimal and high Mn supply, respectively) are significantly different between genotypes within columns at $p < 0.05$. Significance of main and interaction effects F values: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Conclusions

In conclusion, our results showed that shoot, root growth, leaf physiological and root morphological parameters were significantly affected by different Mn rates. Both excessive and insufficient Mn concentrations resulted in reduction of shoot fresh and dry matter, branch number, total leaf area, and leaf total chlorophyll content in the two stated genotypes. Köksal cv. F1 has proven to be Mn-efficient under low Mn rates. While Adana cv. Dolmalık is responsive under high Mn rates. This further indicates that deficit and excess Mn nutrition could negatively affect the crop yield of eggplant.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

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Data availability

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Consent for publication

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Profiling of non-pathogenic bacterial population by MALDI-TOF mass spectrometry in stone fruits

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Abstract
The study was carried out to investigate the status of non-pathogenic bacteria isolated from infected plant tissues in stone fruit orchards including almonds, apricots, cherries, mahaleb, olives and plums in Adıyaman, Diyarbakır and Mardin provinces of Turkey. Surveys were performed in the mentioned provinces between March and August in 2019-2021. Survey studies showed that, 87 samples with typical bacterial disease symptoms were collected from 34 different stone fruit orchards. Hypersensitivity (HR) and host pathogenicity tests were performed following isolation from diseased plant tissues in the samples. A total of 70 isolates, which were found to be non-pathogenic with negative HR and host pathogenicity tests, were definitively diagnosed by MALDI-TOF analysis method. Finally, it was specified that bacteria of *Bacillus* and *Pseudomonas* genera were more densely colonized in different tissues of stone fruits. It was concluded that the most concentrated bacteria in the stone fruits was *Stenotrophomonas rhizophila* with 13 isolates, followed by respectively *Bacillus megaterium* with 9 isolates, *Pantoea agglomerans* with 7 isolates, *Bacillus pumilus* with 6 isolates, *Xanthomonas hortorum* with 5 isolates, *Bacillus mojavensis* and *Rahnella aquatilis* with 3 isolates

Keywords
Drupe, MALDI-TOF, Non-pathogenic bacteria, Bacteria population, Agriculture

Introduction

In many countries of the world, especially in Asian and European countries, peach (*Prunus persica* L.), nectarine (*Prunus persica* var. *nucipersica* Schneid.), cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L.), apricot (*Prunus armeniaca* L.), plum (*Prunus domestica* L.), olive (*Olea europaea* L.), almond (*Prunus amygdalus* Batsch, syn. *Prunus dulcis* (Miller) DA Webb), cranberry (*Cornus mas* L.), silverberry (*Elaeagnus*) and Mahlep (*Prunus mahaleb* L.) is an important type of stone fruit, except for a few, the others are produced economically. Turkey is the 14th country in the world stone fruit production with 1 985 394 tons on area 14 384 953 decares (da). Southeastern Anatolia Region has an important share in this position with 154 875 tons of stone fruit production in 1 091 852 da area (FAO 2020), (TÜİK 2020). Adıyaman, Diyarbakır and Mardin provinces located in the Southeastern Anatolia Region are important production centers of some stone fruit species, especially almonds and cherries. In many countries, diseases such as fungi, bacteria, viruses, viroids and plant plasmas, which limit the yield and quality of stone fruit species, have been recorded. Considering the limited control possibilities of bacteria among these, it turns out to be of great importance. Some of the bacteria infecting stone fruits can be summarized as bacterial canker and leaf blight of stone fruits (*Pseudomonas syringae* pv. *syringae* van Hall, *Pseudomonas syringae* pv. *morsprunorum* (Wormald) Young), bacterial dieback of peach (*Pseudomonas syringae* pv. *persicae*), bacterial leaf spot of stone fruits (*Xanthomonas arboricola* pv. *pruni*), crown gall (*Rhizobium radiobacter*), leaf scorch of almond (*Xylella fastidiosa*), bacterial canker of olive (*Pseudomonas savastanoi* pv. *savastanoi*), bacterial canker of almond

(*Pseudomonas amygdali*) and European yellows of peach (*Candidatus* Phytoplasma prunorum). The detection of these in stone fruits all over the world goes back to the beginning of the 20th century, but this rate is increasing numerically (Wilson 1953). Bacteria that make disease on stone fruits in Turkey are *Pseudomonas syringae* pv. *syringae* on apricot and *Pseudomonas syringae* pv. *morsprunorum* on cherry was identified about 70 years ago (Bremer 1954). However, over time, additional records were made about the presence, prevalence and damage levels of both these pathogens and new bacteria on stone fruits in different regions (Türkoğlu et al. 1974), (Karaca 1977), (Kavak and Çıtır 1995), (Ogawa et al. 1995), (Kotan et al. 2006), (Kavak and Üstün 2009), (Gormez and Sahin 2012), (Bülbül and Mirik 2014), (Mirik et al. 2016).

Plants interact with bacteria in various ways. This connection is not limited to pathogen and host interaction. There are species of endophyte bacteria that can colonize the inner plant tissue and reproduce without harming the plant, or that can reveal strong plant defense mechanisms (Reinhold-Hurek and Hurek 2011). On the other hand Epiphytic bacteria are in contact with plants in various ways such as increasing frost damage in plants, changing plant growth through exogenous phytohormone production, and being a plant disease agent. In general, non-pathogenic epiphytic bacteria that can multiply on the plant surface may not harm the plant they are on but in some cases, they can be beneficial or harmful in various ways (Kinkel et al. 2000), (Gnanamanickam and Immanuel 2007). It can be found in bacteria that colonize plant wounds and become pathogenic when the plant becomes weak, that can compete with pathogenic bacteria and suppress them or increase their activity. Bacteria colonized in plant tissues where there are symptoms of bacterial disease in stone fruits can be effective in many ways such as competition in the pathogenic bacteria-plant relationship, promoting systemic resistance. In this context it is important to diagnose and reveal their status in terms of control strategies.

There are different methods based on many basics such as protein, fatty acid and biochemical properties for the diagnosis of bacteria. MALDI-TOF MS (Matrix-assisted laser desorption ionization time of flight mass spectrometry) is a protein-based technique widely used in the diagnosis and classification of microorganisms (Ernst et al. 2015). MALDI-TOF MS allows rapid identification of bacteria by comparing mass spectra from bacteria with data from the reference library (Ahmad et al. 2012).

Contrary to the common understanding today, apart from the "pathogen x plant" interaction, the effects of microorganisms that are included in the pathosystem but are not pathogenic are evaluated. In this context, the understanding of the effect of the biotic environment is changing our perspective on struggle. In this study, it was aimed to diagnose by MALDI-TOF MS and reveal the status of non-pathogenic bacteria isolated from bacterial disease symptoms in the phyllosphere of plants in stone fruit orchards in Adıyaman, Diyarbakır and Mardin provinces.

Materials and Methods

Bacterial isolates constituting the material of the study were obtained from trees with symptoms of bacterial disease in stone fruit (almond, apricot, cherry, mahaleb, olive and plum) orchards established in Adıyaman, Diyarbakır and Mardin provinces. Samples were taken from the parts where typical symptoms of bacterial diseases such as bacterial ooze, cancer and galls were observed in different organs of the trees such as the main stem, shoot, bud and flower. Survey studies were carried out in the aforementioned provinces between in 2019-2021, during the period between flowering and harvesting of stone fruits.

Isolation method

Isolation study was applied as soon as possible to the samples which were kept in the cooling unit with ice molds and brought to the laboratory. Firstly, the samples were washed in tap water to remove dust etc. factors have been removed. Surface disinfection was done by applying ethanol (70%) to the dried samples and they were placed in a sterile laminar cabinet to dry. After drying, small pieces including healthy and diseased tissue were taken, crushed with a sterile scalpel on a sterile slide, transferred to Eppendorf tubes containing 1 mililitre (ml) of phosphate buffer solution and waited for 1 hour. Then both the plant parts in the eppendorf tube and the buffer solution containing the plant extract were inoculated into King B medium. The planted petri dishes were incubated at 25 ±2 ˚C with daily observation. Purification of bacterial cultures was performed by inoculating on the same medium from the colonies that developed during incubation (Lelliot and Stead 1987), (Popović et al. 2021).

Hypersensitivity (HR) in Tobacco and Pathogenicity Test

Hypersensitivity (HR) test was applied in order to determine whether the bacterial isolates, which were purified, were plant pathogens. The bacterial cultures to be tested were prepared as a suspension at a density of 10^8-10^9 colony forming units (cfu)/ml or at a concentration of 0.3-0.4 absorbance on spectrophotometer (Ultraviolet (UV) visible, %Transmittance: 60% at 600 nanometers (nm) wavelength). Prepared suspension was injected with a hypodermic syringe needle into the mesophyll of the leaf lamina extending along the edge of the lateral veins of the tobacco, in two replications for each isolate. For this procedure, fine needles with an outer diameter of approximately 0.6 millimeter (mm) and a volume of 2 ml were used. Sterile water was used as negative control, and *Pseudomonas syringae* pv *syringae* and *Pseudomonas syringae* pv *morsprunorum* isolates obtained from Van Yüzüncü Yıl University, which caused typical hypersensitive reaction and were molecularly diagnosed, were used as positive control. The injected leaf laminae were labeled by writing the isolate codes on the adhesive labels. The plants were incubated at 25-28 ˚C and 60-80% relative humidity in climate room conditions. In tobacco plants, a collapse and the appearance of water absorption, which was limited to the place where the inoculum was given within 24 hours, followed by dry and light brown necrosis of the tissue within 72 hours was evaluated as a positive reaction. Yellowing or browning without precipitation was not considered a positive reaction

(Klement et al. 1964).

Host pathogenicity test was applied to bacterial isolates with positive HR test and high probability of being plant pathogen. In this context, 3-year-old almond and 2-year-old apricot, cherry, mahalep, olive and plum plants grown under field conditions were used in the pathogenicity test.

For the pathogenicity test, 1-day (24 hours) fresh bacterial cultures were used, which were cultivated in King B medium. Before cutting, the one-year shoot surface was wiped with 70% ethanol and disinfected. Then with the help of a sterile scalpel, a 1 cm long and 0.5 cm deep part of the shoot was cut and the wound was opened. 1 ml of bacterial culture was taken and applied to the opened wound with a sterile toothpick (Klement et al. 1990). The cut bark part was placed on the inoculated shoot part and a sterile cotton piece moistened with sterile water was wrapped on it. The inoculated area was tightly wrapped with parafilm and labeled. At the end of the 5-6 week incubation period of the isolates, parafilm and sterile cotton were opened and the pathogen reaction was evaluated and recorded. The definitive diagnosis of the isolates with negative HR and Pathogenicity test results was made with the MALDI-TOF mass spectrometer device.

Diagnosis of Bacterial Isolates with MALDI-TOF Mass Spectrometer

Fresh colonies (24-48 hour) of pure bacterial cultures inoculated in KB medium were extracted by ethanol-formic acid method. Each bacterial isolate was taken with the aid of a sterile wooden toothpick and placed directly on the corrugated stainless steel plate and covered with 1 microliter (µl) of HCCA Matrix (α-Cyano-4-hydroxycinnamic acid) solution. Following air drying, samples were analyzed using a Bruker Ultraflex II MALDI-TOF-MS (Bruker Daltonics). The mass spectra of the samples were analyzed with Flex Control Software (Bruker Daltonics GmbH, Bremen, Germany) and their definitive diagnosis was made by matching them with the reference spectrum data in the library (BIOTYPERTM 1.1 software) (Pavlovic et al. 2012), (Kara et al. 2017).

Results and Discussion

In the study, 87 samples with typical bacterial disease symptoms were collected from 34 different stone fruit orchards in Adıyaman, Diyarbakır and Mardin. HR and host pathogenicity tests were applied to the bacterial isolates obtained as a result of isolation and purification studies from the collected samples and isolates with negative results were selected. The highest bacterial isolates were obtained from almond (39) plants and followed by apricot (12), mahaleb (9), cherry (6), olive (3) and plum (1) plants, respectively (Table 1). Although bacterial cultures were obtained mostly from shoots and main stems of stone fruit plants, samples from different plant tissues such as gall, bud, flower and leaf were also obtained albeit in small numbers. Colonization of bacteria in stone fruit plants is controlled by many factors of plant, microorganism and environmental origin. The wounds opened by harvesting, pruning etc. in the shoot and main body of stone fruits provide an opportunity for bacteria that have already adapted to the phyllosphere of the plant to enter the plant and colonize it (Manceau and Kasempour 2003). In this respect the presence of bacteria originating from shoots and main stems may be higher in stone fruits.

The definitive diagnosis of bacterial isolates determined to be non-pathogenic by HR and host pathogenicity tests was made by MALDI-TOF analysis method. As a result of the diagnosis it was determined that the majority of bacterial isolates were included in the genus *Bacillus*. A total of 27 isolates were identified from 7 different *Bacillus* species and *Bacillus megaterium* is the main isolated bacteria with 9 isolates in that genus. Aktan and Soylu (2020) obtained similar results in a study they carried out in Diyarbakır province, where the most isolated bacteria on almond trees was *Bacillus* genus.

Pseudomonas spp. with 6 different species and 9 isolates in total is following that. Although it is not a prominent species in the genus *Pseudomonas*, it is generally isolated from 3 different plants such as almond, apricot and cherry. Many bacterial species in the genus *Pseudomonas* are common in stone fruits, especially in almonds (McGarvey et al. 2014).

Following these, many different types of bacteria such as *Stenotrophomonas*, *Pantoea*, *Xanthomonas*, *Acinetobacter*, *Agromyces*, *Erwinia*, *Ochrobactrum* have been isolated. Among the 21 different bacterial species isolated in the study, *Stenotrophomonas rhizophila* was the most isolated non-pathogenic bacterium from stone fruits with a total of 13 isolates (Figure 1). Many studies have documented that bacteria of the genus *Stenotrophomonas* promote plant growth and are antagonistic to soil-borne pathogens (Berg et al. 1994), (Dunne et al. 1998), (Ryan et al. 2009). It also has plant protective properties against abiotic stress conditions (Alavi et al. 2013). At the same time, the aforementioned bacterium was detected in all of the almond, mahaleb, cherry, olive and plum plants in the study, except for apricot. Abiotic and biotic stress occurring in many tissues with typical bacterial disease symptoms such as shoots, leaves and galls in 5 different stone fruit plants creates a suitable environment for the colonization of *Stenotrophomonas rhizophila*.

Pantoea agglomerans with a total of 7 isolates, on the other hand, is the 3rd bacteria isolated from the stone fruits in the study after *Bacillus megaterium*. Six of 39 bacterial isolates isolated from almonds were diagnosed as *Pantoea agglomerans* and it comes first in the presence of non-pathogenic bacteria in the plant in question. *Pantoea agglomerans* which was isolated from almonds in the study, is present in many plants as epiphytic and endophyte. In addition to stimulating plant growth, there are species that can stimulate gall formation in plants such as gypsum and beets (Barash and Manulis-Sasson 2007). Furthermore, in a study conducted by Marchi et al. (2006); they determined that *Pantoea agglomerans* actively helped to increase the population of *Pseudomonas savastanoi*, which is the factor of bacterial canker of olive, which causes tumor formation in the olive plant in the inoculation region. But in the same study; they concluded that when the population of *Pantoea agglomerans* is high, it suppresses the presence of *Pseudomonas savastanoi* in competition for nutrients and space, possibly through antibiotic production.

Bacillus pumilus with a total of 6 isolates obtained from mahlep, olive and almond, is another bacterial that comes to the fore in stone fruits. Five *Xanthomonas hortorum* isolates were identified from the samples taken from shoot parts of almond and apricot plants. There are strains of *Xanthomonas hortorum* bacteria that cause bacterial blight and spot disease on plants such as carrots, lettuce and tomatoes. In addition, non-primary asymptomatic strains of *Xanthomonas hortorum* were isolated from plants such as peony, lavender, pot marigold and avocado (Costa et al. 2021). In this context, some bacteria such as *Xanthomonas hortorum* can be found in different hosts without showing symptoms or as a weakness parasite.

These bacterial species which are more intensively isolated are respectively followed by *Bacillus mojavensis* and *Rahnella aquatilis* with 3 isolates each, *Bacillus niacini*, *Pseudomonas graminis*, *Bacillus altitudinis*, *Bacillus vallismortis*, *Bacillus subtilis*, *Pseudomonas libanensis*, *Pseudomonas orientalis* with 2 isolates each, *Pseudomonas lutea*, *Acinetobacter lwoffii*, *Erwinia herbicola*, *Agromyces mediolanus*, *Pseudomonas aeruginosa*, *Ochrobactrum intermedium* ve *Pseudomonas cedrina* with one isolates each. The presence of bacterial in different tissues in stone fruits may differ in relation to their interactions with each other and with the plant.

Table 1. MALDI-TOF analysis results of non-pathogenic bacteria obtained from different tissues of stone fruits

No	IP	Diagnosis results	MALDI-TOF similarity index	PTI	No	IP	Diagnosis results	MALDI-TOF similarity index	PTI
1	Almond	*Pseudomonas lutea*	1.950	Shoot	36	Almond	*Bacillus megaterium*	1.922	Shoot
2	Almond	*Bacillus niacini*	1.918	Shoot	37	Almond	*Pseudomonas libanensis*	1.989	Shoot
3	Almond	*Pseudomonas graminis*	2.210	Shoot	38	Almond	*Rahnella aquatilis*	1.857	Shoot
4	Almond	*Pseudomonas graminis*	1.673	Shoot	39	Almond	*Stenotrophomonas rhizophila*	1.893	Shoot
5	Almond	*Bacillus pumilus*	2.059	Shoot	40	Apricot	*Bacillus subtilis*	1.643	Flower
6	Almond	*Bacillus niacini*	1.982	Bud	41	Apricot	*Pseudomonas aeruginosa*	1.400	Shoot
7	Almond	*Bacillus pumilus*	1.966	Bud	42	Apricot	*Ochrobactrum intermedium*	1.368	Shoot
8	Almond	*Bacillus altitudinis*	1.912	Bud	43	Apricot	*Bacillus megaterium*	1.934	Shoot
9	Almond	*Bacillus niacini*	1.485	Shoot	44	Apricot	*Xanthomonas hortorum*	2.299	Shoot
10	Almond	*Xanthomonas hortorum*	2.052	Shoot	45	Apricot	*Bacillus megaterium*	2.219	Shoot
11	Almond	*Acinetobacter lwoffii*	2.097	Shoot	46	Apricot	*Bacillus megaterium*	1.897	Shoot
12	Almond	*Erwinia herbicola*	2.190	Shoot	47	Apricot	*Bacillus megaterium*	2.001	Shoot
13	Almond	*Bacillus pumilus*	2.055	Shoot	48	Apricot	*Pantoea agglomerans*	2.169	Shoot
14	Almond	*Bacillus altitudinis*	1.970	Shoot	49	Apricot	*Bacillus vallismortis*	1.580	Shoot
15	Almond	*Xanthomonas hortorum*	1.88	Shoot	50	Apricot	*Bacillus mojavensis*	1.827	Shoot
16	Almond	*Pantoea agglomerans*	2.083	Shoot	51	Apricot	*Xanthomonas hortorum*	2.246	Shoot
17	Almond	*Pantoea agglomerans*	1.912	Shoot	52	Mahaleb	*Stenotrophomonas rhizophila*	2.063	Shoot
18	Almond	*Pantoea agglomerans*	1.930	Shoot	53	Mahaleb	*Stenotrophomonas rhizophila*	2.093	Shoot
19	Almond	*Pantoea agglomerans*	1.834	Shoot	54	Mahaleb	*Bacillus pumilus*	2.019	Shoot
20	Almond	*Xanthomonas hortorum*	2.121	Shoot	55	Mahaleb	*Stenotrophomonas rhizophila*	2.151	Shoot
21	Almond	*Agromyces mediolanus*	1.445	Shoot	56	Mahaleb	*Rahnella aquatilis*	1.906	Shoot
22	Almond	*Bacillus vallismortis*	1.566	Shoot	57	Mahaleb	*Stenotrophomonas rhizophila*	2.150	Leaf
23	Almond	*Bacillus mojavensis*	1.481	Shoot	58	Mahaleb	*Stenotrophomonas rhizophila*	2.037	Shoot
24	Almond	*Xanthomonas hortorum*	2.122	Shoot	59	Mahaleb	*Stenotrophomonas rhizophila*	2.116	Shoot
25	Almond	*Bacillus megaterium*	1.642	Shoot	60	Mahaleb	*Bacillus pumilus*	2.144	Shoot
26	Almond	*Pantoea agglomerans*	2.140	Shoot	61	Cherry	*Pseudomonas orientalis*	2.130	Shoot
27	Almond	*Pantoea agglomerans*	1.737	Shoot	62	Cherry	*Pseudomonas cedrina*	2.197	Shoot
28	Almond	*Bacillus megaterium*	1.523	Shoot	63	Cherry	*Pseudomonas orientalis*	2.053	Shoot
29	Almond	*Rahnella aquatilis*	2.000	Main stem	64	Cherry	*Stenotrophomonas rhizophila*	2.086	Shoot
30	Almond	*Bacillus megaterium*	1.729	Shoot	65	Cherry	*Stenotrophomonas rhizophila*	2.099	Shoot
31	Almond	*Bacillus mojavensis*	1.838	Shoot	66	Cherry	*Stenotrophomonas rhizophila*	2.225	Shoot
32	Almond	*Bacillus pumilus*	2.224	Shoot	67	Olive	*Stenotrophomonas rhizophila*	2.365	Gall
33	Almond	*Bacillus subtilis*	1.629	Shoot	68	Olive	*Stenotrophomonas rhizophila*	1.835	Gall
34	Almond	*Pseudomonas libanensis*	2.038	Shoot	69	Olive	*Bacillus pumilus*	1.964	Gall
35	Almond	*Bacillus megaterium*	1.925	Main stem	70	Plum	*Stenotrophomonas rhizophila*	2.017	Shoot

IP; plant from which is isolated. PTI; Plant tissue from which it is isolated

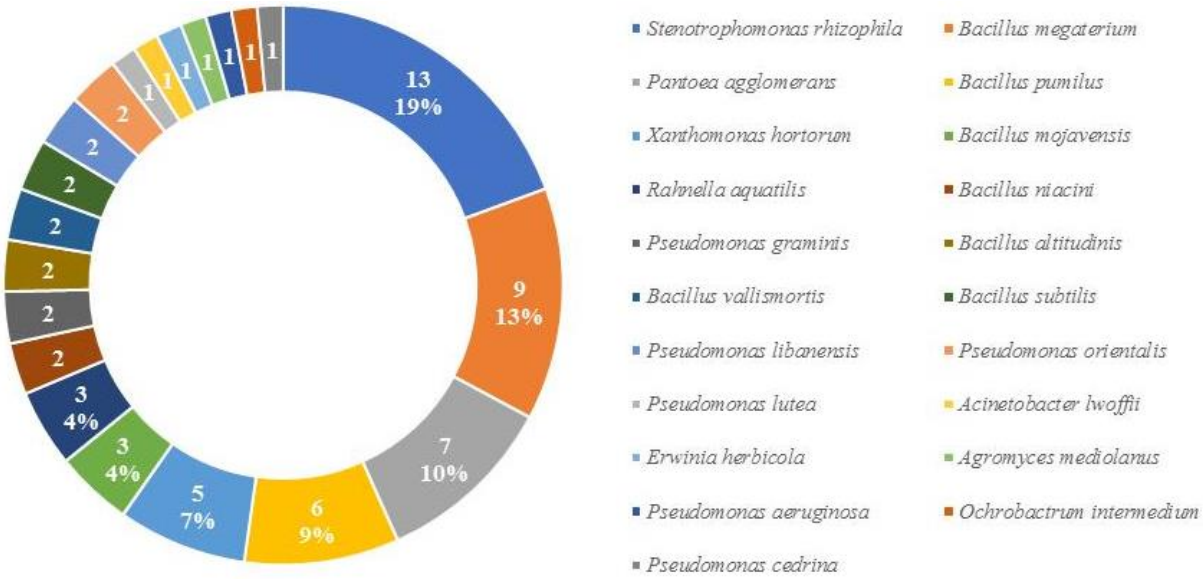

Figure 1. Number of isolates and percent distribution of non-pathogenic bacteria according to MALDI-TOF diagnostic results

Conclusion

Bacillus and *Pseudomonas* genus bacteria came to the fore among the bacteria that were isolated from different tissues of almond, apricot, cherry, mahaleb, olive and plum trees that showed symptoms of bacterial disease and were determined to be non-pathogenic. *Stenotrophomonas rhizophila* bacteria was isolated from all plants of almond, cherry, mahaleb, olive and plum, except apricot. *Stenotrophomonas rhizophila* the most frequently isolated bacteria in the study respectively followed by *Bacillus megaterium*, *Pantoea agglomerans*, *Bacillus pumilus*, *Xanthomonas hortorum*, *Bacillus mojavensis* and *Rahnella aquatilis*. It is important to determine to what extent biotic or abiotic stress conditions affect the presence of *Stenotrophomonas rhizophila* which is intensely isolated from different tissues in stone fruits. How effective the pathogen pressure is in the isolation of bacteria such as *Bacillus* and *Pseudomonas*, which have species that can be used for biological control, can be considered as another research topic. To reveal the effects of differences in plant, bacteria and environment interactions on the presence of bacteria in the plant phyllosphere will be useful to understand the effect mechanisms of bacteria such as *Stenotrophomonas rhizophila*.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Bio-ecology of *Cassida rubiginosa* fed on *Silybum marianum* and *Onopordum boissieri* in laboratory conditions

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Abstract

Cassida rubiginosa Muller (Coleoptera: Chrysomelidae) is a polyphagous pest and is commonly distributed across the world. This species is regarded as natural enemy of weeds by many researchers, while it is also pest of some cultivated plants. In this study, the biological parameters of *C. rubiginosa* fed on *S. marianum and O. boissieri* (Asteraceae) were investigated.

Average periods of egg and 5 larval stages, pre-pupa, pupa and total development of *C. rubiginosa* fed on *S. marianum* and *O. boissieri* were 6.67, 7.71; 2.04, 2.19; 2.05, 2.45; 2.01, 2.74; 2.58, 3.26; 4.41, 4.88; 1.63, 2.12; 5.64, 5.35 and 26.99, 30.35 days, respectively. There were significant differences between all biological stages, except pupa stage between *S. marianum* and *C. scolymus*. Biological parameters were calculated as Intrinsic rate of increase: 0.018, 0.017; Net reproductive rate: 214.16, 357.71 and Mean generation time: 297.41, 337.19 for the populations which used *S. marianum and O. boissieri* as the host, respectively.

Keywords

Weed, Biological control, Thistle tortoise beetle, Milk thistle, Cotton thistle

Introduction

Some species of the Chrysomelidae family are used as biological control agents agains weeds (Batra et al., 1981; Kısmalı and Madanlar, 1990; Kısmalı and Sassi, 1994; Ang et al., 1995; Kok et al., 2000; Van Driesche et al., 2002; Aslan and Özbek, 2002; Koji and Nakamura, 2006; Majka and Lesage, 2008). *Cassida rubiginosa* Muller (Thistle tortoise beetle) is a polyphagous species spreading all over the world (Zwölfer and Eichhorn, 1966; Majka and Lesage, 2008; Cripps, 2013). This species known as natural enemy of weeds also feed on weeds belonging to Asteraceae (=Compositae) family causing yield reduction in many pastures and harm to many animals feeding on these pastures (Batra et al., 1981). Weeds named *Silybum marianum* (milk thistle) and *Onopordum boissieri* (Cotton thistle), which belong to the Astracae family, cause physical damage to the animals fed in these areas with their thorny structures as well as yield losses due to the area they cover in the pasture areas (Batra et al., 1981).

Studies have also reported its feeding activity on cultivated plants (Zwölfer and Eichhorn, 1966; Kısmalı and Sassi, 1994; Jacob et al., 2006).

In this study, the effects of *Silybum marianum* and *Onopordum boissieri* used as hosts on the bio-ecology of *C. rubiginosa* were investigated.

Materials and Methods

Cassida rubiginosa Muller (Coleoptera: Chrysomelidae) individuals used in this experiment were collected from *Silybum marianum* (L.) Gaertner and *Onopordum boissieri* Willk plants in the crop production fields of Çukurova University, Agricultural Faculty in Adana, Turkey. Mating males and females of

C. rubiginosa collected separately from these plants were brought to the laboratory and placed in cages (12 × 8 × 7 cm) covered with gauze to lay eggs. Wetted sponges were placed in the cages to provide sufficient moisture. Young foliated plants about 10 cm in height were placed in the cages and their roots were continuously immersed in water.

Females transferred to a new cage with food to obtain eggs. Development time of all pre adult stages was recorded. The first stage larvae emerging from the eggs were transferred to new culture cages with a soft brush and plastic culture cage labeled by marker. The larvae were fed on fresh plants every two days until pupation. Larval stages, and pre-pupal and pupal periods of these individuals were recorded daily.

Newly hatched adult females were removed and transferred individually into another cage containing test plants. The cages were formed in two parts. Pods (10 × 20 cm) were placed on the bottom part of the cage and a plastic culture box (25 cm × 8 cm) was placed on it. The holes (10 cm x 8 cm) were opened on both sides of the plastic box and covered with gauze. Finally, these cages including adult *C. rubiginosa* individuals were placed in the field. Depending on temperature, feeding adults moved to plant roots for aestivation and wintering periods. After this period, adults came up to soil surface and started to feed on plants. When necessary, each cage was supplied with a new plant. For mating of these females, male individuals were collected from the field and placed in cages after marking of their elytra. Leaves with eggs deposited by female were removed daily from the plants and egg numbers were counted.

The pots were watered every 2 days. The period of *C. rubiginosa* on the plant roots was determined under field conditions; the other periods were determined in laboratory (25±1 °C, 65% ±5 RH and 16 hours light (4000 lux) and 8 hours dark). The experiments were checked every 8 hours (three times per day) to obtain the life table parameters

Life Tables

The life table parameters of *C. rubiginosa* on two host plants were calculated according to the Euler-Lotka equation (Birch, 1948; Kairo and Murphy, 1995; Imura, 1987; Southwood, 1978; Carey, 1993). RmStat-3 was used to calculate all parameters (Özgökçe and Karaca, 2010).

Life table statistics were calculated for the populations on different host plants. The differences of life table parameters (r_m, R_0 and T_0) were tested. For this, jack-knife method was used (Meyer et al., 1986; Sokal and Rohlf, 1995; Özgökçe and Atlıhan, 2005). The mean growth rates in each treatment were subjected to analysis of variance followed by SPSS, (ver. 17; P<0.01) and JMP (ver. 8).

The survivorship data of all individuals of *C. rubiginosa* on different host plants were calculated by using the Weibull frequency distribution

$$S_p(t) = e^{-(\frac{t}{b})^c}$$

(Deevey, 1947; Pinder et al., 1978).

Statistical analyses were done with CurveExpert pro (ver. 1.6.8), SPSS (ver. 17).

Enkegaard equation was used for the number of age-specific eggs laid by a female:

$F(x) = a.x.e^{(-b.x)}$ (Enkegaard, 1993; Hansen et al., 1999; Enkegaard and Broodsgard, 2001)

Analyses were done with CurveExpertPro (ver., 1.6.8), JMP (ver. 9).

Results and Discussion

Development time, preoviposition, oviposition and post oviposition periods of *C. rubiginosa* fed on different host plants are shown in Table 1.

There were some significant differences ($P < 0.05$) in developmental times of *C. rubiginosa* on *S. marianum and O. boissieri* for all individual life stages (Table 1).

In field studies in Erzurum Province (Turkey) by Aslan and Özbek (2002) conducted under temperatures of 20-28 °C, the egg period of *C. rubiginosa* on *Circium arvense was* 7-8 days. In the same study, larval stages and pupal period were 3-4, 5-6, 5-7, 7-8, 7-8 and 5-7 days. *C. rubiginosa* under field conditions and with two hosts (Canada thistle and Cotton thistle) had egg, 1st, 2nd, 3rd, 4th, 5th instar, pre pupa and pupal development times of 5.7, 5.9; 3.2, 3.1; 2.9, 3.1; 2.6 and 2.9; and 3.1, 3.1; 2.8, 2.7; 1.9, 1.8; 6.1, 6.5 days, respectively (Spring and Kok, 1997). Development times of *C. rubiginosa* were found very close each other in all research. The total developmental time of pre adult stage was statistically similar between the 2 hosts.

The total developmental times on *S. marianum* and *O. boissieri* were 26.99 days and 30.35 days approximately. Spring and Kok (1997) reported that the total developmental time of *C. rubiginosa* was 27.4 and 28.5 days on musk and Canada thistle under field conditions, respectively. *Cassida rubiginosa* in the laboratory at constant temperatures had development times of 20, 26, 41 and 60 days at 32.5, 26.6, 21.1 and 17.8 °C, respectively (Ward and Pienkowski, 1978). All study results for the total developmental time were very similar.

Finally, these cages including adult *C. rubiginosa* individuals were placed in the field. Depending on temperature, feeding adults moved to plant roots for aestivation and wintering periods. After this period, adults came up to soil surface and started to feed on plants. Three male released with each female for mating. Preoviposition, oviposition and postoviposition periods of *C. rubiginosa* fed on *O. boissieri* were longer than on *S. marianum.* In addition, longevity of the shield beetle fed on *S. marianum* and *O. boissieri* was 360.40 and 439.50 days, respectively (Table 1).

Kosior (1975) reported that it started to lay eggs between three and seven days after mating, depending on temperature, photoperiod, rain and wind, and the duration of oviposition was 12 weeks. *C. rubiginosa* laid from 36.0 to 61.4 eggs per individual (Koji et al., 2012)

Table 1. Mean development time (days ±SE) of *Cassida rubiginosa* fed on *Silybum marianum* and *Onopordum boissieri*

Stages	n	*Silybum marianum*	n	*Onopordum boissieri*
Eggs	30	6.67±0.09 b*	30	7.71±0.08 a
1st Larvae	30	2.04±0.06 b	30	2.19±0.07 b
2nd Larvae	30	2.05±0.08 b	30	2.45±0.13 a
3rd Larvae	29	2.01±0.06 b	30	2.74±0.17 a
4th Larvae	28	2.58±0.06 a	27	3.26±0.09 a
5th Larvae	28	4.41±0.07 b	25	4.88±0.14 a
Prepupae	28	1.63±0.07 b	25	2.12±0.08 a
Pupae	28	5.64±0.20 a	23	5.35±0.09 a
Total development times	28	26.99±0.22 a	23	30.35±0.27 a
Preoviposition	12	246.42 ± 2.59 b	16	285.19 ± 1.24 a
Oviposition	12	58.08 ± 4.71 b	16	67.69 ± 6.80 a
Postoviposition	12	28.92 ± 4.69 b	16	56.38 ± 10.07 a
Longevity	12	360.40	16	439.50

*Means in the same row followed by a different letter are significantly different (Tukey-Kramer HSD test, P ≤ 0.05).

Life table parameters of *C. rubiginosa* fed on *S. marianum* and *O. boissieri* were calculated with RmStat3 (Özgökçe and Karaca, 2010) and are presented in Table 2.

(r_m), which is a basic parameter for an insect population (Birch, 1948), was significantly different for the two host plants. Other two ecological parameters affected r_m; R_o and T_o of *C. rubiginosa* fed on S. *marianum, O. boissieri* and *C. scolymus* were also significantly different (Table 2).

Table 2. Life table parameters of *Cassida rubiginosa* on *Silybum marianum, Onopordum boissieri* and *Cynara scolymus*

Parameters	*Silybum marianum*	*Onopordum boissieri*
Intrinsic rate of increase, r_m	0,01804±0.0000262 a*	0,01743±0.0000153 b
Net reproductive rate, R_o	214,161±1.413 b	357,708±1.935 a
Mean generation time, T_o	297,406±0.354 b	337,198±0.110 a
Gross reproduction rate, ***GRR***	225,545	383,394
Doubling time, T_2	38,412	39,752
Finite rate of increase, λ	1,0182	1,0176

*Within the first three rows, means with different letters are significantly different (Tukey)

Net reproductive rate (R_0) of *C. rubiginosa,* fed on *O. boissieri* was greater than that fed on *S. marianum*. In contrast, intrinsic rate of increase (r_m) was greater when it was fed on S. *marianum*. Because mean generation time of *C. rubiginosa* was shorter on *S. marianum.*

Indeed, Levontin (1965) stated that r_m depended on the beginning of reproduction time and distribution of the age specific eggs rather than the number of eggs.

The survivorship curve (l_x) and age-specific fecundity rate (m_x) of *S. marianum* and *O. boissieri* are shown in Figure 1. The survivorship curve showed that the mortality rates of *C. rubiginosa* fed on *S. marianum* and *O. boissieri* were zero up to 307th and 331th day, respectively, and then the survival rate started to decrease dramatically, reaching zero by the 406 th and 502nd days, respectively. Similarly, age specific fecundity rates (r_m) of *C. rubiginosa* fed on *S. marianum and O. boissieri* started to increase by the 248 th and 308 th days respectively, and peaked at the 295 th and 321 th days, respectively, and declined in a gradually decreasing trend (Figure 1).

Weibull frequency distribution and the curve shape parameters were shown on Figure 2 and Table 3. Survivorship data can be effectively summarized by using the Weibull frequency distribution (Pinder et al., 1978). In two host plants it is a type I survival curve, as parameter c, is >1. Depend on this results, populations of *C. rubiginosa* fed on *S. marianum* and *O. boissieri* had an increased population.

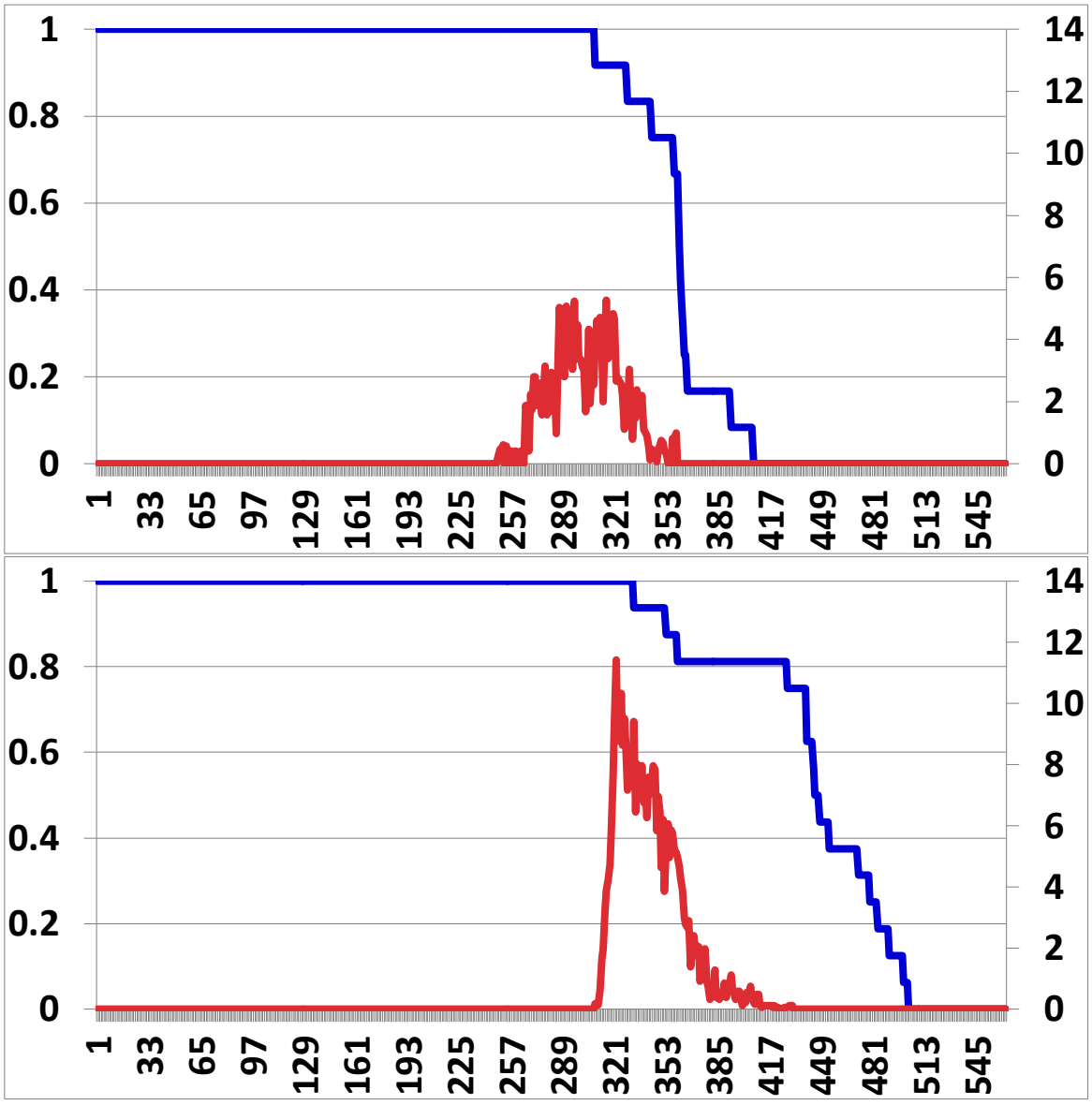

Figure 1. Survival rate (l_x) and fecundity (m_x) of *Cassida rubiginosa* on *Silybum marianum* and *Onopordum boissieri*.

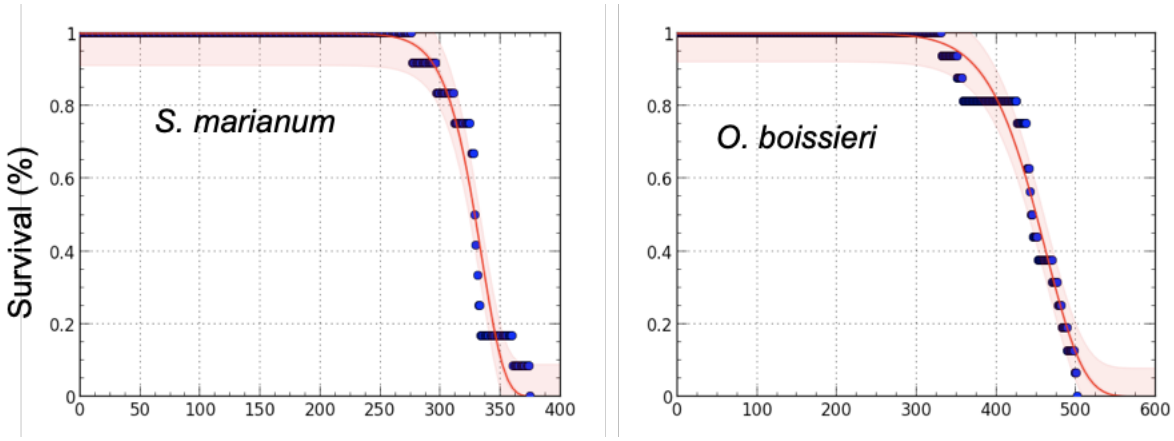

Figure 2. Weibull distribution fitted to survivorship data of *C. rubiginosa* fed on *S. marianum* and *Onopordum boissieri*

Table 3. Weibull parameters for survival curves of *Cassida rubiginosa* fed on *Silybum marianum, Onopordum boissieri* and *Cynara scolymus* (mean ±SE)

Parameters	*Silybum marianum*	*Onopordum boissieri*
b	335.00±0.40	465.67±0.59
c	15.59±0.50	10.66±0.18
Type	1	1
R^2	0.97	0.97

The relationship between two host plants regarding fecundity of *C. rubiginosa* was determined via the Enkegaard equation (Figure 3).

The relationship between days and fecundity was well described by the using the model (for *S. marianum* and *O. boissier;* R^2=0.41, a=0.18, b=0.02 and R^2=0.91, a=1.05, b=0.05 respectively). Most of the eggs were laid within the first half of the oviposition period on *O. boissieri* but on *S. marianum* egg laying was spread over the oviposition period (Figure 3).

Besides reports on biological control of weeds with *C. rubiginosa* (Batra et al., 1981; Tipping, 1993; Spring and Kok, 1997; Aslan and Özbek, 2002; Chaboo, 2007; Cripps, 2013), there have been studies of feeding activities on the cultivated plant *Cynara* discussed used in the current study (Zwölfer and Eichhorn, 1966; Kısmalı and Sassi, 1994; Jacob et al., 2006). Furthermore, Kısmalı and Sassi (1994) also observed feeding activity of *C. rubiginosa* on sugar beet (*Beta vulgaris*).

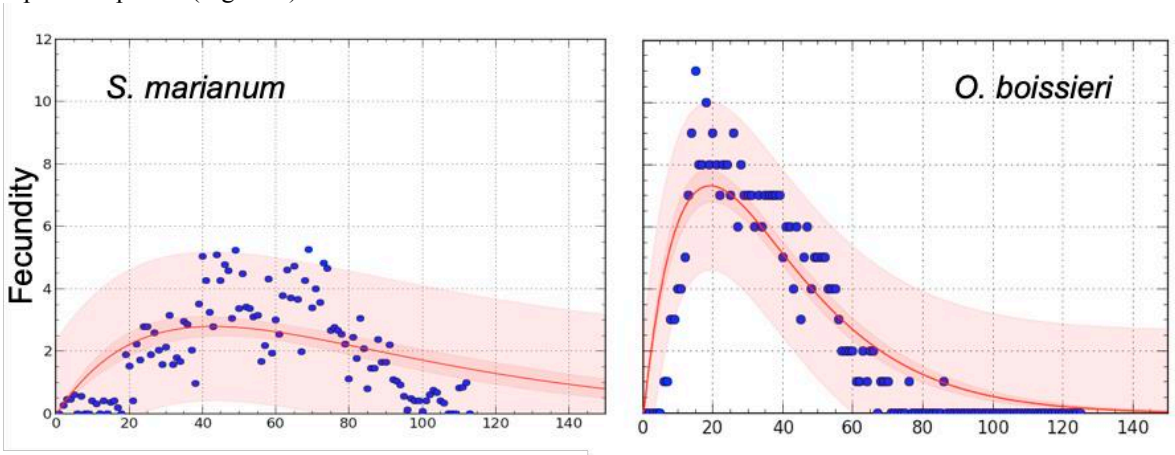

Figure 3. Survival rate (l_x) and fecundity (m_x) of *Cassida rubiginosa* on *Silybum marianum* and *Onopordum boissieri*.

Conclusion

As seen in the present study, *C. rubiginosa* fed on the important meadow pests, *S. marianum, O. boissieri.* Insect development and reproduction were similar on the two plants. This insect can be use as a biological control agent for host weeds in grassland areas but it should also be considered that this insect can cause significant looses in agricultural areas, especially in globe artichoke and sugar beet areas.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Farmers' attitude towards cooperative participation in irrigated vegetables production in Gombe State, Nigeria

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Abstract

The study analyzed the attitudes of farmers towards participation in irrigated vegetable production in Gombe state. Multi-stage sampling technique was used to select 180 farmers from a total population of 904 irrigated vegetable farmers in the study area. The data collected was analyzed using descriptive statistics. The result on the socio-economic characteristics revealed that 42.2% of the respondents were within the age group of 30-39 years. Majority 88.9% of the respondents were male and 60.5% of them had no formal education. Most 91.7% of the respondents were married and 35% of them had 9-15 family members. About 52.8% of the respondents earned between ₦ 101,000 - ₦150,000 as their estimated annual income. Majority 62.2% of the respondents had their farm size between 0.5 -1.4 hectares of land and about 87.2% of the respondents had between 1-9 years of experience in irrigated vegetable production. Majority 66.7% of the respondents belongs to the farmers' association. The mean scores of the attitudinal statements are; cooperative participation in irrigated vegetables production help work done faster (4.75), leads to effective decision (4.78), serve as a basis for getting governmental aids (4.11), yield higher profit (4.65), leads to food security (4.72), enhances access to extension services (4.52), facilitates adoption of innovation (4.52), leads to capacity building (4.67), increase investment (4.68), enhances access to market (4.48), and lastly it brings about agricultural development (M=4.62). The study recommended that vegetable farmers should be encouraged to join and revive the existing cooperative societies for them to raise enough capital for their production activities. Government should make available farm inputs, irrigation facilities and construction of boreholes and tube wells to the farmers at subsidized rates. Farmers need to be educated on their production activities and increase in extension agent-farmer contact in order to facilitate the adoption of improved vegetable production technology.

Keywords

Farmers, Attitude, Irrigated vegetable, Cooperative participation, Gombe state

Introduction

Vegetable is used to describe the tender edible shoot, leaves, fruits and root of plants and spices that are consumed whole or in part, raw or cooked as a supplement to starchy food and meat. They are widely cultivated in most parts of sub Sahara Africa, as a cheap and reliable source of protein, vitamins and minerals constituting between 30% and 50% of iron and vitamin A in resource poor diet. FAO (2011) defines community participation as a process of equitable and active involvement of all stake holders in the formulation of development policies and strategies and in analysis, planning, implementation, monitoring and evaluation of development activities. To allow for a more equitable development process, disadvantaged stakeholders need to be empowered so as to increase their level of knowledge, influence and control over their own livelihoods, including development initiatives affecting them. Furthermore,

Leafy vegetables are an important feature of Nigeria's diet that a traditional meal without it is assumed to be incomplete. In developing countries, the consumption of vegetables is generally lower than the FAO recommendation of 75kg per year or 206g per day per capita (Badmus and Yekini, 2011).

Agricultural co-operatives are agricultural-producer-owned coops whose primary purpose is increase member producers' production and incomes by helping better link with finance, agricultural inputs, information, and output markets.

The purpose of agricultural cooperatives is to help farmers increase their yields and incomes by pooling their resources to support collective service provisions and economic empowerment. Given their primary remit to contribute to smallholder farmer production,

According to International Cooperative Alliance, in Nwankwo (2008), cooperative is an autonomous association of persons united voluntarily to meet their common economic, social and cultural needs and aspirations through a jointly owned and democratically controlled enterprise. Nwankwo (2008) further stated that a cooperative is an independent enterprise, promoted, owned and controlled by members to meet their needs. As an enterprise, cooperatives are active in markets locally, nationally and worldwide Hogeland (2002). At the introduction of formal cooperative in Nigeria over seven decades ago, cooperative was used as a platform for improving agricultural production and farmers' income. The cooperative according to him is one of the most effective vehicles for organizing modernized rural production, which has become one of the most important preconditions for efficient mobilization of production resources and accelerated rural progress. Merrett and Walzer (2001) stated that the original impetus for the introduction of cooperative was in agriculture more precisely the marketing of agricultural products to help fetch better prices and income for cocoa farmers in the Western part of Nigeria. In Nigeria, vegetables production has been ongoing for decades, providing employment and income for the increasing population especially during the long dry season. However, the production is constrained by inadequate infrastructure, agronomic and socio economic variables (Sabo and Zira, 2008). Farmer's participation is an important factor to sustainable agriculture in rural areas through local, state, federal government and donor sponsored programmes.

Gombe state is one of the states where vegetable production is highly practiced. Production of vegetables is largely carried out during dry season under irrigation, although it is also grown under rain fed agriculture. The Gongola river which provides the main drainage system and Benue river basin that cuts across the state and the presence of the 3 dams in the state namely; Dadin kowa, Balanga and Cham dams serves as the potentials for dry season farming of vegetables crops like tomatoes, pepper, water melon, onion, okra and leafy vegetables. Dry season vegetable production has been ongoing in the state for decades providing employment and income for the people in the state.

It has been observed that despite of abundant of natural, physical and human resources that Nigeria is endowed with, there is still high incidence rate of poverty in Nigeria especially in the rural areas. In Gombe state, majority of the people in the state (60%) engaged in agriculture (GSADP, 2013). Year round cultivation is possible in some parts of the state due to favorable weather and extensive irrigation programme. Gombe State is known for production of vegetables like tomatoes, pepper, onions, okra, pumpkin and melon but their production were mainly at subsistence level. Apart from few non-governmental organizations (NGOs) who engaged in capacity building of farmers in irrigated farming through provision of wells, boreholes, improved varieties etc. Several researchers have reported that there is low level of community involvement on irrigated vegetables production in the area (Dauda, Asinbe, Akinbade and Salaha, 2009). Irrigation farming, though high-yielding it is capital intensive, hence the need for community involvement so as to enjoy government support. Also, the scanty work on the determinants of farmer's participation in the area necessitates the conduct of this research. This work is therefore aimed at investigating the farmers attitude towards cooperative participation in irrigated vegetables production in the State.

Materials and Methods

The study was conducted in Gombe State, Nigeria. Gombe State lies between Latitudes 10^0 16' and 11^0 00' North of the equator and Longitude $11^0$00' E and $11^0$11' E of the Greenwich meridian distance above sea level within the Sudan savannah ecological zone of Nigeria. It shares boundary with Bauchi in the west, Taraba and Adamawa in the south west, Borno in the east and Yobe to the North.

The State covers a land area of 158,998,569 m^2 with a population of 2,365,040 inhabitants based on 2006 National Population Commission (NPC) census with a 2.8 percent annual growth rate. Gombe State is divided into eleven (11) Local Government Areas. The climatic condition of the state is characterized by two distinct season's dry and wet season. The wet season begins from April and ends in October, while, the dry season starts in November and lasts up to March. The annual rainfall ranges from 600mm to 1200mm, with the minimum and maximum temperatures of 22.7^0C and 33.5^0C. (Gombe State Economic Empowerment and Development Strategies, GOSEEDS, 2006).

The vegetation of Gombe State is described as open savannah woodland with trees of up to six meters high or more. The trees normally occurred singly or in cluster, while space in between is occupied by herb layers of non- woody species up to three meters high (GOSEEDS, 2006). The State is a multi-ethnic predominantly occupied by the Fulani, Hausa, Tera and host of others all juxtaposed together.

Gombe is an agrarian State with most of the 20,266 km^2 land mass is cultivable. About 60% of the population is engage in agriculture. Year round cultivation is possible in some parts of the state due to favourable weather and an extensive irrigation programme. There are 3 dams in the state, namely, Dadinkowa, Balanga and Cham with a capacity of about 1.85 million cubic metres of water. These dams would significantly enhance the total land area available for irrigation and increase the tonnage of fish that is harvested from the permanent bodies of water in the state.

Dadin-kowa Dam which is the biggest of the three existing dams irrigates about 6,200 hectares of farm land yearly and can support the production of more than 30,000 tonnes of grains including rice, maize and groundnuts as well as with output of 50,000 metric

tonnes per annum (GSADP, 2013). The major income activities of the people are agriculture (farming and livestock rearing), trading and public service. Major irrigated vegetable crops grown in the state includes onion, spinach, amaranths, okra, tomatoes, pepper, onions, pumpkin, cabbage, lettuce, water melon and sweet melon etc. (GOSEEDS, 2006).

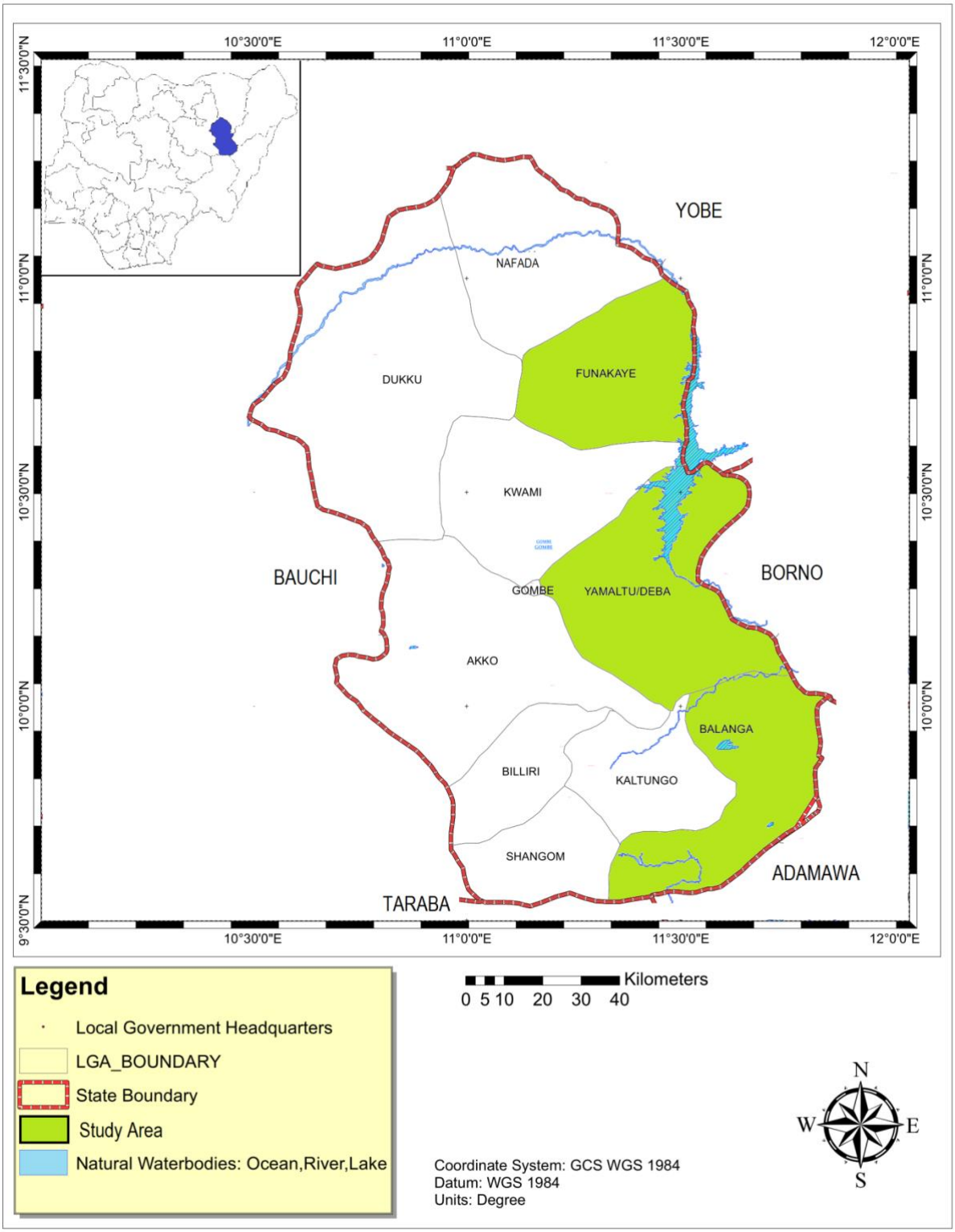

Figure 1. Ressearch area map.

A multi-stage sampling technique was employed. The first stage was purposive selection of three (3) local governments from the state based on their participation in production of irrigated vegetables namely; Yamaltu-Deba, Balanga and Funakaye. The second stage involved purposive selection of three (3) villages based on types of irrigated vegetables production from the three (3) LGAs making a total of nine (9) villages (Table 1).

The third stage was random selection of 20 % of the respondents across each villages based on the list of farmers provided by the Gombe State Agricultural Development Programme (GSADP) to make a total sample of one hundred and eighty (180) farmers.

Table 1. Vegetable Irrigation Farmers in the Study Area

Local Govt. Areas	Villages	Population	Number of farmers to be selected (Sample)
Balanga LGA	Mai Dara	102	20
	Daban Magariya	98	20
	Dasa Mai Buzu	87	17
Yamaltu –Deba LGA	Dadin kowa	155	31
	Kwadon	107	21
	Dumbu	82	16
Funakaye LGA	Ashaka Gari	96	19
	Juggol Borkono	93	19
	Gwangila	84	17
Grand Total		**904**	**180**

Source**:** Field survey, 2017

Method of Data Collection

Data for this study were derived mainly from primary source, with the use of a well structured questionnaire. The questionnaire was used to collect primary data on the socio-economic characteristics of the respondents in the study area. The data for the study was collected through a field survey with the help of Agricultural extension officer and farmers' leaders.

The analytical tools that were used in this study were descriptive statistics, where mean, frequency and percentages was used to analyze the socio-economic variables of the respondents. Five points likert scale was used to determine attitudes of farmers towards cooperative participation.

Results and Discussions

The mean age of the respondents was approximately 40 years. In general the result implies that there is presence of young and middle aged individuals known to be expected to participate more actively in irrigated vegetable production in the study area. The dominance of young persons among the respondents might be due to the fact that irrigation requires a lot of management practices and high labour. The result also indicates an availability of labor required for the farm activities. These results compared favorably with the findings of Taher and Gholam, (2008) who noted that the mean age 41 years of respondents in farmer participation in irrigation management in Doroodzan Dam irrigation Network in Iran.

Furthermore, 88% of the respondents were male and 11% female (Table 2). The result revealed that vegetable production in the study area was dominated by males. This implies that men are more actively involved in irrigated vegetables production than their female counterpart. This could be attributed to the fact that irrigation farming requires rigorous labor which male farmers can provide easily than their female counterpart. The dominance of male farmers in vegetables production in the study area may also be due to upper hand enjoyed by male farmers in terms of accessibility to farm land and production inputs. These results agree with the findings of Shettima et al (2016) who noted that 99% of the respondents in economic efficiency of irrigated vegetables production in Borno State Nigeria were males.

Also 60.5% of the respondents had no formal education, 16.7% of the respondents had primary school education as their highest level of education, 13.9% of them had secondary school education, while, and only 8.9% of the respondents had tertiary education as indicated on Table 2. The result revealed that there is low level of formal education among irrigated vegetables farmers in Gombe state. It is generally agreed that education is a factor that could enable a person to think properly and make reasonable decisions. Mazza et al (2012) were of the view that education enhances communication among farmers and participation in fadama II project. These result agree with the findings of Musa *et al.* (2013) who noted that 53% of farmers had no formal education in economic analysis of crop production under jibya irrigation project Katsina state Nigeria.

Table 2 shows that about 42% of the respondents were in the age group ranging between 30 and 39 years,31% between 40-49 years.11% between 50-59 years.while,4.5% 60 years and above.

Table 2 reveals that majority (91.7%) of the respondents were married, 4% were single, and 2% were divorced, whereas, only1.7% were widowed. The findings imply that there may be abundance of labor supply in irrigated vegetables production in the study areas since most of the people of the area were married and they are likely to have children. This is similar to the finding of Mazza et al (2012) which revealed that 96.5% of the farmers they sampled in Imo state were married. Okafor et al (1994) stated that marital status influences the size of the family and rate of population growth. This in turn, determines the number of hands to help in farm work.

About 35% of the respondents had between 9-10 family members. Whereas 34.4% had between 16-22 family members 8.9% had 23-29 family members. While, 6.1% had 30 family members and above. The mean household size of the respondents was approximately 15 people (Table 2). The study reveal that majority (35%) had 9-15 as members of their families which in turn means the larger the size of family of a farming community, the greater the availability of hands to participate in irrigated vegetables production in the study area.

Majority (68.3%) of the respondents owned their farms, whereas (12.2%) of the respondents acquired farms by renting before they cultivate. While, 19.4% of the respondents combined both their own farms and renting for their farming activities (Table 2). The study revealed that farmers in the study area have enough land for their farming activities.

Table 2. Distribution of the Respondents based on Socio-Economic Characteristics

Variable	Frequency	Percentage (%)	Mean
Age (Years)			
20-29	20	11.11	
30-39	76	42.2	
40-49	56	31.1	40
50-59	20	11.1	
60-69	5	2.8	
70 and above	3	1.7	
Sex			
Male	160	88.9	
Female	20	11.1	
Level of Education			
No formal	109	60.5	
Primary	30	16.7	
Secondary	25	13.9	
Tertiary	16	8.9	
Marital Status			
Single	8	4.4	
Married	165	91.7	
Widow	3	1.7	
Divorce	4	2.2	
Household size			
2 – 8	28	15.6	
9 – 15	63	35	
16 – 22	62	34.4	15
23 – 29	16	8.9	
30 and above	11	6.1	

Table 2. Distribution of the Respondents based on Socio-Economic Characteristics (Cont.)

Variable	Frequency	Percentage(%)	Mean
Land Ownership			
Own	123	68.3	
Rent	22	12.2	
Own and Rent	35	19.4	40
Income (₦)			
10,000 - 50,000	32	17.8	
51,000 – 100,000	31	17.2	
101,000 – 150,000	95	52.8	N102,638.67
151,000 – 200,000	15	8.3	
201,000 and above	7	3.9	
Farm size (Ha)			
0.5 - 1.4	112	62.2	
1.5 - 2.4	59	32.8	
2.5 and above	9	5	
Farming Experience (Years)			
1-9	157	87.2	
10-18	9	5	
19-27	8	4.4	
28-36	3	1.7	7
37 and above	3	1.7	
Extension Contact (Season)			
None	66	36.7	
Once	106	58.9	
Twice	3	1.7	
Trice	2	1.1	
Four times	3	1.7	
Association Membership			
Not belong	60	33.3	
Belong	120	66.7	
Source of water			
On-farm bore hole	147	81.7	
Off-farm bore hole	18	10	
Dam	13	7.2	
Stream	2	1.1	

About 52.8% of the respondents earned from 101,000 -150,000 naira per annum as an annual farm income. While, 17.8% earned from10, 000 – 50,000 naira, 17.2% earned from 51,000 – 100,000 naira and 8.3% earned from151, 000 - 200,000 naira.3.9% earned 201,000 naira and above (Table 2). The mean annual income was approximately 102,638.67 naira. The result is similar to the finding of Dauda et al. (2009) reported

the average net income of 109,750 naira earned by farmers per annum in their study of an assessment of the roles of irrigation farming in the millennium development goals in Oyo and Ogun state, Nigeria.

Majority 62.2% of the respondents had a total farm size ranging from 0.5- 1.4 hectares of land, 32.8% of them had a total farm size ranging from 1.5 – 2.4 hectares of land, and 5% had a total farm size of 2.5-3.4 hectares of land (Table 2). The mean farm size was approximately 1.37 hectares. The study revealed that there was a considerable little problem of land in the study area. The result is similar to finding of Musa et al (2013) which revealed that 42% of the farmers had a total farm size of 0.56 - 1.6 hectares of land in economic analysis of crop production under Jibiya irrigation project in Katsina state, Nigeria.

Table 2 shows that 87.2% of the respondents were found to posses between 1-9 years of experience in irrigated vegetables production. and 5% had 10-18 years of experience,and4.4% of the respondents had 19-27 years of experience in irrigated vegetables production.while,3.45% had 28 years and above. The mean year of experience was approximately 7 years. The study revealed that there is less number of years of experience in irrigated vegetables production in the study area.

About 58.9% of the respondents indicated that they received extension visit once in a season.36.7% of them did not received any extension visit. While, 1.7% of the respondents received extension visit twice in a season and 2.8% received extension three times and above in a season (Table 2). The study revealed that the rate of visit of extension agents in the study area is very low. And it also indicated that farmers in the study area will lack some vital information with regard to their farming activities.

About 66.7% of the respondents were members of farmers' associations/ cooperative societies. 33.3% of them were not members of any farmers associations /cooperative society as shown on Table 2. The result revealed that majority of the respondents was members of farmers associations/ cooperative society. This implies that the respondents may enjoy the benefits derived from being a member of associations, cooperative such as loan, information, inputs among others, which will enables them to participate actively in irrigated vegetables production in the study area.

Majority 81.7% of the respondents sourced their irrigation water from on-farm bore hole. And 10% of them sourced their irrigation water from off-farm borehole, while, 7.2% of them sourced their irrigation water from dam and 1.1% sourced water from stream (Table 2). The study revealed that water for irrigation in the study area is not a problem and it also indicated that they have the opportunity to participate actively in irrigated vegetables production in the study area.

Table 3. Attitude of Farmers towards Cooperative Participation in Irrigated Vegetables Production in Gombe State

Variables	(SA)	(A)	(U)	(D)	(SD)	Total	Mean	S.D
Help the work done faster	151	18	9	-	2	856	4.75	0.64
Leads to effective decisions.	154	16	9	-	1	862	4.78	0.57
Serves as a basis for getting governmental aids and interventions.	114	23	9	17	17	740	4.11	1.38
Yields to higher profits from the investment.	147	16	9	4	13	838	4.65	0.85
Enhances household food security.	150	17	9	2	12	851	4.72	0.70
Enhances access to extension services.	137	20	9	9	5	815	4.52	0.99
Enhances adoption of improved production technologies.	138	18	11	6	7	814	4.52	1.02
Enhances capacity building.	145	19	10	4	2	841	4.67	0.77
Improves investment in agriculture.	146	18	12	1	3	843	4.68	0.76
Enhances access to market.	125	32	12	8	3	808	4.48	0.92
Enhances agricultural sustainability and development	140	22	12	3	3	833	4.62	0.81

NB: Strongly Agree=SA, Agree=A, Undecided= U, Disagree=D and Strongly Disagree=SD

Attitude of Farmers towards Cooperative Participation in Irrigated Vegetables Production in Gombe State

Attitude toward cooperative participation in irrigated vegetables production was measured using a five point likert- type attitude scale all items means are above the median score of 3 as shown on Table 3.These findings shows that farmers generally had positive attitude toward cooperative participation in irrigated vegetables production. The mean scores of the attitudinal statements are cooperative participation in irrigated vegetables production help work done faster (4.75), leads to effective decision (4.78), serve as a basis for getting governmental aids ((4.11), yield higher profit(4.65), leads to food security (4.72), enhances access to extension services(4.52), facilitates adoption of innovation (4.52), leads to capacity building(4.67), increase investment(4.68), enhances access to market(4.48), and lastly it bring about agricultural development(4.62). The findings agreed with those of Ozowa (1995) and Bothoko and Oladele (2013) who found that access to extension services, capacity building, agricultural development and access to land improve farmers' participation in agricultural projects.

Conclusion

Based on the findings of this study it can be concluded that armers have positive attitude towards cooperative participation in irrigated vegetable production in the study area.

Recommendations

The following recommendations were made:

i. Provision of Adult literacy programme to farmers in order for them to acquire basic skills and knowledge for better decisions and improved irrigated vegetables production and marketing.

ii. Extension agents should increase the number of visit to farmers in order to facilitate the adoption of improved agricultural technology.

iii. Government should provide necessary facilities such as irrigation facility, agrochemicals etc. to further enhance farmers' participation in irrigated vegetable production.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Ethics committee approval is not required.

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Utilization of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] on plant growth and nutrient uptake of garden cress (*Lepidium sativum* L.) grown in different fertilizer applications

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Abstract

This study was employed as a pot experiment in the controlled greenhouse conditions in order to investigate the effect of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] application on plant growth and nutrient uptake of garden cress (*Lepidium sativum* L.) grown in different doses of mineral fertilizer applications. Sieved soil in 3-liter pots was used as the growing medium. Equal amount of irrigation was applied to all pots during the period from seed sowing to the end of the experiment. Microalgae application was applied twice (100 ml and 150 ml per pot) to the seedling-growing medium. As chemical fertilizer, 0%, 50% and 100% of NPK (160 mg N kg^{-1}, 80 mg P_2O_5 kg^{-1}, and 100 mg K_2O kg^{-1}) were applied. As the parameters in garden cress, shoot height, shoot fresh weight, total soluble content (TSS), chlorophyll amount (SPAD value) and some nutrients element (K, Ca, Fe, Zn, Cu and Mn) contents were examined. At the end of the study, microalgae applications were found to have a promising effect on plant growth and some nutrient uptake. It was observed that the values of the studied traits generally increased in the microalgae application compared to the control group.

Keywords

Bio-fertilizer, Garden cress, Microalgae, Nutrient content, Plant growth

Introduction

In response to the constantly increasing population in the world, the need for food is also increasing rapidly (Sencar, 1988). Due to the continuous increase in the world population, agricultural areas should also be increased, which is not so feasible. Therefore, the amount of product obtained from the unit area should be increased or the existing areas used should be sustainable (Midmore, 1993). The soil structure of Turkey is generally poor in terms of organic matter (Kaçar and İnal, 2008). It is also known that the intensive use of artificial chemical fertilizers in agricultural lands deteriorates the soil structure and pollutes the underground water resources, and the excessive use of these chemicals disrupts the ecological balance (Turan, 2007). The sustainability of the solutions to be produced in response to the problems encountered in production is an important criterion (Koru and Cirik, 1999). Moreover, it is important to use sustainable resources correctly in terms of environment and human health (Kut et al., 2007).

Garden cress (*Lepidium sativum* L.), is included in the Brassicaceae family. It is a rather fast-growing, edible herb; it is an annual herbaceous plant; it is used as an appetizer with its pleasant peppery, tangy flavor and aroma (Vural et al., 2000). It is also prized as a

medicinal herb especially for diabetes (Tahraoui et al., 2007).

Chlorella, a genus of single-celled green algae and defined as the oldest living thing known for 2.5 billion years, is an important food source. *Chlorella* is dark green. Its greenery is high in chlorophyll. It is known as green freshwater algae (Kut et al., 2007). In recent years, different microalgae have been used as plant nutrients in agricultural production (Okur et al., 2001). Today, the widespread use of microalgae fertilizers is getting prominent (Eşiyok et al., 2001). It is known that microalgae have positive effects on plant growth and development. Moreover, it increases the yield and quality of the agricultural crops and is used as a biofertilizer in agriculture (Engin et al., 2019). Schreiber et al. (2018) reported that algal biomass could also support crop growth on marginal soils.

This study aimed to investigate the effects of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] use on plant growth and nutrient uptake in garden cress (*Lepidium sativum* L.) in different doses of mineral fertilizer applications to see if it can compensate for the use of artificial fertilizers to some extent.

Materials and methods

In the present study, the effect of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] use on plant growth in garden cress [*Lepidium sativum* L. cv. Helen (Sim Arzuman Seeds)] was investigated. The study was carried out as a pot experiment in controlled greenhouse conditions (15± 4 Cº night and 28± 4 Cº day). Microalgae application was applied twice (100 ml and 150 per pot) to the seedling growing medium and as mineral fertilizer, 0%, 50% and 100% of NPK (160 mg N $kg^{-1,}$ 80 mg P_2O_5 $kg^{-1,}$ and 100 mg K_2O kg^{-1} as 20:20:20 NPK, Potassium sulfate, and urea) were applied as base fertilizer and 40 mg N kg^{-1} as urea two weeks after seedling emergence (Table 1). The soil properties used in the experiment are given in Table 1.

Sieved soil in 3-liter pots was used as the growing medium, and 40 seeds were sown in each pot. Equal amount of irrigation was applied to all pots during the period from seed sowing to the end of the experiment, is about 6.5 weeks.

Table 1. Applications and soil properties

#	Application	
1	Control (no microalgae and no fertilizer)	
2	%50 NPK fertilizer	
3	%100 NPKfertilizer	
4	Microalgae	
5	%50 NPK fertilizer + Microalgae	
6	%100 NPK fertilizer + Microalgae	
	Soil properties	
Potassium (K_2O) kg ha^{-1}	580.2	High
Phosphor (P_2O_5) kg ha^{-1}	31.2	Little
Lim (%)	11.025	Medium
Organic Matter (%)	1.98	Little
Total salt (%)	0.036	No salt
pH	7.21	Neutral

Cultivation and application of microalgae

The microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] used in the present study were obtained from Cukurova University, Faculty of Fisheries and cultured in a tissue culture laboratory at Van Yuzuncu Yil University. Bold Wynne nutrient medium ($NaNO_3$-0.250 g; $MgSO_4.7H_2O$-0.075 g; K_2HPO_4-0.075 g; KH_2PO_4-0.0175 g; NaCl-0.025 g; $CaCl_2.H_2O$-0.025 g; 1000 ml of Distilled Water) was employed in the production of bulk cultures (Duygu et al., 2017). The nutrient medium prepared in 1000 ml was equally separated into two 500 ml flasks, autoclaved at 121˚C for 20 minutes for sterilization. Microalgae were cultured with 9 ml of medium +1 ml of suspended culture. The sowing of the bulk cultures was initiated with 500 ml and then transferred to 1000 ml nutrient medium. To prevent contamination and for air circulation, the mouth of the flasks was not closed, but the sterilized cotton was placed (Ağırman, 2015). After the first two days, the cultures were aired with an aquarium pump to keep the bulk cultures in suspension. The light needs of the cultures were met artificially with a light source (150 µmol $m^{-2}.s^{-1}$) placed horizontally at a distance of 22 cm from the cultures. Cultures were treated with a 16:8 light/dark period and grown at 22-25 °C. Cell counts were performed using a Thoma slide. *Chlorella vulgaris* Beyerinck (Beijerinck) algae were applied at the rate of $2x10^7$ algae l^{-1} to the half of the application twice (at seed sowing and 20 days after as 100 ml and 150 ml, respectively).

The studied parameters are as follows:

Shoot fresh weight (g)

The shoot weight was determined with a weighing scale with a precision of 0.1 g.

Shoot height (cm)

The shoot length was measured with a ruler with a precision of 1 mm.

Total Soluble Solid Content (TSS brixº)

Garden cress samples were extracted with a blender, then TSS was determined with a hand refractometer (Atago, Tokyo, Japan).

Chlorophyll amount (SPAD value)

Chlorophyll content in garden cress leaves was determined with a SPAD meter (Minolta SPAD-502, Osaka, Japan).

Plant Nutrient analysis

The plant samples were put in an oven (65 ºC) for constant weight. The 0.5 grams of dried and grinded samples were pre-burned with 1 ml of ethyl alcohol in

crucibles and the samples were burned in a muffle furnace at 500 ºC for 9-12 hours. 4 ml of 3 N HCl was mixed with the obtained ash. The samples were left on the hot plate and kept on it until they turned yellow. When the yellow color was formed, the crucibles taken from the hot plate were transferred to the volumetric flask with the help of the filtering set and the samples were made ready for reading (Kaçar and İnal, 2008).

Statistical analysis

IBM SPSS 21.0 package program was employed for variance analysis to compare the data obtained from the average of different doses of fertilizer applications and microalgae applications. T-test was applied for the differences between microalgae applications, and Duncan multiple range test was used for fertilizer applications (Duncan, 1955).

Results and Discussion

The effect of different doses of NPK fertilizer and microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] application on the growth of garden cress (*Lepidium sativum* L.) was investigated in the present study. It has been determined that microalgae applications have a positive effect on plant growth and some mineral matter contents in general and some noticeable parameters are discussed below. The data about shoot fresh weight, shoot height, Total Soluble Solid Content (TSS) and chlorophyll amount (SPAD value) are given in Table 2; the values of some nutrients (K, Ca, Fe, Zn, Cu and Mn) were also indicated in Table 3.

NPK fertilizer application had mostly higher and more significant values than the control application on plant growth parameter of garden cress (Table 2). Microalgae application had also largely higher and significant values than the control application on plant growth parameter of garden cress (Table 2). Microalgae application caused a significant increase in the shoot fresh weight and shoot height values compared to control microalgae application. Microalgae application caused significant ($p \leq 0.01$) increases (about 129% and 36%) in the shoot fresh weight and shoot height values in garden cress, respectively (Table 2). Similarly, chlorophyll amount (SPAD value) was affected and increased significantly by NPK fertilizer ($p \leq 0.01$) and microalgae ($p \leq 0.05$) applications. Microalgae application had about 27 % more chlorophyll amount (SPAD value) than the control application. On the other hand, the increases caused by NPK fertilizer and microalgae on TSS content of garden cress were insignificant (Table 2).

Table 2. Effect of microalgae application on plant growth of garden cress grown with different doses of NPK fertilizer

	Shoot fresh weight (g pot^{-1})			Shoot height (cm)		
NPK Fertilizer	Microalgae -	Microalgae +	Mean	Microalgae -	Microalgae +	Mean
0% (Control)	2.21	6.13	4.17 B**	9.5	11.7	10.6 C**
50%	3.84	8.53	6.18 A	12.0	16.2	14.1 B
100%	4.39	9.23	6.81 A	13.0	18.2	15.6 A
Mean	3.48 B**	7.96 A		11.5 B**	15.3 A	

	Chlorophyll amount (SPAD value)			Total Soluble Solid Content (TSS brix^{o})		
NPK Fertilizer	Microalgae -	Microalgae +	Mean	Microalgae -	Microalgae +	Mean
0% (Control)	28.65	39.02	33.83 C**	3.40	3.50	3.45 ^{ns}
50%	34.55	42.70	38.62 B	3.45	4.45	3.95
100%	35.32	43.55	39.43 A	4.10	4.52	4.31
Mean	32.80 B*	41.75 A		3.65 ^{ns}	4.15	

^{ns}: not significant, *: Significant at $p \leq 0.05$, **: Significant at $p \leq 0.01$

In the present study, some nutrient (K, Ca, Fe, Zn, Cu and Mn) contents of garden cress grown with different doses of NPK fertilizer and microalgae application were determined and listed in Table 3. The effect of microalgae use on nutrient content was especially positive for potassium ((K), zinc (Zn), and copper (Cu). Microalgae application caused a significant ($p \leq 0.01$) increase (about 33%) in K content of garden cress (Table 3). Similarly, microalgae application caused a significant ($p \leq 0.01$) increase (about 17%) in Zn content of the plants (Table 3). Likewise, in Cu content, there was about 24% significant ($p \leq 0.01$) increase due to microalgae application (Table 3). Moreover, fertilizer applications significantly increased the contents of K, Cu, and Zn. However, both applications caused no significant differences for calcium (Ca) and manganese (Mn) contents. Bio-fertilizers could possess a significant place in the development of plant growth, soil fertility and environmental factors in sustainable agriculture (Vernieri et al., 2006; Singh et al., 2016; Godlewska et al., 2019). It has been reported that microalgae application in agriculture might play an important role in the amelioration of nutrient content and plant growth (Jardin, 2015).

Table 3. Effect of microalgae application on some nutrient contents of garden cress grown with different doses of NPK fertilizer

	K (ppm)			Ca (ppm)		
NPK	Microalgae			Microalgae		
Fertilizer	-	+	Mean	-	+	Mean
0% (Control)	5098	6486	5792 C**	4244	4300	4277 ns
50%	5873	7491	6797 B	4325	4408	4366
100%	7407	9741	8574 A	4506	4696	4601
Mean	6023 B **	8018 A		4358 ns	4468	

	Fe (ppm)			Zn (ppm)		
NPK	Microalgae			Microalgae		
Fertilizer	-	+	Mean	-	+	Mean
0% (Control)	441.0	462.9	451.9 ns	5.56 e**	9.79 d	7.67 C*
50%	427.3	475.7	451.5	10.47 c	10.57 c	10.52 B
100%	449.8	489.6	469.7	12.66 b	13.26 a	12.96 A
Mean	439.3 ns	476.0		9.56 B**	11.20 A	

	Cu (ppm)			Mn (ppm)		
NPK	Microalgae			Microalgae		
fertilizer	-	+	Mean	-	+	Mean
0% (Control)	2.89	3.30	3.12 B**	25.29	25.66	25.47 ns
50%	3.01	3.94	3.57 AB	26.94	26.08	26.49
100%	3.21	4.26	3.66 A	25.13	24.82	24.97
Mean	3.06 B**	3.78 A		25.78 ns	25.52	

ns: not significant, *: Significant at p≤0.05, **: Significant at p≤0.01

Microalgae, as a bio-fertilizer or bio-stimulant, could especially play important role in particular infertile soils. Organic matter produced by microalgae might also enrich the soil structure. The results of Schreiber et al. (2018) studied microalgae on wheat and confirmed that algal biomass can support crop growth on marginal soils. These researchers determined that microalgae fertilization increased the shoot size to 87 and 107% of that of sand supplied with mineral fertilizer. Michalat et al. (2015) suggested that algal extracts might induce stronger seed germination and plant growth parameters in garden cress because they are rich in nutrients and polyphenols. Michalat et al. (2016) also reported that algal extracts enhanced chlorophyll and carotenoid content in the shoots, as well as root thickness and aboveground biomass in garden cress and wheat. It was also indicated that *Chlorella vulgaris* as a bio-fertilizer is effective and cost-effective in improving soil nutrients for greater yield in okra (Agwa et al., 2021). Anitha et al. (2016) studied the effect of microalgae use on the nutrient content in tomato seedlings and determined that microalgae ameliorated the zinc, potassium and calcium contents significantly. Zodape et al. (2011) also examined the effect of microalgae on tomatoes and determined that nutrient contents of the microalgae applied plants improved significantly compared to the control plants.

Similarly, Grouch et al. (1990) investigated the microalgae effect on the lettuce seedlings and reported that microalgae usually improved the yield and the contents of Ca and K. Sayed Ahmed et al. (2021) accounted that *Cholorella* might raise mineral matter content in garden rocket by upregulation of key genes in their biosynthetic pathway.

Conclusion

Parallel to the expanding populace in the world, those require to nourishment also increases. An aftereffect of expanded pressures, the nonstop-escalated utilization of artificial chemical fertilizers might cause pollution in nature and could deteriorate the ecological balance. In the face of this situation, agricultural lands would be destroyed, and therefore, the amount and quality of agricultural crops declines. With the utilization of claiming bio-fertilizers such as microalgae, the employment of artificial chemical fertilizers could be decreased. In the present study, in which the impact of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] utilization on plant growth in garden cress (*Lepidium sativum* L.) was investigated, the effect of microalgae, defined as bio-fertilizer, was determined to be by and large encouraging for plant development and nutrient uptake. It was seen that there were important ameliorations of microalgae applications in many examined traits, and microalgae might compensate for the use of artificial fertilizers to some extent. In the future, it is thought that as the number of investigations on the association of microalgae and agricultural plants increases, newer knowledge will be revealed for more sustainable agriculture.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

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Not applicable.

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Sustainable food alternative in gastronomy: edible insects (entomophagy)

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Abstract
The increase in the world population, the inefficient use of resources and the constant change in living conditions constitute the problems of our age. Besides all these, the sustainability of food resources is gaining more importance day by day due to problems such as climate changes and insufficient agricultural areas. In the future, especially meeting the protein requirement is foreseen as a fundamental problem. Edible insects are considered as sustainable food sources due to the advantages in their production when compared to traditional protein sources. It is predicted that the way of eating and drinking will change in the coming years due to problems such as population growth, climate change and food crises. A literature review was conducted in this study on gastronomy trends, etymology and historical development of entomophagy, edible insects as a sustainable food alternative, the advantages of edible insects, and insect cultivation and consumption in the world and in Turkey.

Keywords
Sustainable food, Edible Insects, Entomophagy, Gastronomy, Food trends, Nutritive value

Introduction

The inadequacy of the resources to meet the food needs of the increasing world population, the inability of the people to use food efficiently and the constant change in living conditions bring humanity face to face with some problems. The most important of these problems is the difficulties experienced in accessing food. Human nutrition has been affected by the world food crisis that emerged in 2007-2008. Due to this crisis, there was a great increase in food prices, a global crisis was experienced, and political and economic instability affected all countries negatively (Gürlük and Turan, 2008). Apart from these, the sustainability of food resources is gaining more importance day by day due to problems such as climate changes and insufficient agricultural areas. Especially meeting the protein requirement is foreseen as a fundamental problem in the future. Edible insects are considered as sustainable food sources due to the advantages in their production when compared to traditional protein sources. Edible insects, which are traditionally consumed frequently in countries such as South American countries or Asia, are not accepted in western societies in terms of causing disgust (Erdoğan, Görür, Peksever, Sümer and El, 2021).

Aim and sources

The purpose of this study is, in the future, meeting the protein requirement is foreseen as a main problem. Edible insects are considered as sustainable food sources due to the advantages in their production when compared to traditional protein sources. In the study, a literature review was conducted on etymology and historical development of entomophagy, gastronomy trends, edible insects as a sustainable food alternative. Furthermore, a review of the advantages of edible insects, and insect cultivation and consumption has been carried out.

Gastronomy trends

Gastronomy is a multi-faceted discipline that concerns nations and communities, contributes to human nutrition with raw materials, creativity, traditions, different techniques and recipes, and can develop with new technologies from the perspective of the historical development of human nutrition. Gastronomy trends are affected by many factors. Trends emerge as the types of foods, the materials used in food preparation and cooking, the way food is prepared and cooked, its service and marketing. Considering this situation, it is necessary to develop a different perspective to examine gastronomy trends. It would be appropriate to associate gastronomy trends with the type of innovation that constitutes its source with this new perspective. On the other hand, it will be possible to determine the sources of the innovation motivation of the gastronomy ecosystem by examining the gastronomic trends depending on the innovation type in question and to make a mutual comparison of the trends in these innovation areas.

Eating and drinking, which is accepted as a sociological phenomenon, has paved the way for eating out to become a culture due to reasons such as not having enough time to cook, leisure time or business trips (Yıldız and Yılmaz, 2020). This change in eating habits has revealed many different trends in the field of gastronomy. Some of these flows can be seen in Table 1. The edible insects, one of the gastronomy trends, will be examined in this study. This change in eating habits has revealed many different trends in the field of gastronomy. Some of these flows can be seen in Table 1. In the study, edible insects, one of the gastronomy trends, will be examined.

Table 1. Some gastronomy trends

1.	Entomophagy/edible insects
2.	Edible flowers
3.	Fusion cuisine
4.	Functional foods
5.	Raw food
6.	Molecular gastronomy
7.	Fast food
8.	Slow food
9.	Surf & Turf
10.	Green restaurants
11.	Vegetarian cuisine
12.	Gluten free diet
13.	Blood type diets
14.	Authentic, black, and purple foods
15.	Local cuisine
16.	Neurogastronomy
17.	Organic farming
18.	Vertical farming
19.	Cellular agriculture
20.	Clean supreme
21.	Hyper-local sourcing
22.	Silicon valley & food
23.	Fast casual, Fast casual 2.0
24.	3D printed food
25.	Theme restaurants

Source: (Kurgun, 2017; Nizamlıoğlu, 2018; Oğan, 2021)

Etymology and historical development of entomophagy

Looking at the etymology of the word "Entomophagy", which is derived from Greek, "Entomo" means insect, "Phagein" means food, and the combination of these two words means "insect eating" (Pal and Roy, 2014). The use of insects as a food source has existed for centuries. Humans have been collecting and consuming eggs, larvae, pupae, and adults of some insect species from nature for many years.

We see in cave drawings and other records of ancient civilizations that people have been consuming insects for centuries. Insects have also been used in medicine, religious rituals and for nutritional purposes in the historical process (Ramos-Elorduy, 1998). It is seen that locusts strung on skewers were served at royal banquets until the 8th century BC in the Middle East (Van Huis, Van İtterbeeck, Klunder, Mertens, Halloran, Muir, and Vantomme, 2013). Eating cicadas was considered graceful in Greece, and Ethiopians were called locust eaters by Sicilians. Cossus, the longhorn beetle, which is the larva of Cerambyx cordo living in oak trees, was considered a favorite food by the Romans. Materia Medica is a book on Chinese medicine written by Li Shizhen and contains information on the use of many insects as food (Van Huis et al., 2013).

Parallel to the increasing population in the world in recent years, insects are on the agenda as an alternative food source because the demand for animal protein has not increased enough, but the production has not increased sufficiently (Güneş, Sormaz, and Nizamlıoğlu, 2017). Along with the increasing population growth, factors such as food waste, lack of awareness about nutrition, not giving the necessary importance to agriculture and animal husbandry, domestic waste and migration also affect the nutrition model of societies. For this reason, people are trying to reach many alternative new food sources in order to access sustainable and safe foods. Insects appear as an alternative food source in this context (Becker, 2007; Post, 2012; Van Der Spiegel, Noordam and Van Der Fels-Klerx, 2013; Van Huis, 2015; Karaman, 2019).

The use of insects as food has increased especially after the 2000s and has been the subject of many fields from food festivals to international conferences, and from documentaries to film festivals (Güneş, Sormaz, and Nizamlıoğlu, 2017). In his article titled "Future foods: what will we be eating in 20 years' time?" in BBC NEWS Magazine, Winterman (2012) interpreted the characteristics of entomophagy as a kind of win-win relationship. Edible insects are considered an important trend in terms of being the food of the future as a sustainable environment and food alternative for all humanity.

Edible insects also have environmental benefits with their features such as less harmful gas emission and less land requirement and economic benefits due to their low technology requirements and low capital investment. Although insect consumption is mostly seen in Asian countries, it is thought that its consumption will increase globally with the decreasing world resources over time (Kurgun, 2017).

Insects, which offer many advantages as food, are very rich in protein, vitamins, and minerals. The use of insects as food is thought to have less negative impact on the environment. This situation creates positive psychological feelings on people as the consumption of insects as food causes less damage to the environment compared to traditional livestock farming. Insects, which are considered as food, can either be collected from their natural habitats or grown in insect farms. Employment in rural areas is increasing in both of these methods. (Hanboonsong, Jamjanya, and Durst, 2013).

Considering today's conditions, it is seen that insect consumption is widespread in many countries in the world. Insect consumption becoming more common means that many restaurants include insects on their menus, thus bringing people closer to consumption of them (Baker, Shin, and Kim, 2016). Table 2 shows the edible insect species in the world and the numbers of these insect species (Ramos-Elorduy, 2005):

Table 2. Edible insect species and numbers of these species in the world

Order	Common name	No. of species
Thysanura	Silverfish	1
Anoplura	Sucking lice	3
Ephemeroptera	Mayflies	19
Odonata	Dragonflies	29
Orthoptera	Grasshoppers, crickets, and cockroaches	267
Isoptera	Termites	61
Hemiptera	True bugs	102
Homoptera	Cicada and leafhopper, mealybugs	78
Neuroptera	Net-winged insects, dobsonflies	5
Lepidoptera	Butterflies and moths	253
Trichoptera	Caddisflies	10
Diptera	Flies and mosquitoes	34
Coleoptera	Beetles	468
Hymenoptera	Bees, ants, wasps	351
Total		1681

Source: (Ramos-Elorduy, 2005)

As seen in Table 2, Ramos and Elorduy (2005) recorded 1681 edible insect species in their study. It is seen that this number is 2111 in the 'List of edible insect species of the world' published by Jongema (2017). Figure 1 shows the registered edible insect species by country:

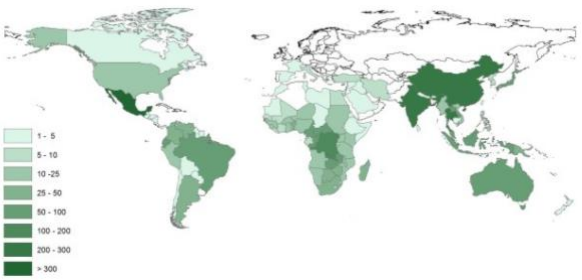

Figure 1. Registered edible insect species by country

Looking at Figure 1, it is thought that the most edible insect species are found in Asia, while there are 5-10 edible insect species in Europe and Turkey.

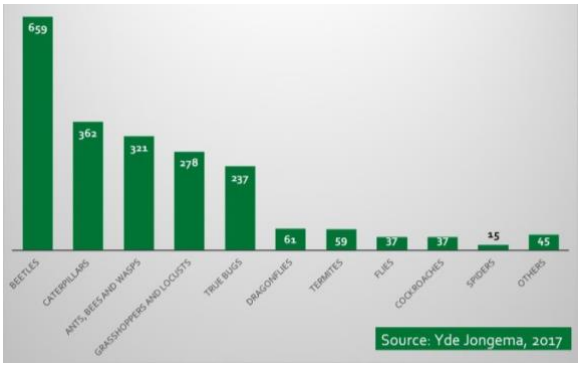

Figure 2. Grouping of edible insect species recorded in the world

When Figure 2 is examined, there are 2111 edible insect species that are most consumed and registered. The numbers of these insect species are as follows, respectively: beetles (659), caterpillars (362), ants, honeybees, and wasps (321), grasshoppers (278), hemipteras (237), dragonflies (61), termites (59), flies (37), cockroaches (37), spiders (15) and other insect species (45). Although the types of edible insects vary according to the regions, the most frequently consumed insect species are listed as mealworms, cockroaches, grasshoppers, crickets, ants, butterflies, moths, and flies. (FAO, 2013).

Edible insects as a sustainable food alternative

It is very important for sustainability that insects produce less greenhouse gases and consume less water compared to other animals. It is considered an advantage that they need smaller areas for their breeding and maintenance than another animal husbandry. Insects have both economic advantage and feed conversion efficiency by obtaining their feed from industrial by-products and agricultural residues (Baş Aksoy, and El, 2021). It is known that the feed conversion efficiency of crickets (Acheta domesticus) is 12 times higher than cattle, 4 times higher than pigs and 2 times higher than chickens (Van Huis et al., 2013). Most insects use unsuitable plant material as nutrients for agricultural by-products. Therefore, insects do not play a negative role in the consumption of food sources compared to cows, chickens and pigs that consume human food (Dossey, Tatum, and McGill, 2016; Baş Aksoy, and El, 2021). Studies have shown that while 25 kilograms of feed is spent to produce 1 kilogram of beef, 2 kilograms of feed is spent to produce 1 kilogram of edible insects (Guine, Correia, Coelh, and Costa, 2021).

While few people consumed snails, shrimp, crab, lobster, frog legs and raw fish in the early 1980s, many people today consume such products in a variety of ways. The term 'new sushi' is also used for the increasing trend towards edible insects in recent years. For example, some of the raw fish consumption patterns can be listed as follows (Uzun, 2017):

- *Sushi:* It is a traditional Japanese dish in which cooked vinegared rice and various ingredients are served on or inside raw fish such as seafood, vegetables.

- *Sashimi:* It is another Japanese dish consisting of thinly sliced raw fish or meat.

- *Poke:* It is a Hawaiian salad made with pieces of raw fish mixed with spices and vegetables.

- *Ceviche (Seviche):* It is a traditional Peruvian dish that is served cold and is marinated by chopping large fish with white meat, the bones of which can be easily removed, and by keeping them in lime or lemon juice (citric acid) for 2-3 hours in a glass bowl.

- *Gravlax:* It is a Scandinavian dish made by marinating raw salmon with sugar, salt and dill. It is consumed with mustard sauce.

- *Koi Pla:* It is a Southeast Asian dish made by mixing finely chopped fish with lime juice, fish sauce, herbs, and vegetables.

Considering the examples given above, the fact that people who used to refuse to eat raw fish consume raw fish frequently in different ways supports that this situation may one day be valid for insects as well. In his study, Shapla (2012) describes his amazement when he saw that children were playing with their friends in the garden, excitedly consuming ants and saying that it was a very tasty thing. The fact that 2 billion people around the world eat insects today shows that this situation should not be surprising.

Advantages of Edible Insects

The entomophagy movement, which is described as a strong solution for climate challenges and health, is becoming widespread day by day (Müller, Evans, Pane, and Roberts, 2016). Narzari and Sarmah (2015) emphasize that the widespread use of insects as a natural resource is necessary. It is predicted that edible insects will play an important role in tackling a possible food crisis or depletion of natural resources. Their superiority in nutrition is effective in the inclusion of edible insects in the menus and their being considered as attractive elements as food (Kurgun, 2017).

The nutritional advantages of edible insects are: they are good sources of protein, they contain more calcium than milk, they contain 20 times more B12 than steak, saturated fat ratios in insects are quite low, magnesium content of insects is 5 times higher than steak, they are rich in beneficial probiotics that are beneficial for the intestines, they contain 9 essential amino acids that provide repair and development of muscles, they do not contain GMOs, chemicals, and sugar, they are rich in chitin, which provides support in the fight against tumors, viruses, and allergies, they contain more iron than spinach and they do not include animal-borne diseases transmitted to humans.

Scientific research on the reasons for the preference of edible insects still continues today. It is seen that there is a need to develop new processing methods or take precautions in order to deepen the researches on some of these advantages mentioned above and to eliminate the problems such as animal-based diseases transmitted to humans. For this purpose, it should be carried out in a transparent manner, taking into account the guidance of the scientific research results of the necessary legal regulations within the framework of the producer, processor, and consumer. Such studies will be able to transform edible insects into a safe and natural gastronomic ecosystem food (Kurgun, 2017).

In the study titled "Edible Insects: Future Food and Feed Safety Perspectives" published by the United Nations Food and Agriculture Organization (FAO) in 2013, 3 main reasons for consuming insects are mentioned (Van Huis, Van Itterbeeck, Klunder, Mertens, Halloran, Muir, and Vantomme, 2013; Kurgun, 2017):

Health

- Insects are healthier and more nutritious alternatives to pork, beef, fish, and chicken.

- Most insects are very rich in calcium, good fats, iron, zinc, and protein.

- Insects are a traditional part of many regional and national diets.

Environment

- While growing edible insects is not a land-based activity, there is no need to expand land to expand production.

- Edible insects emit less greenhouse gases than many animals. For example, methane gas is produced by several groups of insects, such as cockroaches and termites.

- Because insects are cold-blooded, they are more successful at converting food into protein. For example, crickets need to eat 12 times less than cattle, 4 times less than sheep and half as much as pigs and chickens to produce the same amount of protein.

- Edible insects can also feed on organic waste.

- Consumption of insects as food is also a healthy and safe way to help reduce insects without the need to use pesticides (Holland, 2013).

Livelihood (Social and Economic Factors)

- Edible insect farming (mini livestock) provides a livelihood in both urban and rural areas.

- The edible insect farming business is a low-tech and capital investment option. It provides the opportunity to participate in the economy even in the poorest sections of the society.

In addition to the negative effects of land and water use, climate change and increasing world population, new strategies should be developed in malnutrition and food production. It is predicted that edible insect cultivation will contribute significantly to the solution of hunger in a sustainable environment (Nadeu, Nadeu, Franklin, and Dunkel, 2014).

Insect breeding and insect consumption in the world and in Turkey

In recent years, interest in edible insects, especially crickets, has increased considerably in Western countries. More than 30 edible insect companies were established in North America in 2012. Edible insect production and its integration into the gastronomic ecosystem have been progressing and developing effectively in recent years (Kurgun, 2017). Thailand is one of the important countries in insect farming and 7500 tons of edible insects are produced annually in 20 thousand farms (Hanboonsong, Jamjanya, and Durst, 2013; Karaman, 2019).

In order to promote insect breeding, an international project is carried out in Africa, especially on crickets. Insect farming in the West is mainly for the purpose of supplying pet food, and in some countries this situation varies. For example, special production areas for crickets, grasshoppers and mealworms have been

established by some companies in the Netherlands for human consumption (Van Huis, 2015; Karaman, 2019).

There are businesses and farms producing edible insects in many countries around the world. Some of these businesses are listed in Table 3 (Kurgun, 2017):

Table 3. Edible insect producing businesses and farms in the world

Business/farm	Country
Jimini's	France
Snack İnsect	Germany
Beetle Jelly	Belgium
Gran Mitla	Mexico
Exa Foods	Canada
Edible Bug Farm	UK
Edible Bug Shop	Australia
Entomos	Switzerland
Chapul	USA
Bugsolutely	Thailand
Primal Future	New Zealand
Haocheng	China
Insektenessen	Austria
ArhtroFood	Colombia
EntoCube	Finland
Livin Farms	Hong Kong
Big Cricket Farms	USA
Nutrinsecta	Brasil
Ento Factory	Chile
Nutrition Technologies	Vietnam
Mira Livestock Insect	Turkey

Source: Kurgun, O. A. (2017)

As can be seen in Table 3, there is a facility for insect breeding in Turkey. Although the Mira Livestock Insect Farm in Antalya currently produces insects as animal feed, they deliver the insects live to their customers when an order is placed for human consumption. Insects such as mealworms, grasshoppers and crickets are produced in this farm (Karaman, 2019). There is no detailed scientific study on insect consumption in Turkey. Considering the production amount of mollusks and crustaceans in Turkey between 1985 and 2005, the main mollusks are listed as Mediterranean mussel, octopus, sea snail, sand mussel, hairy mussel, cuttlefish, stone mussel. Crustaceans can be listed as lobster, crab, crayfish, shrimp, and some insect species. While insect production was 47 tons in 1985, it was determined that this amount decreased to 30 tons in 2005 (Başçınar, 2007; Mankan, 2017; Karaman, 2019).

Some examples of the use of edible insects in the kitchen

In Karaman's (2019) thesis study, the preparation stages of food or beverages to be made with edible insects were mentioned. It was mentioned that insects should be washed before use, and that the pheromones responsible for the taste and odor of insects will disappear due to washing. It has been said that after the washing phase, their flavor can be increased with various seasonings and spices. Insects such as grasshoppers should not be fed for 12 hours before being consumed because insects can also feed on bitter plants. Since the bitter taste passing through such plants also affects insects, it takes time for the insects to get rid of this bitterness from their digestive systems. In addition, edible insects should be kept in the freezer for 15 minutes before being prepared for consumption, and their vital functions should be expected to end. It is known that if the insects are left in the freezer for more than 15 minutes, the insects will freeze and there will be negative changes in the taste of the insects thanks to freezing. In Oaklander's (2015) article titled "20 Delicious Bug Recipes from Chefs" published in the Times, various restaurants in different states of America were researched and 20 recipes made with edible insects were exemplified. Some of these recipes can be listed as follows:

Chef Karen Barroso's Garlicky Grasshopper Mix

There are 398 different species of edible insects in Mexico. Grasshoppers or chapulins (a type of locust native to Mexico) are among the most commonly consumed insects. Chef Karen Borroso cooks the garlicky grasshopper while she prepares the chapulins by sautéing them with garlic, cloves, Chili de Arbol hot pepper oil, sea salt, and Spanish peanuts. It is then served at the bar, accompanied by Mezcal (a Mexican high-alcohol drink made from the sap of the agave plant grown in Mexico - Karen Barroso, Guajillo, Arlington, Virginia (Oaklander, 2015).

Figure 3. Chef Karen Barroso's Garlicky Grasshopper Mix

Chef Laurent Quenioux's Ant Larvae

Blini (mini pancakes) are made with ant larvae (escamole), quail eggs and salmon eggs. Later, when the eggs are frozen, the meringue is made with albumen (egg white), which flows out. They serve Mexican tea leaf (epazote), ant larva (escamole), serrano pepper and shallot on a nasturtium leaf, sautéed in butter, and served on a corn tortilla accompanied by Mexican beer and lime gel (Laurent Quenioux, Bistro LQ, Los Angeles (Oaklander, 2015).

Figure 4. Chef Laurent Quenioux's Ant Larvae

Chef Zack Lemann's Lightly Fried Dragonflies

One tablespoon each of butter, dijon mustard and soy sauce are heated in the pan for a few minutes. Sliced Portobello mushrooms and a pinch of garlic powder are sautéed in a small amount of butter in another pan. After the oil is thoroughly heated, the dragonflies are cooked for 30 seconds on one side for 30 seconds on the other side (Zack Lemann, Audubon Butterfly Garden and Insectarium, New Orleans, Louisiana (Oaklander, 2015).

Figure 5. Chef Zack Lemann's Lightly Fried Dragonflies

Chef Hugo Ortega's Mescal Worm Tacos

After frying in butter, olive oil and white onion, Mezcal worms are lightly sautéed and finally served with fresh parsley and serrano pepper. (Hugo Ortega, Hugo's, Backstreet Cafe and Caracol, Houston, Texas (Oaklander, 2015).

Figure 6. Chef Hugo Ortega's Mescal Worm Tacos

Daniella Martin's Mealworm Slaw

After the mealworms are baked in the oven until golden brown, they are salted and served over the cabbage salad. (Daniella Martin, insect-eating expert (Oaklander, 2015).

Figure 7. Daniella Martin's Mealworm Slaw

Paul Landkamer's Marinated Stink Bugs

Skunk bugs marinated for a day in cajun sauce (a kind of sauce prepared with mayonnaise, cajun seasoning, tomato paste, onion, and garlic powder) are boiled for 5 minutes. Just before draining, they are cooked in cajun sauce, dried until they reach a crispy taste and served (Oaklander, 2015).

Figure 8. Paul Landkamer's Marinated Stink Bugs

Chef Gordon's Deep-Fried Tarantulas

First, tarantulas are frozen, ending their vital functions. After removing the liquid-filled bellies of the frozen tarantulas, their body hair is burned with the help of a blowtorch. The tarantulas, which are ready to be cooked, are dipped in tempura dough, and fried in deep oil to be served (Oaklander, 2015).

Figure 9. Chef Gordon's Deep-Fried Tarantulas

Discussion and Conclusion

Sustainability of food resources is becoming more and more important every day for various reasons. Especially meeting the protein requirement is considered as one of the main problems in the future. Insect nutrition comes to the fore as an alternative food source due to reasons such as the decrease in protein sources due to population growth and the inability to reach sufficient and healthy food.

Factors such as food waste, decreased interest in agriculture and animal husbandry, unconscious nutrition, domestic waste, and migration also cause changes in nutrition patterns. Consumption of seafood is common in Turkish culinary culture, especially in coastal areas. For example, shrimp and grasshoppers are insect species that are similar to each other in terms of their skeletal structure and nutritional content. It is very important for people to be aware of insect consumption. Edible insects have benefits such as less harmful gas emissions, less land requirement, low technology, and low capital investment. Insects, which also offer many advantages as food, are very rich in vitamins, proteins, and minerals.

In this context, it is thought that the use of edible insects as a sustainable food alternative will be a useful solution for possible future problems such as famine,

crises, etc. FAO wants insect farms to be established and insect consumption to be supported to solve the food problem. However, factors such as people's dietary habits, religious beliefs, nutritional culture and not finding insects very safe in terms of hygiene will affect the edibility of insects in different ways. It is also very important to choose the right storage conditions and cooking methods for insects.

Considering the contribution of edible insects to the ecosystem, insect consumption should be planned in a way that does not disturb the ecological balance. Insects can carry biochemical, microbiological, and allergic risks and may be carriers of various diseases. Detailed scientific studies on insects are not available in the current literature. It is important to carry out the necessary scientific studies in the dissemination of insect consumption as an alternative food source.

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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Estimating crop yield under conditions of soil water deficit and salinity stress with crop water productivity model

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Abstract

The aim of this study was to simulate grain yield, biomass production, canopy cover and water productivity of winter wheat grown under soil water deficit and salinity stress by AquaCrop model. Five different irrigation strategies (S_{100} - S_{75} - S_{50} – S_{25} and S_0) and 5 different irrigation water salinity levels (T_1 = 0.3 dS m^{-1}, T_2 =5 dS m^{-1}, T_3 = 7.5 dS m^{-1}, T_4 = 10 dS m^{-1}, T_5 = 15 dS m^{-1}) were used with the model to estimate deficit irrigation and salinity stress scenarios. According to estimation of the model the grain and biomass yields were fluctuated in the range of 5.43-8.00 t ha^{-1} and 12.84-17.67 t ha^{-1} at irrigation treatments. The application of 25%, 50% and 75% level of deficit irrigation, grain yield reduction was obtained 5%, 13% and 26% respectively. It was compared to the T_1 (control) treatment, a low value of 3% was obtained for the T_2 treatment. Yield loss of T_3 and T_4 salinity treatments were found to be 19% and 43% respectively. The crop yield reduction was dramatically (86%) at 15 dS m salinity level of irrigation water. The lowest yield was obtained at all salinity levels in I_{25} treatment, where 75% water saved. The highest and lowest water productivity was 1.28 kg m^{-3} and 1.20 kg m^{-3} respectively. It is possible to irrigate much more areas saving water with deficit irrigation and also the yields obtained from these areas were 2.17, 6.17 and 17.2 tons more than the yields obtained from areas irrigated with full irrigation. For, sustainable water management in agriculture area, using simulation model such as AquaCrop is useful tolls to estimate effect of applied water depth and quality of irrigation water on crop yield.

Keywords

Deficit irrigation, Salinity stress, Wheat yield, AquaCrop

Introduction

The population of the world is estimated to reach 9.8 billion people in the next 30 years, according to the United Nations, and global water and food demands can also be foreseen to increase accordingly. The agricultural sector uses 70% of the world's fresh water. Water is a limited resource and climate change has accelerated the depletion of the natural resource. The growing population has also increased per capita water use, compounding the global situation of freshwater scarcity (Maysoun et al., 2021). In order to manage over this problem, field management strategies such as using marginal water resources for irrigation, choosing convenient planting techniques, planting salt resistance genotypes are suggested (Dastranj and Sepaskhah, 2021).

The global wheat production came to about 778.6 million tons in 2021-22 growing season (Shahbandeh, 2022). Wheat is one of the strategic crops in Turkiye where wheat cultivation area has a value of 2.4% in the world as of 2020/21 production season (USDA, 2021). According to 2020 United States Department of Agriculture data, Turkey ranks 9th in world wheat exports and its self-sufficiency level is between 95-100% over the years about wheat production. Wheat being the winter season crop (vegetation period; 270 days) needs about 350 to 500 mm irrigation water throughout the growing period in Central Anatolia Region of Turkey.

The decrease in freshwater resources and winter precipitation due to the effect of climate change makes it even more necessary to determine irrigation strategies in wheat. In determining the effects of irrigation strategies and irrigation water qualities on crop yield,

computer models are a very useful tool to see the results that may arise in the future.

The FAO's AquaCrop water productivity model is well known simulation model to estimate the effects of different irrigation applications and irrigation water quality parameters on crop yields (Steduto et al., 2012). The model has been tested to simulate yield response to water for most of the major field crops cultivated worldwide (Steduto et al., 2009). This model was used to simulate to effects of deficit irrigation and irrigation water salinity level on winter wheat yield and biomass in this study.

Material and Methods

Experimental field

A field research project was conducted in Ankara/Turkiye (40º 04'N and 32º 36'E) to calibrate and validate the AquaCrop (Ver. 6.1) model for semi-arid climate conditions between 2008 and 2012 (Kale Çelik et al., 2018). According to the results of the project, the prediction accuracy of the model for arid and semi-arid regions was found to be statistically acceptable. In this study, the field data of this project was used as input in the AquaCrop model and the model was run for different irrigation and salinity scenarios.

Experimental field soils are non-saline and mostly silty clay loam and clay loam textures. Average field capacity on the volume basis of soil is 36%, wilting point 21% and bulk density 1.22 gr cm^{-3}. *Bayraktar-2000* wheat variety was cultivated during the experimental studies.

The research area has typical continental climate which summers are dry and hot, winters are rainy and cold. The daily temperature differences are quite high. The lowest temperature measured in the region is -4.7 °C, the highest temperature is 34.3 °C, and the annual average temperature is 9.1 °C. The average annual total precipitation is 398.6 mm, most of which falls during the winter months.

Model description

AquaCrop version 6.1 was used in this study and it was obtained from the official website of FAO via https://www.fao.org/aquacrop/software/aquacropstandardwindowsprogramme/en/ link. AquaCrop is a crop simulation model which describes the interactions between the plant and the soil. From the root zone, the plant extract water and nutrients. Field and irrigation management are considered since it affects the interaction. The described system is linked to the atmosphere through the upper boundary which determines the evaporative demand (ET_o) and supplies CO_2 and energy for crop growth. Water drains from the system to the subsoil and the ground water table through the lower boundary. If the groundwater table is shallow water can move upward to the system by capillary rise (Raes et al., 2012).

Method

In order to simulate grain and biomass yield of wheat under drought and salinity stress with AquaCrop model, five irrigation strategies and five different irrigation water salinities scenarios were created. For irrigation scenarios; Fixed irrigation dose (90 mm) was applied in the stem elongation, heading and milk stages for full irrigation treatment (I_{100}). In deficient irrigation treatments (I_{75} - I_{50} – I_{25}) 75%, 50% and 25% of the full irrigation amount was applied on the same day as I_{100} treatment. No irrigation water applied for rainfed treatment (I_0). Initial soil moisture contents during model run were taken from the project carried out between 2011-2012. For salinity scenarios; five irrigation water salinity levels (S) were used as 0.3, 5, 7.5, 10 and 15 dS m^{-1}.

Input data for AquaCrop (Ver. 6.1) was *i)* climate file; daily rainfall, minimum and maximum air temperature, CO_2 amount and ET_o, *ii)* crop file; emergence, start of flowering time, duration of flowering, canopy senescence, maximum canopy cover and maturity time, *iii)* soil file; saturated hydraulic conductivity, wilting point and field capacity, *iv)* management file; field management practices, irrigation schedule, irrigation water quality, *v)* initial condition; initial soil water content, initial soil salinity. Crop inputs includes conservative and user-specific parameters (Table 1).

Experimental field soil parameters which were given in Table 2 were used as soil inputs in the model. Total applied irrigation water amount according to irrigation treatments were 270 mm, 203 mm, 135 mm and 68 mm for S_{100} - S_{75} - S_{50} – S_{25} respectively.

Result and Discussion

Effect of deficit irrigation treatments on yield and biomass of wheat

The lowest average grain yield and biomass amounts were found at the rainfed treatment, which did not apply irrigation water during the growing period, with a value of 5.13 t ha^{-1} and 12.84 t ha^{-1}. The highest average grain yield and biomass values of 8.43 t ha^{-1} and 17.67 t ha^{-1} were obtained respectively at the control (S_{100}) treatment which was fully irrigated during the stem elongation, heading and milk stages. The grain yields and biomass were fluctuated in the range of 8.00 t ha^{-1} – 6.28 t ha^{-1} and 17.00 t ha^{-1} - 14.53 t ha^{-1} at the deficit irrigation treatments. According to variance analysis there is significant negative relationship ($P<0.05$) between treatments (Figure 1). The application of 25%, 50% and 75% level of deficit irrigation, grain yield reduction was obtained 26%, 13% and 5% respectively. According to the study was conducted by Tari (2016) that deficit water application in the stem elongation and heading stages of winter wheat crucially diminish the yield. However other studies have indicated that the application of 40-60% deficit irrigation causes only in average 15% reduction in wheat yield (Pereira et al. 2002; Memon et al. 2021). There is a positive and significant relationship between grain yield and biomass with the determination coefficient (R^2) 0.98 (Figure 2).

Model simulates water productivity (WP) as a function of evapotranspiration (ET). WP expresses the yield which was produced per cubic meter of water loss by ET at field level. WP=Grain yield / ΣET where GY is the grain yield in kg ha^{-1}, and ET is the crop evapotranspiration (mm). Table 3 showed WP values under deficit irrigation treatments.

The results indicated that, there was not significant differences between water productivity values of full

irrigation and 25% and 50% deficit irrigation treatments. However applied irrigation amount was half of the full irrigation treatment at 50% deficit irrigation treatment. The relationship water productivity and grain yield for different water deficit irrigation strategies were presented in Figure 3.

Table 1. Crop inputs for winter wheat

Conservative Crop parameters	*Values*
Temperature - base and upper ˚C (LE)	0 -27
Canopy cover (CC_o; per seedling at 90% emergence) % (M)	6.46
Maximum canopy cover (CC_x) % (C)	90
Canopy growth coefficient (CGC;incease in CC relative to existing CC per GDD*) %(C)	2.7
Canopy decline coefficient (CDC) % (C)	0.35
Growth threshold of leaf (p_{upper}) (D)	0.21
Growth threshold of leaf (p_{lower}) (D)	0.64
Curve shape of leaf growth stress coefficient (D)	5.0
threshold of stomatal conductance (p-upper) (D)	0.64
Curve shape of stomata stress coefficient (D)	2.46
Senescence stress coefficient (D)	0.71
Curve shape of senescence stress coefficient (D)	2.49
Harvest index %(M)	36
Water productivity $g_{(biomass)}$ m^{-2} (D)	15.1
User-Specific Crop Parameters	*Values*
Sowing rate, kg seed ha^{-1} (M)	170
1000 seed mass, g (M)	33.50
Germination rate, % (M)	85
Cover per seeding, cm^2 $plant^{-1}$ (M)	1.5
Plant density, plants m^{-2} (M)	431.3
Sowing date (M)	October 20
Plant emergence date and as a GDD (M)	123 (October 31)
Day of Max canopy cover and as a GDD (M)	1276 (May 12)
Day of maximum root depth and as a GDD (M)	775 (March 16)
Day of start senescence and as a GDD (M)	1768 (June 10)
Day of maturity and as a GDD (M)	2605 (July 20)
Time to reach flowering and as a GDD(M)	1320 (May 15)
Duration of flowering stage and as a GDD (M)	179 (May 25)
Effective root depth (minimum and maximum), m (M)	0.3 and 1.5
Saturated hydraulic conductivity for 0-30 and 30-150 cm soil depth, mm day^{-1} (M)	125-230

LE; local experience, M; measured, C; calibrated (Kale Celik et al. 2018), *GDD; growing degree days, D; default (Steduto et al., 2012)

Table 2. Soil inputs used in AquaCrop model

Soil depth (m)	Soil moisture contents (%)			Soil texture	Soil salinity (dS m^{-1})	Bulk density (g cm^{-3})	K_{sat} (mm day^{-1})
	Field capacity	Wilting point	Saturation				
0.0 – 0.30	33.78	16.67	44.60	SiCL	1.02	1.26	230
0.30 – 0.60	35.56	22.01	46.52	CL	0.72	1.27	175
0.60 – 0.90	36.24	21.94	47.19	CL	0.68	1.20	125
0.90 – 1.20	37.12	22.71	48.85	CL	0.65	1.21	125

SiCL; Silty Clay loam, CL; Clay Loam, K_{sat}; Saturated hydraulic conductivity

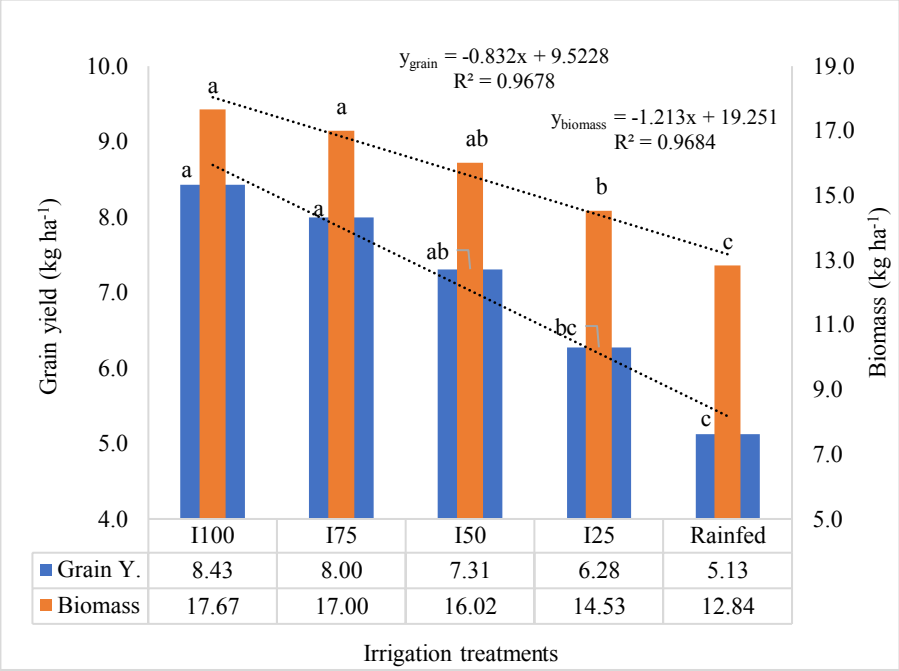

Figure 1. Estimated grain yield and biomass for irrigation treatments

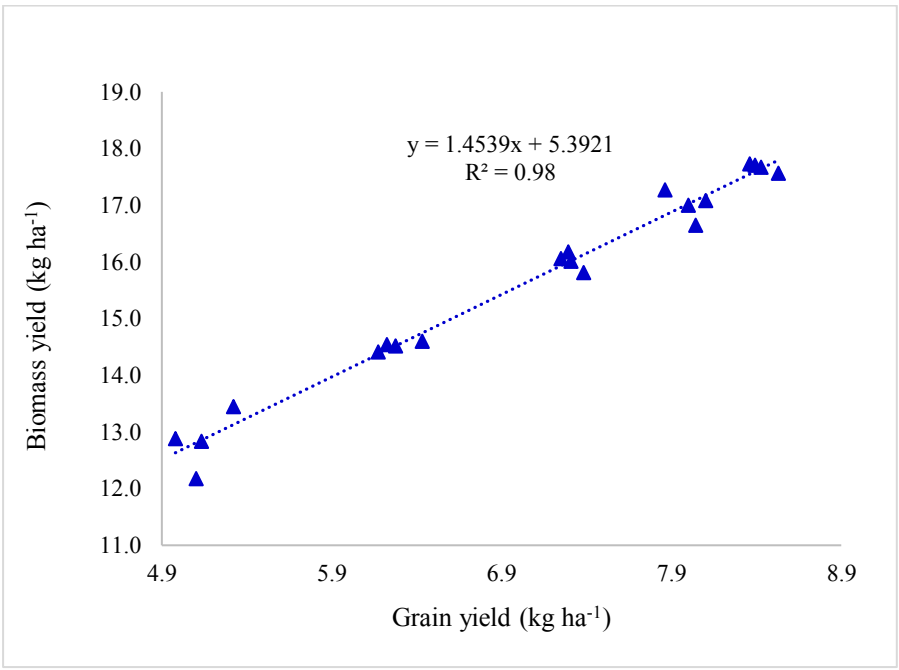

Figure 2. Grain yield and biomass relationship

Table 3. Water productivity of irrigation treatments

Treatment	Irrigation amount (mm)	Yield (kg ha^{-1})	ET (mm)	WP (kg m^{-3})
I_{100}	270	843	659	1.28
I_{75}	204	800	630	1.27
I_{50}	135	731	585	1.25
I_{25}	66	628	523	1.20

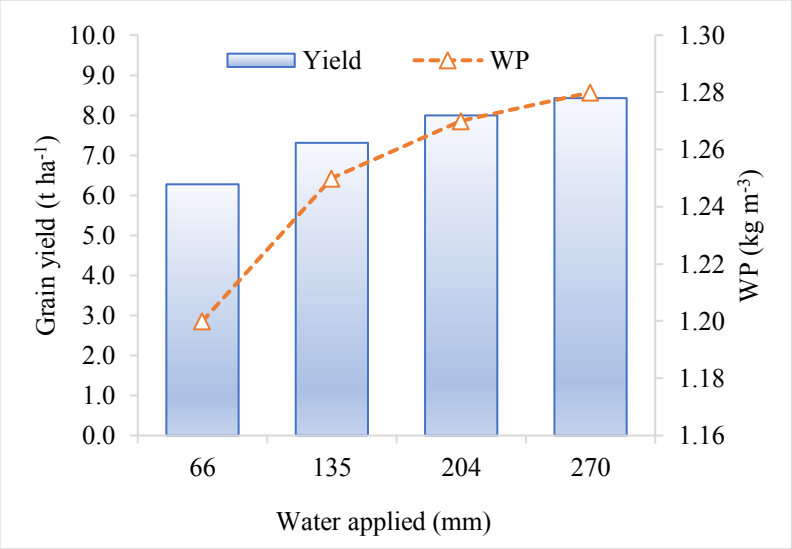

Figure 3. Water productivity and grain yield relations for deficit irrigation strategies

It is possible to irrigate much more areas saving water with deficit irrigation. As an example; the yields were calculated that the areas will be irrigated with saved water for every 2700 m^3 of available irrigation water (Table 4). According to calculation results; since larger areas are irrigated when 25, 50 and 75 percent irrigation savings are made, the yields obtained from these areas were 2.17, 6.17 and 17.2 tons more than the yields obtained from areas irrigated with full irrigation.

Table 4. Additional irrigated areas and grain yield with saved water

Treatments	100% full irrigation	25% saved water	50% saved water	75% saved water
Irrigated area (ha)	1.00	1.32 (=2700/2040)	2.00 (=2700/1350)	4.09 (=2700/660)
Grain yield (t)	8.43	10.56 (=8.00 x 1.32)	14.62 (=7.31 x 2.00)	25.68 (=6.28 x 4.09)

A research study was carried out by Mustafa et al. (2017) to determine the effects of deficit irrigation applications on wheat yield and water productivity. According to results of this study the highest yields were obtained at yield formation and ripening stages and water saved about 35% compared to full irrigation application.

Effect of irrigation water salinity on grain and biomass yield of wheat

Obtained the grain and biomass yields under different irrigation water salinity levels and statistical classifications on related treatments were given in Figure 4. The increase in irrigation water salinity caused a significant decrease in grain yield and biomass value. It was compared to the yield of T_1 (control) treatment, 3% lower yield was obtained at the T_2 treatment. Yield loss of T_3 and T_4 salinity treatments were found to be 19% and 43% respectively. The crop yield reduction was dramatically (86%) at 15 dS m^{-1} salinity level of irrigation water. The similar results were also presented regarding the decrease in yield as a result of the increase in the salinity of the applied irrigation water (Tekin et al. 2014; Mostafazadeh-Fard et al. 2009; Gowing et al. 2009; Kumar, 2020; and Hammami et al. 2020).

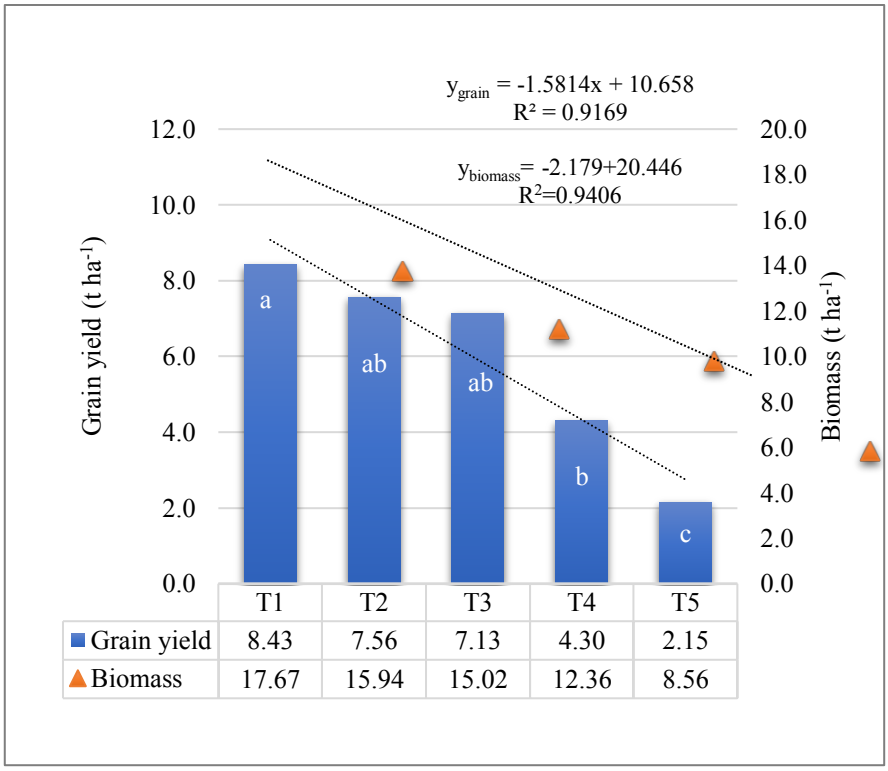

Figure 4. The grain yields and biomass values for different irrigation water

Canopy cover (CC) of wheat

Hammani et al. (2020) was reported that the maximum 85% and minimum 30% CC were obtained in the sub-humid areas. The canopy cover values of all treatments showed the same trend until early spring.

The highest CC value was obtained as 82.5% on S_{100} treatment. Figure 5 shows that water deficit stress effects on canopy cover of winter wheat.

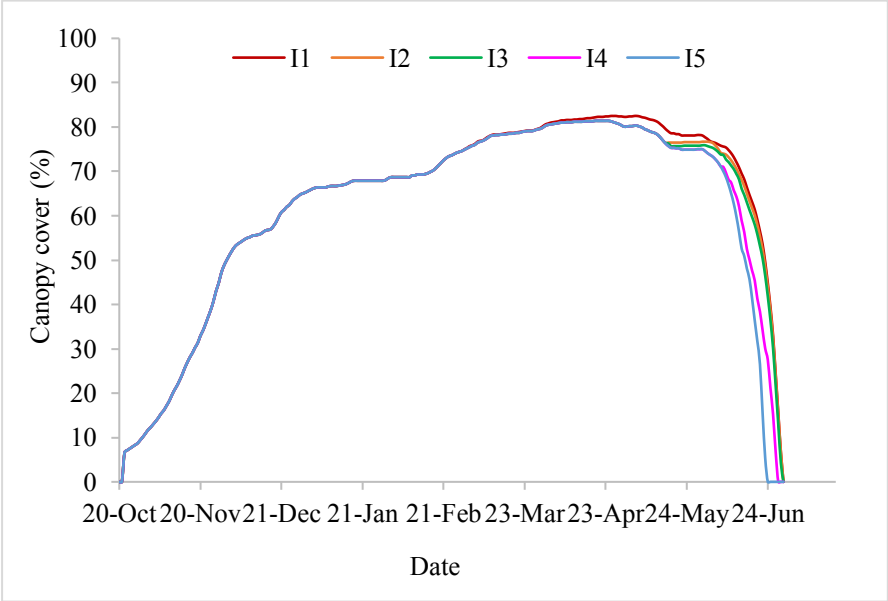

Figure 5. Canopy cover of wheat under different irrigation water amount

The simulation results showed that the application of saline irrigation water in semi-arid conditions such as the Central Anatolia region caused a decrease in CC of 18.8% (Figure 6). Similar result was obtained by Hammani et al. (2020) such as the salinity induces a 10% reduction in the CC in the sub-humid environment and 5–30% in the dry climate condition.

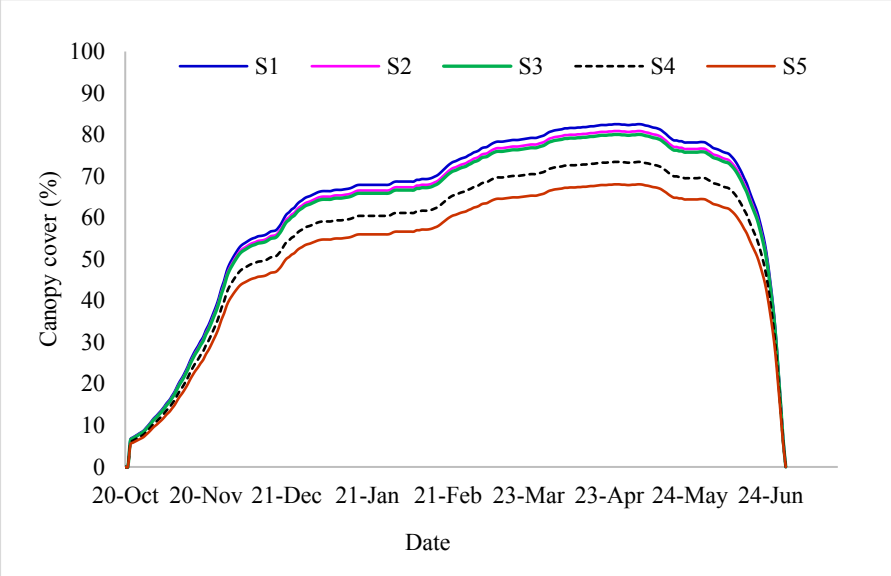

Figure 6. Canopy cover of wheat under different irrigation water salinity

Drought and salinity interactions

When all treatments were evaluated together, it was observed that the yield increased proportionally as the amount of irrigation water applied increased (Table 5). On the other hand, the lowest yield was obtained at all salinity levels in I_{25} treatment, where 75% water saved. When both the decrease in the amount of irrigation water and the increase in the salinity level come together, decline in yield were significant.

Table 5. Grain yield amount (t ha^{-1}) under drought and salinity stress

Treatments	S_1	S_2	S_3	S_4	S_5
I_{100}	8.43	7.56	7.13	4.30	2.15
I_{75}	8.00	6.65	5.67	3.89	1.91
I_{50}	7.31	5.93	4.62	3.30	1.85
I_{25}	6.28	4.91	3.62	2.66	1.19

Interaction between irrigation water salinity and irrigation water amount on wheat grain yield were found to be statistically significant at the level of 1%. The statistical evaluations were given in Table 6.

Table 6. Variance analysis table of grain yield

Sources	SD	SS	AS	F values	F Table	
					0.05	0.01
Salinity	4	204497904	215909047	155.40**	2.42	3.32
Irrigation	4	130111354	130111354	93.65**	1.56	2.40
Salinity * Irrigation	16	14.80	0.30	23.68**	1.32	2.34
Error	65					
Total	73					

**; Significant level of 0.01, SD; Standard deviation, SS; Sum of square, AS; Average of square

The change in the amount of irrigation water also changed the effects of irrigation water salinity on crop yield. The results are in agreement with previous studies which was conducted by Juis et al. (2003) and Mostafazadeh-Fard et al. 2009. Also, Gowing et al. (2009), reported that there were small but statistically significant effects of the interaction between the salinities of the irrigation and water use of wheat.

Conclusion

The deficit irrigation with water reduction of more than 75% of full irrigation was applied at growth stages of wheat, revealed the significant reduction in grain yield, biomass, water productivity and canopy cover as compared with full irrigation practice. Irrigation water salinity is one of the most important factors in limiting crop growth and reducing crop yield in arid and semiarid regions. In this study results showed that highest irrigation water salinity caused highest crop yield

reduction. Also, increasing irrigation water depth in saline treatments resulted in increased grain and biomass yield. For sustainable water management in agriculture area, using simulation model such as AquaCrop is useful to estimate effect of applied water depth and quality of irrigation water on crop yield.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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The effect of different climatic zones on fatty acid profile of *Ricinus communis* seed oil

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Abstract
Castor bean has not been well studied in different genotypes and geographic zones despite its application in industry and medicine. Recently, the use of castor beans as biodiesel and industry makes this plant a point of interest for researchers. However, more studies are needed for evaluating genotypes from different ecologies. The effect of climatic zones, Adana and Mersin, on the fatty acid profile of chaster bean seed oils was investigated. It was found that locations significantly influenced the fatty acid content. The main fatty acid was ricinoleic acid with 84.63% and 86.87% in both Adana and Mersin locations, respectively. Despite ricinoleic acid, Adana had higher concentrations of Palmitic acid (1.97%), Stearic acid (2.1%), Oleic acid (4.4%), and Palmitoleic acid (2.29%) whereas Linolenic acid (5.83%), and Ricinoleic acid (86.87%) was high in Mersin. These results showed that climate affects the fatty acid contents of studied caster oil. This study will help in the selection of proper caster oil cultivars not only in these regions but in other regions of the world as well.

Keywords
Ricinus communis, Fatty acids, Location, Ricinoleic acid

Introduction
The limited conventional energy resources besides the harmful and destructive effect of diesel fuels on the environment have accelerated the search for alternative fuels. Concerns about global warming of pollutants lead to using biodiesels as a solution. Biodiesel is an alternative non-toxic, environmental-friendly, and biodegradable fuel obtained from renewable sources such as animal or vegetable oils. Therefore, it is very important to evaluate plant species with potential applications as biodiesel. On the other hand, heavy metal pollution caused by releasing mine waste to open areas is another concern of ecological problem and makes it necessary to select the suitable plant species for planting on mine waste which accelerates the revegetation process, increasing biodiversity and stabilizing nutrient cycling (Olivares et al 2013). One of these special plant species is the castor bean.

Castor bean also known as castor oil plant (*Ricinus communis*) belongs to the Euphorbiaceae family. The origin of the castor plant is debatable, but the strong evidence shows that its origin is in the tropical belt of India and Africa. However, today, the castor plant is cultivated worldwide in many tropical and subtropical regions and even warm temperate regions (Kaur and Bhaskar 2020; Yusuf et al 2015). Castor bean is a fast-growing perennial shrubby plant 8-10 meters high. It can be self- and cross-pollinated and worldwide studies reveal low genetic diversity among castor bean germplasm. The roots of the castor bean are fibrous and scarcely ramified; the trunk is upright and highly branched, and its color varies from bright green, pale

green, or red to purple and is covered with a waxy layer that makes the plant high drought-resistant. The leaves are alternate green or reddish formed in long stalks, alternate and palmate, divided into 5-12 lobes. The stems have different colors. The flowers are monoecious the flowers formed in the upper parts are female and the ones form in the lower part are male. The fruits are set in upper branches in three boxes, these boxes separate into ripen fruits and each contains one oval, bean-like seed which is swelled in the center. The seeds are caruncle and have a warty appendage at one end (Jena and Gupta 2012). The Castor bean is a precious plant cultivated for industrial and medicinal purposes. Phytochemicals extracted from different organs of caster bean have numerous pharmacological uses such as antibacterial, cytotoxicity, antioxidant and anticancer, antiasthmatic, anti-inflammatory, laxative-cathartic reagent, and many other applications (Javanshir et al 2020; Vasco-Leal et al 2020, Franke et al 2019; El-Naggar et al 2019; Ribeiro et al., 2016; Jena and Gupta 2012, Salimon et al 2010).The castor bean also was reported as an efficient pesticide application by researchers (Carolina et al 2019, Adeniyi et al., 2018; Soni and Dhiman, 2017; Rampadarath and Puchoa, 2016). Castor oil is extracted from the grains, and the residue, and can be used for nematode control (Gahukar, 2017) and, as fertilizer (Lima et al., 2011). The industrial application of castor bean is in cosmetic production, production of PU foams, elastomers, surface coating materials, adhesives, and interpenetrating polymer networks (IPNs), in paint, print, and textile industries, production of soap, preparation of brake fluids as a lubricant, (Yusuf et al 2015). Castor bean is also an ideal plant for phytoremediation (Rehn et al 2020; Yeboah et al 2020, Palanivel et al 2020, Olivares et al 2013). However, castor bean is a tolerant plant grown in every ecological condition especially in semi-arid and arid regions even in metal-polluted sites (Rajkumar and Freitas 2008). Studies showed that not only *R. communis* is resistant to urban roadside air pollution, but also antioxidant activity and total free amino acids have tremendously increased in this condition (Khalid et al 2019).It is proved by many studies that chaster bean is an ideal biodiesel alternative for sulfur-based diesel due to its unique oil structure (Roy et al 2020; Carrino et al 2020; Awais et al 2020; Osorio-González et al 2020, Chan et al 2010, Perdomo et al 2013). However, what makes the castor bean a unique plant is the structure of its seed oil. Castor oil is rich in hydroxy fatty acid with one double bond, ricinoleic acid (cis-12- hydroxyoctadeca-9-enoic acid), the only commercial source of a hydroxylated fatty acid. Ricinoleic acid counts for unique properties of castor oil including high specific gravity, high boiling point(3130C), excellent solubility in alcohols, and unusual versatility. The presence of a hydroxyl group on C-12 of ricinoleic acid makes castor oil unusually polar, thus promoting hydrogen bonding. Unlike other oils, it is mixable with alcohol, but only slightly soluble in petroleum ether at room temperature. It is reported that the oil content of castor seed is about t 46-55% that 87-90% of its content is ricinoleic acid (Gupta et al. 1951; Foglia et al. 2000; Puthli et al. 2006; Ogunniyi 2006; Conceicao et al. 2007). Akpan et al., 2006; Ogunniyi, 2006; Conceicao et al., 2007), with only about 4.2% linoleic, 3.0% oleic, 1.0% each stearic, and palmitic, 0.7% dihydroxy stearic, and 0.3% each linolenic and eicosanoic acids (Dave, 2002). This high level of purity (by single fatty acid content) makes the oil unique among all naturally occurring fats and oils. Although caster seeds contain a toxic protein called ricin and toxic allergen and are poisonous to humans and animals, none of the toxic components is carried into the oil (Nangbes et al 2013). However, oil content and fatty acids composition in castor seed vary based on genetic characteristics, genotypes, geographical origin/climatic conditions, agricultural operations such as irrigation and foliar application of nutrients, and the oil extraction method(s) used (Sadeghi-Bakhtavari & Hazrati2020; Yusuf et al 2015). Therefore, it is important to have knowledge about different genotypes grown in different geographical zones. Because as mentioned above phytochemical composition of castor bean oil is affected strongly by geographical origin/climatic conditions.

The purpose of this study was to evaluate the effect of different ecological zones on the composition of fatty acids in naturalized wildly grown castor beans from Turkey which there is little information regarding its use, adaptation, and characterization.

Materials and methods

Plant Material

Ricinus communis plants were collected from Mersin, Atakent Municipality Kapızlı Camp, 8 m (36˚24′14″N, 34˚04′33″E), and Adana Cukurova University, Ali Nihat Gökyiğit Çarkıpare Şarıçam, 112 m, (37˚03′02″N,35˚21′14″E) with a one-week interval. Then seeds were removed and dried at 65˚C for 8 h. For each location, three samples were used.

Oil extraction

The oils of all samples were extracted immediately after harvest. The oil of samples was extracted via an automatic soxhlet device (Gerhardt GmbH & Co. KG). Ten grams of dried seeds were used for oil extraction. Hexane (Merck KGaA, Darmstadt, Germany) was used as a solvent and extracted oil was weighted for the determination of the oil percent in the samples. The oil content of seeds was expressed as g 100 g-1 in dry samples. Obtained fresh oil was analyzed determination of fatty acids composition.

Determination of fatty acids

Esterification of Fatty acids was done using the method described by Perdomo et al (2012). One hundred mL of oil was mixed with 1 mL of NaOH methanolic solution. Then, samples were heated up to 100˚C for 25 min followed by adding 6 mL of HCl methanolic solution. After heating the obtained solvent again up to 80˚C for 10 min, 75 mL of equimolar hexane was added. The upper phase was removed and mixed with 9 mL of NaOH solution, and after 1 min standing solution was used for injection to gas chromatography (GC, Perkin Elmer, Auto system GLX, Shelton, USA). Chromatographic separation was performed using a Supelco SPTM-2380 (30 m 0.25 mm inner diameter, 0.25 mm film thickness) column equipped with a flame ionization detector (FID). The injector and detector temperatures were 280˚C and

260˚C respectively. The carrier gas was helium with a flow rate of 0.5 mL/min. The initial temperature of the oven was adjusted at 120˚C for 2 min, increased at 58ºC/min to 220ºC, and held for 10 min. Data was collected and quantified with a TotalChrom Navigator and the results were expressed as percent concentration (Demirtas et al. 2013).

Statistical Analysis

The experiment was conducted as a completely randomized design using two replications. The results were expressed as average and standard deviation. The correlation analysis was done among fatty acids. The principal component analysis (PCA) was done using the XLSTAT software program (Addinsoft, USA).

Figure 1. A view of *Ricinus communis* plants and seeds (A: Mersin, B: Adana)

Results and Discussion

The fatty acid composition of castor oil from two studied geographic zones is shown in Table 1. The results showed that the major part of seed oil includes ricinoleic acid in both samples. These results are in agreement with previous studies on castor beans (Gupta et al. 1951; Foglia et al. 2000; Puthli et al. 2006; Ogunniyi 2006; Conceicao et al. 2007; Salimon et al, 2010; Pedremo et al 2013; Yusuf et al 2015; Sadeghi-Bakhtavari & Hazrati 2020).

Table 1. The fatty acid composition of castor oils from studied geographic zones [%]

Compound name	Adana	Mersin	Mean Difference
Total fat	46.36	47.41	
Capric acid (C10:0)	0.06±0.00	N.D.	0.06
Caprylic acid (C8:0)	0.08±0.00	0.07±0.00	0.01
Undecanoic acid (C11:0)	0.08±0.01	N.D.	0.075
Myristic Acid (C14:0)	0.09±0.01	0.04±0.00	0.045
Palmitic acid (C16:0)	1.97±0.04	1.44±0.01	0.53
Margaric Acid (C17:0)	0.04±0.01	0.04±0.01	0
Stearic acid (C18:0)	2.10±0.02	1.23±0.01	0.87
Arachidic acid (C20:0)	0.08±0.01	0.05±0.00	0.025
Behenic acid (C22:0)	0.31±0.01	0.04±0.00	0.265
Tricosanoic acid (C23:0)	0.04±0.02	0.02±0.00	0.015
Σ SFA	**4.81**	**2.92**	**1.895**
cis-Pentadecan Acid (C15:1)	0.02±0.00	0.03±0.00	-0.01
Palmitoleic acid (C16.1)ω−7	0.07±0.03	0.02±0.00	0.05
Oleic acid (C18:1n9c)ω−9	4.40±0.01	3.86±0.03	0.54
Eicosenoic acid (C20:1n9c)ω−9	0.50±0.01	0.49±0.00	0.005
Ricinoleic acid [C18:1(OH)]	84.63±0.13	86.87±0.05	-2.235
Σ MUFA	**89.62**	**91.27**	**-1.65**
Linoleic acid (Cl 8:2n6c) ω−6	5.20±0.04	5.38±0.03	-0.185
γ-Linolenic Acid (C18:3n6) ω−6	0.03±0.00	0.01±0.00	0.02
a-Linolenic acid (C18:3n3) ω−3	0.37±0.01	0.45±0.01	-0.08
Σ PUFA	**5.59**	**5.84**	**-0.245**

Due to the unique characteristics of ricinoleic acid with high purity in castor bean oil, castor oil is characterized as an inexpensive and environment-friendly biodiesel and precursor in industry. However, results revealed that samples from Mersin had a higher amount of ricinoleic acid compared to Adana samples. In terms of total oil percentage, the seeds from Mersin (47.41%) location contained higher oil than Adana

(46.36%). While the oil contents of seeds in the present study were found to be higher than those reported by Sadeghi-Bakhtavari & Hazrati 2020 (40.75% in full water and 37.83% in underwater stress), the obtained results are confirmed by the findings of Ogunniyi, (2006), Goytia-Jime´nez et al. (2011) Román-Figueroa et al (2020) (46-55%). Under natural conditions, the average oil content in castor beans fluctuates between 42.0% and 49.0% but in Mediterranean conditions increased up to 55.0% (Román-Figueroa et al 2020). However, previous studies emphasized that oil content in castor oil is extremely affected by variety and environmental conditions, especially during the seed-filling stage as seen in this study (Salmon et al 2010, Onemli 2012). Başalma and Pashazade (2011) reported that castor oil needs an optimum range of temperature (20-26 ºC) and humidity. At temperatures lower than 15 ºC and higher than 35 ºC the oil content of seeds decreases.

Mersin and Adana show the typical characteristics of the Mediterranean climate in the same geographical area. However, the sampling locality in Mersin is expected to be more open to the sea effect and high water table. So, it can be generalized that the castor seeds in Mersin have grown in more warm, humid, less volatile (in terms of air temperature and humidity), and wetland conditions. Although the average temperature of Mersin and Adana is the same which is 19.1°C, the temperature volatility is higher in Adana. In detail, the difference between the average maximum and average minimum temperatures are 8.6 °C and 12.5 °C for Mersin and Adana, respectively. In addition, the monthly average rainfall is 615.8 mm in Mersin and 671.3 mm in Adana which is concentrated during the winter season. The altitude of the Mersin sample collection point is 6 m in a marsh area, while it is 173 m for Adana in rugged terrain. This may lead to higher water-deficit stress for the Adana sample collection point despite higher rainfall. Moreover, Mersin sample collection point is near the seaside but the distance between the Adana sample collection point and the sea is approximately 55-60 km. Sufficient water supply during the growing period leads to extended flowering and pollination periods. Therefore, the number of seeds, seed weight, and oil content increased. It was reported that water deficit stress reduces the concentration of linolenic and linoleic acid but increases the concentration of oleic acid in oilseed crops (Alyari et al., 2000). In line with that, higher oleic acid and lower linoleic and a-Linolenic acids contents were observed in the seeds from Adana, associated with higher water deficit. In water-deficit stress, the uptake of nutrients decreases causing an increase in soluble salts and osmotic pressure around the root system (Sadeghi-Bakhtavari & Hazrati 2020).

The total SFA is higher in Adana location than Mersin which is caused by the higher rate of palmitic, stearic, and behenic acid. While the rate of MUFA is higher in the Mersin location compared to Adana samples. Although oleic acid was higher in Adana, the higher rate of ricinoleic acid in Mersin caused Mersin to have higher Total MUFA. The rate of total PUFA in Mersin is also higher than Adana due to higher linoleic acid and a-linoleic acid (Table 1 and Figure 2).

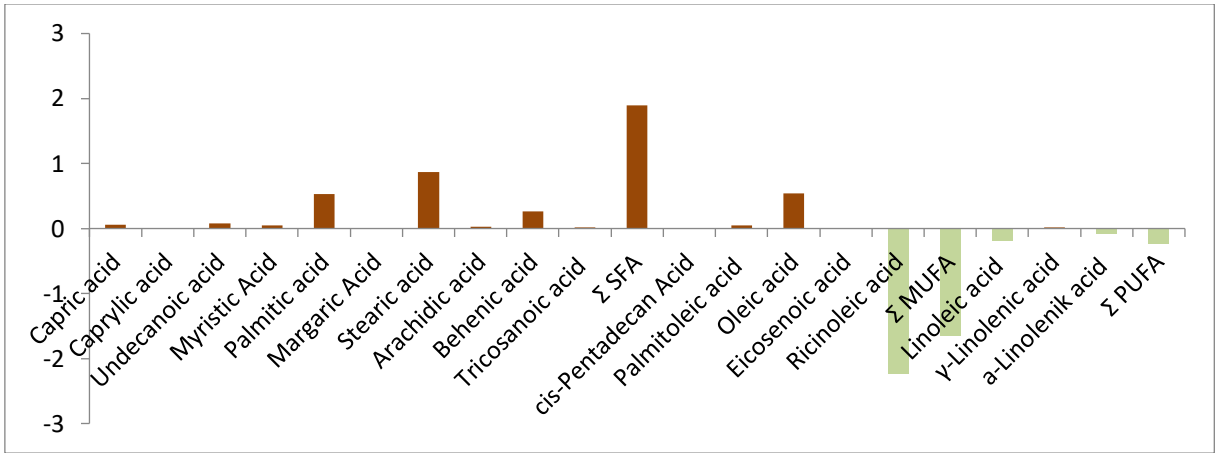

Figure 2. Differences among fatty acid compositions of seeds from Adana and Mersin-%.

In PCA analysis if the first two principal components (PCs) are able to explain a significant portion of the total variance, it is possible to visualize the experiment results. The variable chart indicates the correlations between the components and the variables. The correlations can be seen in the following matrix apparently (Table 2). The blue points are the observations and the vectors are the variables. In the figure 3, PC1 and PC2 can explain 86.61 % and 11,59% of the total variance, respectively. The location of the samples separated significantly from one another. As a result, using the *Ricinus communis* seeds from different locations as explant may create a difference in the fatty acids compositions even in the same climate and geographical area (Figure 3).

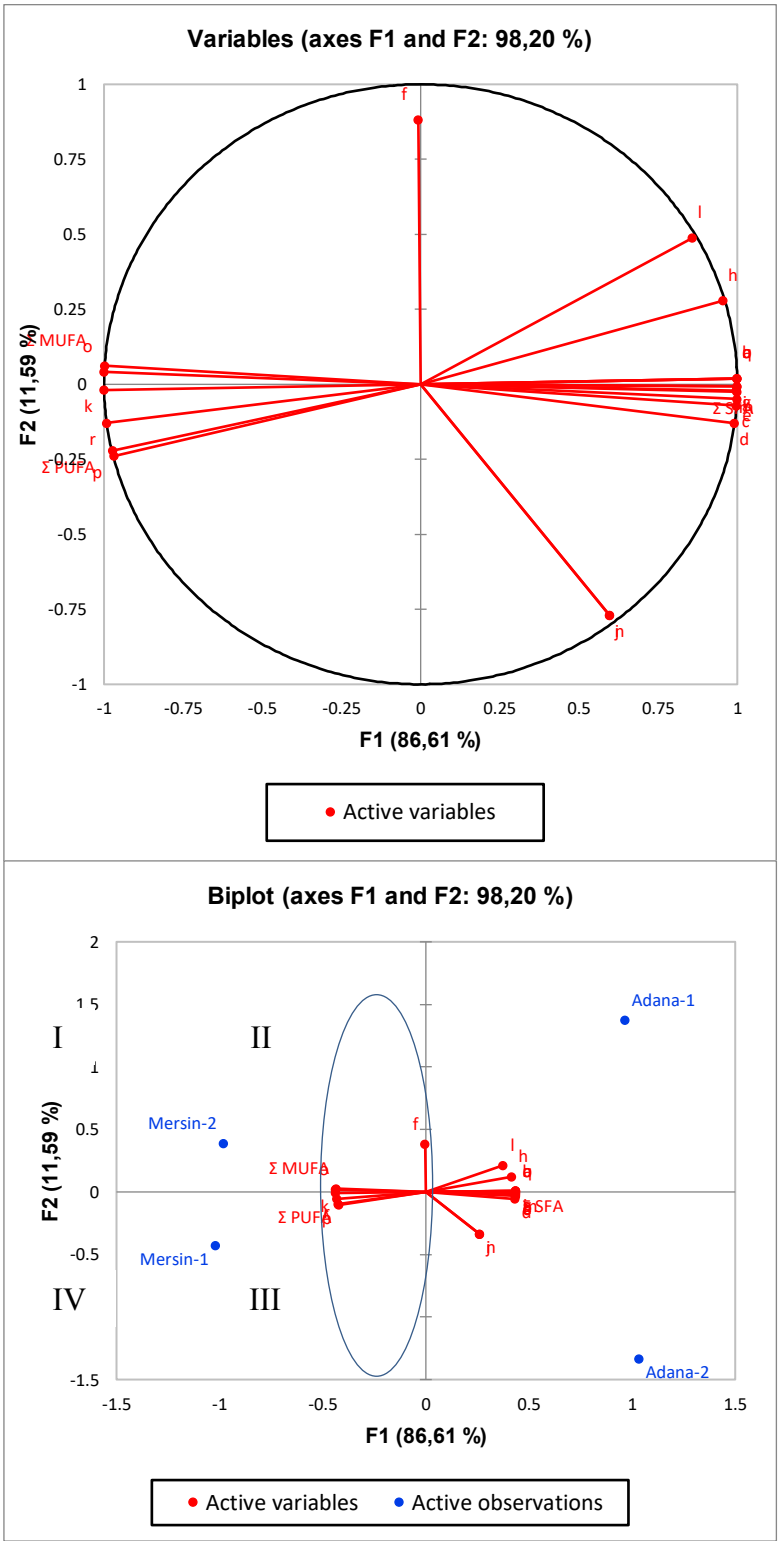

Figure 3. Correlation Circle Chart and Biplot Graph Obtained by The Principle Component Analysis (PCA) showing the interrelations of sample locations and fatty acids.

Capric acid a, Caprylic acid b, Undecanoic acid c, Myristic Acid d, Palmitic acid e, Margaric Acid f , Stearic acid g, Arachidic acid h, Behenic acid i, Tricosanoic acid j, cis-Pentadecan Acid k, Palmitoleic acid l, Oleic acid m, Eicosenoic acid n, Ricinoleic acid o, Linoleic acid p, γ-Linolenic Acid q, a-Linolenik acid r

On the Biplot graph Fatty acids observed in Area I: f, o, ΣMUFA, Area II: I, h, q, b, a, Area III: g, m, ΣSFA, c, I, e, d, n, j, Area IV: k, r, ΣPUFA, p

Table 2. Correlation Matrix (Pearson (n))

Variables	a	b	c	d	e	f	g	h	i	j	Σ SFA	k	l	m	n	o	Σ MUFA	p	q	r	Σ PUFA
a	**1**	**1.000**	**0.996**	**0.988**	**0.998**	**0.000**	**1.000**	**0.962**	**1.000**	**0.577**	**0.999**	**-1.000**	**0.870**	**0.998**	**0.577**	**-0.998**	**-0.997**	**-0.971**	**1.000**	**-0.992**	**-0.976**
b	**1.000**	**1**	**0.996**	**0.988**	**0.998**	**0.000**	**1.000**	**0.962**	**1.000**	**0.577**	**0.999**	**-1.000**	**0.870**	**0.998**	**0.577**	**-0.998**	**-0.997**	**-0.971**	**1.000**	**-0.992**	**-0.976**
c	**0.996**	**0.996**	**1**	**0.998**	**1.000**	**-0.066**	**0.998**	**0.932**	**0.998**	**0.651**	**0.999**	**-0.996**	**0.820**	**0.996**	**0.651**	**-0.999**	**-1.000**	**-0.950**	**0.996**	**-0.980**	**-0.955**
d	**0.988**	**0.988**	**0.998**	**1**	**0.996**	**-0.110**	**0.991**	**0.908**	**0.992**	**0.697**	**0.994**	**-0.988**	**0.783**	**0.990**	**0.697**	**-0.995**	**-0.997**	**-0.931**	**0.988**	**-0.967**	**-0.937**
e	**0.998**	**0.998**	**1.000**	**0.996**	**1**	**-0.056**	**0.999**	**0.942**	**0.999**	**0.630**	**1.000**	**-0.998**	**0.836**	**0.998**	**0.630**	**-1.000**	**-1.000**	**-0.955**	**0.998**	**-0.983**	**-0.960**
f	**0.000**	**0.000**	**-0.066**	**-0.110**	**-0.056**	**1**	**-0.023**	**0.192**	**-0.019**	**-0.577**	**-0.034**	**0.000**	**0.348**	**-0.055**	**-0.577**	**0.056**	**0.066**	**-0.236**	**0.000**	**-0.124**	**-0.219**
g	**1.000**	**1.000**	**0.998**	**0.991**	**0.999**	**-0.023**	**1**	**0.955**	**1.000**	**0.597**	**1.000**	**-1.000**	**0.858**	**0.999**	**0.597**	**-0.999**	**-0.998**	**-0.965**	**1.000**	**-0.989**	**-0.970**
h	**0.962**	**0.962**	**0.932**	**0.908**	**0.942**	**0.192**	**0.955**	**1**	**0.955**	**0.333**	**0.949**	**-0.962**	**0.972**	**0.953**	**0.333**	**-0.945**	**-0.937**	**-0.985**	**0.962**	**-0.979**	**-0.985**
i	**1.000**	**1.000**	**0.998**	**0.992**	**0.999**	**-0.019**	**1.000**	**0.955**	**1**	**0.599**	**1.000**	**-1.000**	**0.857**	**0.999**	**0.599**	**-0.999**	**-0.998**	**-0.966**	**1.000**	**-0.990**	**-0.971**
j	**0.577**	**0.577**	**0.651**	**0.697**	**0.630**	**-0.577**	**0.597**	**0.333**	**0.599**	**1**	**0.613**	**-0.577**	**0.101**	**0.598**	**1.000**	**-0.623**	**-0.642**	**-0.409**	**0.577**	**-0.501**	**-0.425**
Σ SFA	**0.999**	**0.999**	**0.999**	**0.994**	**1.000**	**-0.034**	**1.000**	**0.949**	**1.000**	**0.613**	**1**	**-0.999**	**0.847**	**0.999**	**0.613**	**-1.000**	**-0.999**	**-0.961**	**0.999**	**-0.987**	**-0.966**
k	**-1.000**	**-1.000**	**-0.996**	**-0.988**	**-0.998**	**0.000**	**-1.000**	**-0.962**	**-1.000**	**-0.577**	**-0.999**	**1**	**-0.870**	**-0.998**	**-0.577**	**0.998**	**0.997**	**0.971**	**-1.000**	**0.992**	**0.976**
l	**0.870**	**0.870**	**0.820**	**0.783**	**0.836**	**0.348**	**0.858**	**0.972**	**0.857**	**0.101**	**0.847**	**-0.870**	**1**	**0.856**	**0.101**	**-0.841**	**-0.827**	**-0.937**	**0.870**	**-0.907**	**-0.932**
m	**0.998**	**0.998**	**0.996**	**0.990**	**0.998**	**-0.055**	**0.999**	**0.953**	**0.999**	**0.598**	**0.999**	**-0.998**	**0.856**	**1**	**0.598**	**-0.999**	**-0.998**	**-0.957**	**0.998**	**-0.984**	**-0.962**
n	**0.577**	**0.577**	**0.651**	**0.697**	**0.630**	**-0.577**	**0.597**	**0.333**	**0.599**	**1.000**	**0.613**	**-0.577**	**0.101**	**0.598**	**1**	**-0.623**	**-0.642**	**-0.409**	**0.577**	**-0.501**	**-0.425**
o	**-0.998**	**-0.998**	**-0.999**	**-0.995**	**-1.000**	**0.056**	**-0.999**	**-0.945**	**-0.999**	**-0.623**	**-1.000**	**0.998**	**-0.841**	**-0.999**	**-0.623**	**1**	**1.000**	**0.956**	**-0.998**	**0.984**	**0.961**
Σ MUFA	**-0.997**	**-0.997**	**-1.000**	**-0.997**	**-1.000**	**0.066**	**-0.998**	**-0.937**	**-0.998**	**-0.642**	**-0.999**	**0.997**	**-0.827**	**-0.998**	**-0.642**	**1.000**	**1**	**0.951**	**-0.997**	**0.981**	**0.957**
p	**-0.971**	**-0.971**	**-0.950**	**-0.931**	**-0.955**	**-0.236**	**-0.965**	**-0.985**	**-0.966**	**-0.409**	**-0.961**	**0.971**	**-0.937**	**-0.957**	**-0.409**	**0.956**	**0.951**	**1**	**-0.971**	**0.993**	**1.000**
q	**1.000**	**1.000**	**0.996**	**0.988**	**0.998**	**0.000**	**1.000**	**0.962**	**1.000**	**0.577**	**0.999**	**-1.000**	**0.870**	**0.998**	**0.577**	**-0.998**	**-0.997**	**-0.971**	**1**	**-0.992**	**-0.976**
r	**-0.992**	**-0.992**	**-0.980**	**-0.967**	**-0.983**	**-0.124**	**-0.989**	**-0.979**	**-0.990**	**-0.501**	**-0.987**	**0.992**	**-0.907**	**-0.984**	**-0.501**	**0.984**	**0.981**	**0.993**	**-0.992**	**1**	**0.995**
Σ PUFA	**-0.976**	**-0.976**	**-0.955**	**-0.937**	**-0.960**	**-0.219**	**-0.970**	**-0.985**	**-0.971**	**-0.425**	**-0.966**	**0.976**	**-0.932**	**-0.962**	**-0.425**	**0.961**	**0.957**	**1.000**	**-0.976**	**0.995**	**1**

Values in bold are different from 0 with a significance level alpha=0

Conclusion

Castor bean is a precious plant in terms of various applications in medicine and industry. The oil extracted from seeds is high-quality to be considered biodiesel. The plant can grow almost everywhere in the world because it has high competitive power and endurance to various climate and soil conditions. More than half of the oil contains ricinoleic acid a unique saturated fatty acid with excellent properties which makes it a good candidate for application in industry and biodiesel. However, studies proved that the location where the plant is grown affects the rate of fatty acids and quality of the oil as the results obtained in this study from different locations. Therefore, this study will help international researchers on determining the optimum cultivation conditions for the exploitation of qualified oil for applications both in industry and biodiesel production.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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Response of some carnation varieties to *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne arenaria*

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Abstract
Carnation is one of the most crucial cut flowers in Turkey. Root-knot nematodes (RKNs) cause significant damage on carnation production and quality. Therefore, determination of response of carnation varieties to RKNs is required for management practices. In this study, four carnation cultivars, Turbo, Betsy, Nirvana and Glaciar, were inoculated with second stage juveniles (J2s) of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* under controlled conditions. *Meloidogyne incognita*, *M. javanica* and *M. arenaria* did not reproduce on Turbo variety. Therefore, Turbo variety was found to be resistant to these nematodes. Nirvana variety was moderately resistant to *M. incognita* and *M. javanica*, but highly resistant to *M. arenaria*. Betsy and Glaciar varieties were susceptible to *M. incognita*, *M. javanica* and *M. arenaria*. Results showed that the response of carnations was different based on root knot nematodes. Determining resistant carnation varieties could be an advantage for the control of RKNs. Resistant carnations could be used to control the RKNs and develop new carnation varieties.

Keywords
Carnation, Resistance, *Meloidogyne incognita, Meloidogyne javanica, Meloidogyne arenaria*

Introduction
Carnation (*Dianthus caryophyllus* L.) is one of the major cut flowers and has economic value in the world. It belongs to the genus *Dianthus* which is a member of the family Caryophyllaceae. More than 300 species have been reported (Galbally and Galbally, 1997; Jurgens et al., 2003). Cut flowers of carnation are classified into two types as standard and spray. The standard and spray types have one flower and multiple flowers on a stem, respectively (Satoh et al., 2005). Carnation, rose and chrysanthemum form more than 50% of cut flower market in the world. The main carnation importer countries in the European market are the United Kingdom, the Netherlands and Germany. The major suppliers of carnations to the EU are Colombia, Turkey, Kenya, Morocco, and Ethiopia, respectively (Anonymous, 2017). In Turkey, carnations are widely grown and produced cut flowers with 607.070.350 pieces. Also, Turkey is the 3rd country to export the largest amount of carnation with $32,4 million (Trademap, 2018; Turkish Statistical Institute, 2018).

Carnation production is affected by many diseases and pests (Trujillo et al., 1989; Marroquin and Arbelaez, 1992; Sharma and Sharma, 2008;). However, there are limited studies on interaction between carnation and nematode. RKNs are major pathogens in carnation areas (Lamberti et al., 1987; Çelik et al., 2019). Nagesh and Parvatha Reddy (2000) showed that *Meloidogyne incognita* caused 26% yield loss in the carnation production in India. In another study, Phyllis (1997)

reported that yield loss caused by RKNs was 20% worldwide.

Management of the RKNs is very difficult since they can survive in soil and plant tissues. Various cultural, physical, biological and chemical control methods have been used against *Meloidogyne* spp. (Thomason and Caswell, 1987). Nematicides application against root knot nematodes is commonly used but the use of highly toxic nematicides adversely affects human health and the environment (Stirling, 1991; Rich et al., 2004). Therefore, host plant resistance to root-knot nematodes provides a successful alternative management strategy. Fawzy et al. (1991) reported two carnation cultivars were resistant to *M. incognita* among twelve carnation cultivars tested. In another study, Cho et al. (1996) investigated thirty-three carnation cultivars to determine the resistance to *M. incognita* and determined that seven carnation cultivars were resistant. Since carnation production is the most significant cut flower sector in Turkey, a survey was carried out and reported that RKNs were present widespread in carnation production areas (Çelik et al., 2019). However, there is no study on resistance of carnation cultivars to root knot nematode in Turkey. Therefore, in this study, the response of some carnation cultivars that are widely grown in Turkey was investigated against *M. incognita*, *M. javanica* and *M. arenaria* isolates under controlled conditions.

Materials and Methods

Plant material

Carnation varieties used in this study are listed in Table 1. Cuttings obtained from each carnation cultivar were treated with humic acid for better rooting. The rooted cuttings were transplanted to 125 ml plastic pots containing sandy loam soil sterilized with an autoclave. The seedlings were watered daily for the duration of the experiment.

Table 1. The type and company of carnation varieties used in this study

Variety	Type	Company
Turbo	Standard	Santa Maria
Betsy	Standard	Selecta
Nirvana	Spray	Selecta
Glaciar	Spray	Dümmen Orange

Root-knot nematodes

Meloidogyne incognita, *M. javanica* and *M. arenaria* isolates were used in this study. They were identified in the previous study (Devran and Söğüt, 2009). The isolates have been cultured in our laboratory for a long time.

Nematode culture

The isolates were multiplied on susceptible tomato cv. Tueza F1. Tomato seedlings were inoculated with 1000 J2 according to Özalp and Devran (2018). Then, tomato plants were maintained under controlled conditions (25 ± 1 °C temperature, 16-h light/8-h dark photoperiod and 60 ± 5% relative humidity). Eight weeks later, plants were removed from soil. The egg masses were collected from roots of tomato infected using a needle and hatched at room temperature. After then J2s were counted under a light microscope They were stored in the refrigerator at 4ºC for 2 days until inoculation.

Nematode inoculation and experimental design

Carnation cultivars were separately inoculated with 500 *M. incognita*, *M. javanica* and *M. arenaria* J2. The studies were performed as a completely randomized block design with 5 replications and repeated twice. Carnation cultivars were incubated in the growth chamber under the conditions mentioned above and harvested 8 weeks after inoculation. The roots of plants were washed free of soil individually under tap water. Each root system was stained with phloxine B and assessed based on 0-10 Zeck-scale (0=no galls, 1=very few small galls, 2=numerous small galls, 3=numerous small galls of which some are grown together, 4=numerous small and some big galls, 5=25% of roots severely galled, 6=50% of roots severely galled, 7=75% of roots severely galled, 8=no healthy roots but plant is still green, 9=roots rotting and plant dying, 10=plant and roots dead) (Zeck, 1971).

Statistical analyses

Data on nematode infestation scores were analyzed by ANOVA. The significance of the differences among mean values was tested with Tukey's test at the $P \leq 0.05$ significance level. The analysis was carried out with SAS (v. 9.0 for Windows; SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Production of quality carnation against RKNs is quite important for commercial flower growers. In this study, the response of four carnation varieties grown widely in Turkey was investigated to isolates of *M. incognita*, *M. javanica* and *M. arenaria*. Reactions of Turbo and Betsy, which are cultivars of standard type, were found to be significantly different to *M. incognita*, *M. javanica* and *M. arenaria*. Turbo was resistant to three root-knot nematodes, but Betsy cultivar was susceptible to these species (Table 2). Therefore, Turbo cultivar may provide positive contribution to the management and can be used for infected fields with three *Meloidogyne* species. The reaction of *M. incognita* on Betsy cultivar was statistically different than *M. javanica* and *M. arenaria. M. incognita* and *M. javanica* developed slightly on Nirvana cultivar but *M. arenaria* did not develop on this variety and did not produce egg mass or gall on Nirvana (Table 2). These results indicated that the *M. incognita* and *M. javanica* penetrated the roots of the plant but few individuals completed their life cycle. Glaciar cultivar was susceptible to *M. incognita*, *M. javanica* and *M. arenaria.* Glaciar cultivar was more susceptible than Betsy cultivar to root-knot nematodes tested (Table 2).

Table 2. Response of carnation varieties to root-knot nematodes

Nematodes	Carnation variety			
	Standard type		Spray type	
	Turbo	Betsy	Nirvana	Glaciar
Meloidogyne javanica	0.00 a*	2.70 a	0.20 a	5.12 a
Meloidogyne incognita	0.00 a	5.70 b	0.40 a	6.00 a
Meloidogyne arenaria	0.00 a	2.66 a	0.00 a	5.44 a

*Data are means of ten replications. Mean values within a column followed by the same lower case letter are not significantly different (P=0.05) according to Tukey's test.
Plant roots were evaluated according to 0–10 scale proposed by Zeck (Zeck, 1971)
0=no galls, 1=very few small galls, 2=numerous small galls, 3=numerous small galls of which some are grown together, 4=numerous small and some big galls, 5=25% of roots severely galled, 6=50% of roots severely galled, 7=75% of roots severely galled, 8=no healthy roots but plant is still green, 9=roots rotting and plant dying, 10=plant and roots dead.

It is necessary to have knowledge about the reactions of carnation cultivars to *Meloidogyne* species for the management practices. However, there is limited information about the screening of carnation cultivar to root knot nematodes. Fawzy et al. (1991) reported that two carnation cultivars were resistant to *M. incognita*. In another study, Cho et al. (1996) showed that seven, twelve and fourteen carnations were resistant, moderately resistant and susceptible to *M. incognita*, respectively. In this study, we determined that standard carnation variety Turbo was resistant to *M. incognita*, *M. javanica* and *M. arenaria* and spray carnation variety Nirvana was resistant to *M. arenaria*.

Turkey is one of the most significant countries in terms of carnation production. In this study, relationship between nematode species and mostly used carnation cultivars was determined. Resistant carnation cultivars can be effective for the management of RKNs. The data could be used to develop new carnation cultivars, resistant to *Meloidogyne* species in breeding programs. Therefore, more detailed studies are required for development of resistant carnation cultivars against nematodes.

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 they have not been published before.

Ethical approval

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Investigation of appropriate grafting method and plant applications to increase grafting success in cucumber

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Abstract
In grafted seedling production, in addition to the compatibility and performance of the rootstock, the correct selection of the grafting method and the treatments to the rootstocks nd scion are effective on the success of the graft. A three-stage trial was conducted to determine the appropriate grafting method, the effect of root cutting, and some treatments on grafting success in cucumber (*Cucumis sativus* L.). In Experiment I, it was aimed to determine the most appropriate grafting technique for cucumber by using single cotyledon, hole insertion, and tube grafting techniques. The effect of rooted and rootless grafting on grafting success and seedling growth in Experiment II was determined by using the most appropriate grafting technique determined in Experiment I. In experiment III, the effect of sucrose, IBA (Indole-3-butyric acid) and antitranspirant applications on rootstocks on the success of grafting was determined. The graft success rate of the grafted plants was evaluated 14 days after grafting. While the most appropriate grafting technique was the single cotyledon grafting method with a success rate of 76%, the grafting success rate was 67.8% and 55.6% in hole insertion and tube grafting methods, respectively. The effect of grafting with rooted or rootless rootstock on grafting success was found to be insignificant. The highest stem fresh and dry weight were recorded in rooted grafting with 28.00 and 2.30 g/plant, respectively. The highest root fresh and dry weights were found in rooted grafting with 19.30 and 1.93 g/plant, respectively. In Experiment III, the highest grafting success was obtained from sucrose+antitranspirant (98.82%) and sucrose+antitranspirant+IBA (97.65%) applications, respectively. The lowest grafting success was determined in antitranspirant (74.86%) and control (78.24%) applications. According to the results te highest grafting success was achieved by using rooted rootstocks and single cotyledon grafting method. In addition, the combined application of sucrose and antitranspirant and the triple combination of sucrose, antitranspirant, and IBA to rootstocks before grafting is recommended because they increase the success of grafting in cucumber.

Keywords
Cucumis sativus, Rootstock, Auxin, Carbohydrate, Grafting success rate

Introduction
Cucumber (*Cucumis sativus* L.) is an important vegetable belonging to the Cucurbitacea family, which is grown in greenhouses and open fields worldwide. World cucumber production is 91.258.272 tons on an area of 2.261.318 hectares. The cucumber production of Turkey is 1.926.883 tons (FAO, 2020). In greenhouse and open field cucumber cultivation, especially in arid regions, some abiotic stress factors are encountered due to excessive mineral fertilizer application, high pH water use, soil salinity and high temperatures in summer (Fan et al., 2020; Phogat et al., 2020). The negative impact of such stress factors is expected to increase day

by day due to climate change. Abiotic stresses, in general, cause yield losses in agricultural areas of the world and this makes agricultural areas unusable. Up to now in cucumber plants; the effects of many abiotic stresses such as alkaline stress (Nie et al., 2018), drought (Li et al., 2018), salinity (Zhang et al., 2020) and low-high temperature stress (Ali et al., 2018) were examined. However, while these studies focus on a single stress, global climate changes cause multiple stresses in various combinations (Thomas-Barry et al., 2021). Plants are stable organisms and have gradually developed defense systems to protect themselves from environmental stresses (Isah, 2019). In addition, various agricultural practices contribute to adapting to environmental stresses (Singh et al., 2021). One of the approaches used in agriculture to overcome abiotic stresses is grafting on suitable rootstocks (de Oliveira et al., 2021). Inoculation is considered one of the most important cultural techniques that can save time and cost in the fight against abiotic stress factors in breeding programs (Bithell et al., 2013). Vegetable grafting; it is a technique that enables special plant parts (rootstock, scion) to be grown as a single plant by combining them with a suitable technique under appropriate conditions. For this reason, both the scion and rootstock have an effect on the performance of the plant (development, yield, quality, tolerance to stress conditions) in different conditions (Etehadnia et al., 2008). Although grafting is a cultural process that is usually applied to edible vegetables (watermelon, tomato, cucumber, eggplant, melon and pepper), it can also be done in plants whose leaves are consumed recently, such as basil. As rootstocks, genotypes with the desired characteristics within the species can be used, as well as different species and hybrid rootstocks within or between species. Watermelon, melon and cucumber can be grafted onto their wild forms or pumpkin rootstocks (bottle gourd, pumpkin, winter squash, luffa, ficifolia) (Lee, 1994; Yetişir et al., 2004). Inoculation in cucumber plants can increase tolerance to abiotic stress factors, as well as increase the uptake and more effective use of water and nutrients (Sallaku et al., 2019). The choice of grafting method depends significantly on the plant type, the grower's judgment and experience, as well as the facilities available. In the world, generally the methods of approach graft (Sakata et al., 2005), curved cut graft (single cotyledon) (Lee et al., 2010) and punch graft (Lee et al., 2010) are used in cucumber. In Turkey, puncture graft and single cotyledon method are generally used as grafting method in cucumber. The grafting method that is widely used in cucumber seedling plants in our country is the single cotyledon grafting method (Yetisir, 2017). The single cotyledon grafting method is preferred more than other grafting methods because the grafting technique is simple and the grafting can be done faster, and the labor cost is less than other grafting methods. In cucurbits, different grafting techniques are also used, such as using rooted and rootless rootstocks. However, using these grafting techniques (rootless rootstock or rooted rootstock) has several advantages and disadvantages (Lee & Oda, 2002). Rootless grafting method has advantages such as enabling fast and easy grafting for some vegetables, adjusting the rootstock size during grafting for seedling homogeneity and being more hygienic. On the other hand, the disadvantages of this method are; the prolongation of the graft union period, the delay in root formation during the healing of the graft, and the slow vegetative growth in general (Ulaş et al., 2019). Because both graft union and root growth require energy, cutting the roots can allow the energy stored in the rootstock to be used for graft union, resulting in increased graft success (Penny et al., 1976; Lee, 1994; Memmott & Hassell, 2009).

For graft combination, newly grafted plants are kept in low light conditions for at least 3 days, under which conditions the synthesis of new carbohydrates is limited; therefore, the grafted seedling is dependent on stored carbohydrates for survival (Memmott & Hassell, 2009). When both the rootstock and cuttings are cut, the transport of water, phytohormones and carbohydrates stops. The success of grafting depends on the development of vascular tissue (xylem and phloem) and reconnection between rootstock and scion (Pina & Errea, 2005; Aloni et al., 2010; Melnyk, 2017). Carbohydrates in rootstock play a crucial role in callus formation and cellular differentiation that connects vascular bundles at the graft interface (Ogata et al., 2005); According to Rapaka et al. (2007) removing both cotyledon leaves during grafting causes the plant to decrease the amount of stored carbohydrates. The decrease in the amount of carbohydrates reduces the rate of cell division, and as a result, the fusion of tissues is prevented and the graft retention rate decreases (Asahina et al., 2002). On the other hand, since tomato (*Solanum lycopersicum*) and other Solanum species have higher carbohydrate levels in the hypocotyl, the graft retention rate with the tube grafting method is quite high (>95%) (Davis et al., 2008). By applying sucrose solution to rootstock seedlings before grafting, the carbohydrate level in hypocotyl of rootstock could be increased, resulting in an increased percentage of graft retention in watermelon. Dabirian and Miles (2017) reported that sucrose and antitranspirant solution applications to rootstock seedlings before grafting process increased the success of grafting. Abscisic acid (ABA) and antitranspirant products can close the stomata by penetrating the leaf and manipulating the ABA signaling pathway and reduce Scion transpiration (Nitzsche et al., 1991). Reducing scion transpiration during the vascular connection between rootstock and scion during graft fusion is crucial for grafted plant survival (Dabirian & Miles, 2017; Devi et al., 2020). Phytohormones such as auxin and cytokinin are reported to affect the formation and proliferation of callus and new vascular tissues by promoting cell division and/or cell differentiation (Salisbury & Ross, 1992; Preece & Read, 2005). The cotyledons are an important source of auxin, and auxin produced in the cotyledon affects the percentage of graft retention in plants (Bhalerao et al., 2002; Procko et al., 2014; Nanda & Melnyk, 2018). The aim of the study is to determine the effects of three different grafting methods and rooted and rootless grafting, as well as sucrose, auxin and antitranspirant applications to the rootstock before grafting on the success of grafting in cucumber.

Materials and Methods

The study was carried out in the seedling unit of the climate-controlled and fully automated Venlo type glass R&D greenhouse of Kırşehir Ahi Evran University in 2022. Research subjects; grafting method (Experiment I), rooted and rootless grafting (Experiment II), and the effects of different applications on rootstocks on grafting success (Experiment III). The detailed information of the study subjects and the number of plants that were grafted are given in Table 1.

Table 1. Detailed information on the factors used in the experiments and the number of grafted plants.

Experıment	Treatments	Number of grafted plants (number)
Experıment I	Single cotyledon graft	90
	Hole insertion graft	90
	Tube graft	90
Experıment II	Rooted grafting	90
	Rootless grafting	90
Experıment III	Control (Tap water)	60
	Sucrose (S) 2% (w/v)	60
	Antitranspirant (A) 2% (v/v)	60
	Auxin-Indole-3-butyric acid (IBA) 20 mg/l	60
	S+IBA	60
	S+A	60
	A+IBA	60
	S+A+IBA	60

Antitranspirant 2%(V/V): Prohexadione-Calcium 'Velonta' Trade Name (10% Pro-Ca, Basf, Ludwigshafen, Germany), Auxin-Indole-3-Butyric Acid 20 Mg/L (IBA), Waltham, Ma,USA), S+A:(Sucrose 2% (W/V)+Antitranspirant 2% (V/V)), S+IBA:(Sucrose 2% (W/V)+Auxin-Indole-3-Butyric Acid 20 Mg/L), A+IBA:(Antitranspirant 2% (V/V)+Auxin-Indole-3-Butyric Acid 20 Mg/L), S+A+IBA:(Sucrose 2% (W/V)+ Antitranspirant 2% (V/V)+ Auxin-Indole-3-Butyric Acid 20 Mg/L). Sucrose antitranspirant and auxin solutions were applied by dissolving them in tap water.

Plant material and grafting process

The study, Meloni hybrid cucumber cultivar was used as a scion and Kublai hybrid (*C. maxima* x *C. moschata*) was used as rootstock. Scion and rootstock seeds were sown in 45-cell viols, in a mixture of 2:1 peat and perlite (EC: 0.36 dS m-1, pH: 6.0-6.5) with 5-7 days intervals, with the rootstock and the scion being the scion first. Grafting was done when the rootstock and scion reached the stage of 1-2 true leaves.

Grafting methods (Experiment I)

In experiment I, single cotyledon graft, hole insertion graft and tube graft method were used as graft methods. In single cotyledon graft, the growth point of the rootstock, which has reached the grafting stage, was removed and a 8-10 mm long cut with a slight inclination was made at the growth point of the cotyledon leaf from one to the other. The hypocotyl of the scion was cut with the same slope and the cutting surfaces were attached with the help of a grafting forceps and the grafting process was completed (Oda et al., 1993; Davis et al., 2008).

Hole insertion graft, the first true leaf and growth point of the rootstock were removed, a 1.4 mm diameter metal hole was drilled with a pointed tip, and both sides of the hypoquitila were cut at an angle of 35°-45° 1-2 cm below the cotyledon leaves. It was built by placing it (Yetisir, 2001). The tube graft was cut inclined from under the cotyledon leaves of the rootstock at the 1-2 leaf stage, and then the graft tube was placed on the cut rootstock, half empty from above. The scion, which was cut at the same angle from about 1 cm below the cotyledon leaves, was made by placing it on the empty part of the tube in such a way that the cutting surfaces were completely in contact (Figure 1).

Rooted and rootless grafting (Experiment II)

In experiment II, the rooted and rootless grafting method was used, according to the results of experiment I, the single cotyledon graft method, which had the highest success rate of grafting. In rooted grafting, grafting was done on the viol where the rootstocks were. In rootless grafting, rootstock roots were cut from the point where the hypocotyl ended and replanted in viols containing a 2:1 mixture of peat and perlite after the grafting process was completed (Figure 1).

Sucrose, Antitranspirant and IBA application (Experiment III)

In Experiment III, single choyledon and rooted grafting methods were used according to the results of Experiments I and II. Experiment III, sucrose, antitranspirant and Auxin-IBA were applied before grafting on rootstock seedlings. 6 days before grafting on rootstock seedlings, 50 ml of sucrose (2%) was applied per plant. The sucrose application was diluted with water containing sucrose (2%) in 3 times, 20 ml 6 days before the grafting, 20 ml 4 days before and 10 ml 2 days before the grafting. Antitranspirant (2%), which slows down the plant transpiration rate, was used at 50 ml per plant before grafting on rootstock seedlings. Antitranspirant was given by spraying on rootstock leaves in 20 ml 6 days before grafting, 20 ml 4 days before and 10 ml 2 days before grafting. Auxin (IBA) 10 mg/l was used. Auxin application was given by spraying 4 ml 6 days before grafting, 4 ml 4 days before and 2 ml 2 days before grafting. The leaves of the control plants were sprayed with the same amount of distilled water.

Figure 1. Grafting techniques, a) single cotyledon grafting, b) hole insertion grafting, c) tube grafting, and d) rootless grafted seedling

After all grafting processes completed, the grafted plants were placed in the post-grafting care unit, which was shaded with 90-95% relative humidity, 22-25 ºC temperature and 50% light transmission shade material by spraying water on the grafted plants. three days after grafting, the shade material and plastic inoculum containers were opened in the morning and evening, this exercise was done for 7-10 days, and on the 10th day the plants were removed from the grafting unit.

Plant Measurements and Data Analysis

Determination of grafting success rate (%) values

The graft retention rate of the grafted plants was evaluated 14 days after grafting. Scion and rootstock leaves erect or parallel to the stem and robust marketable plants were considered viable. Plants with severely wilted scion and rootstock were considered dead. Grafting success rate was calculated with the following formula = (Number of live plants/total number of grafted plants) × 100%.

Researching of hypocotyl, Cotyledon, First true leaf, stem, root, fresh and dry weight characteristics

Researching of hypocotyl, cotyledon, first true leaf, stem, root fresh and dry weights features was carried out in three replications and 20 plants in each replication, in a total of 60 plants.

Hypocotyl length (mm)

The distance between the soil surface and the horizontal cotyledon leaves was measured with a digital caliper and recorded as the length of the hypocotyl when the zucchini seedlings were at the grafting stage.

Hypocotyl thickness (mm)

The cotyledon, which is the graft junction, was measured with a digital caliper just below the leaves and parallel to the leaves and recorded as hypocotyl thickness.

Cotyledon length (mm)

The distance from the junction of the cotyledon leaves with the plant body to the tip of the leaf was measured with a digital caliper and recorded as the cotyledon length.

Cotyledon width (mm)

The leaf width at the midpoint of the cotyledon leaves was measured with a digital caliper and recorded as the cotyledon diameter.

First true leaf width (mm)

Just before the first true leaf grafting, the width in the middle part of the leaf was measured with a digital caliper.

Root and stem fresh and dry weights (g)

14 days after grafting, the grafted plants were separated into their organs as roots and stems, their fresh weight was measured with the help of a digital scale Dry weights were determined after three days in an oven at 65°C.

Data Analysis

The data obtained in the research were subjected to variance analysis according to the randomized plot design with the IBM SPSS statistical program, and the parameters with significant F values were grouped according to Duncan test at 5% and 1% significance level. The averages shown with the same letter in the tables are statistically in the same group. Correlation analysis was performed using SPPS computer package statistical program on the data obtained from seedling characteristics and grafting success measurements (SPSS, 2013).

Results and Discussion

In the study carried out to determine the most suitable grafting technique for cucumber (Experiment I), single cotyledon, hole insertion, and tube grafting techniques were compared. For each grafting method, totally 90 plants were grafted. The grafting was successful in 69 plants in the single cotyledon grafting method, 50 plants in the tube grafting method and 61 plants in the perforated grafting method. The highest grafting success rate was 76.7% in the single cotyledon grafting method, followed by the hole insertion graft method with 67.8%. The lowest grafting success was determined in the tube grafting method with 55.6% (Table 2). Since the cost of grafted cucumber seedlings is higher than that of ungrafted seedlings, the survival rate after grafting should be high. The grafting techniques used, rootstock/scion combination, pre- and post-grafting care conditions affect the success of the graft in cucumber. Single cotyledon grafting method is preferred in cases where rootstocks have thin stems, especially watermelon, cucumber and melon (Sakata et al., 2007). This method is done when the rootstock and

scion are of similar size and the first true leaf of the rootstock has started to develop (Oda, 1999; Lee & Oda, 2002). Most cucurbits can be grafted onto several rootstocks, and incompatibility can be corrected using appropriate grafting methods and growing media. Rojas and Riveros (1999) reported that the grafting method and the variety used significantly affect the success of the graft. Oda et al. (2001) reported that grafting methods in cucumber, the number of cotyledons left on the rootstock, the width of the graft surface cutting area and the compatibility of the diameters of the rootstock and scion affect the success of the graft. Oda et al. (2001) reported that grafting methods in cucumber, the number of cotyledons left on the rootstock, the width of the graft surface cutting area and the compatibility of the diameters of the rootstock and scion affect the success of the graft.

Table 2. Experiment I, grafting method, Number of grafted plants (number), Grafting success and rate

Grafting Method	Number of grafted plants (number)	Grafting success (number)	Grafting success rate (%)
Single cotyledon graft	90	69	76.70a
Tube graft	90	50	55.60c
Hole insertion graft	90	61	67.80b
F values:			**
F values: p < 0.01 (**) and N.S. Non Significant			

According to experiment I results, it was determined that the most appropriate grafting method in cucumber was the single cotyledon grafting method. In experiment II, the plants were grafted with and without roots, with 90 plants in each, using the single cotyledon grafting method. While grafting of 69 plants was successful in rooted grafting, grafting of 70 plants was successful in rootless grafting. However, the effect of rooted and rootless grafting on the success of the grafting was not found to be statistically significant. When the effect of rooted and unrooted grafting on stem fresh and dry weight was examined, the highest stem fresh weight (28.00 g) and stem dry weight (2.30 g) were obtained in rooted grafting. In rootless grafting, the fresh weight of the stem was 16.30 g and the dry weight of the stem was 1.33 g. When root fresh and dry weights were examined, the highest values were obtained in root grafting in the same way. In rooted grafting, root fresh weight was measured as 19.30 g and root dry weight was measured as 1.93 g. In rootless grafting, these values were determined as 13.00 g and 1.33 g, respectively (Table 3). Rootless grafting increases the amount of primary roots and as a result, the plant develops vigorously as it increases the plant's tolerance to cold and heat (Lee & Oda, 2002). Today, more than 40% of watermelon grafting is done by this method (without roots) in Japan (Devi et al., 2021). The survival rate in rootless grafted plants is 11% lower than in rooted grafting and rootless grafting has advantages such as preventing the risk of contamination with grafting machines and rapid grafting (Devi et al., 2021). Ulaş et al. (2019) suggested that grafting on rootless rootstocks for growth and physiology of watermelon plants grown under hydroponics conditions is not a useful implementation strategy, even if they are grafted on strong rootstocks. In our study, it was observed that rootless grafting of cucumber did not have a negative effect on the success of the graft. For this reason, rootless grafting method can be recommended as it will be beneficial in preventing the risk of contamination in grafting machine/robot applications and will reduce the aged/rolled root ratio.

Table 3. Experiment II, Number of rooted and rootless grafted plants, Grafting success and rate, Stem fresh weight, Stem dry weight, Root fresh weight and Root dry weight.

Rooted, Rootless Grafting	Number of grafted plants (number)	Grafting success (Number)	Grafting success rate (%)	Stem fresh weight (g/plant)	Stem dry weight(g)	Root fresh weight (g)	Root dry weight (g)
Rooted graft	90	69	76.70	28.00a	2.30a	19.30a	1.93a
Rootless graft	90	70	77.70	16.30b	1.33b	13.00b	1.33b
F values:			N.S	**	**	**	**
F values: p < 0.01 (**) and N.S. Non Significant							

Considering the effects of the applications on the success of grafting in experiment III, the highest graft success was obtained from sucrose+antitranspirant (98.82%) and sucrose+antitranspirant+IBA (97.65%) applications, respectively. The lowest grafting retention rate was obtained from antitranspirant (74.86%) and control (78.24%) applications (Figure 2). In the effect of applications on hypocotyl thickness, the highest hypocotyl thickness was determined in IBA application alone (4.61 mm), while the lowest hypocotyl thickness was determined in plants with sucrose application alone (3.66 mm) and in rootstocks that were not treated (3.69

mm). The highest hypocotyl length was measured in untreated (control) rootstocks (40.91 mm) and antitranspirant +IBA (40.81 mm) application, while the lowest was measured in antitranspirant alone (31.89 mm) application. The highest cotyledon width was obtained in sucrose+antitranspirant (43.69 mm), antitranspirant+IBA (42.52 mm) and sucrose+antitranspirant +IBA (40.56 mm) applications. The highest cotyledon length was obtained from antitranspirant alone (58.32 mm) and antitranspirant+IBA (57.58 mm) applications. The first true leaf width was highest in IBA alone (80.05 mm) application, while the lowest first true leaf width was determined in sucrose+antitranspirant (40.78 mm) application (Table 3).

Karaağaç (2013) reported that the short hypocotyl length is due to the genetic structure of the plants. Hypocotyl length is less important than hypocotyl thickness in terms of grafting success. However, the hypocotyl lenght should not be too short for easier grafting. In addition, there are disadvantages such as slipping in the grafting site and lying on its side, since the grafting apparatus will be attached higher in rootstocks with long hypocotyls (Oda, 1994; Hamamoto & Oda, 1997; Yang et al., 2012; Yıldız & Balkaya, 2016). Hypocotyl lengths can be controlled by applying different chemicals and regulating environmental conditions (Yang et al. 2012). Many researchers reported that the difference between the hypocotyl thickness and the number of vascular bundles among rootstock and scion did not have a significant effect on graft success (Edelstein et al., 2004; Yetişir & Sarı, 2004; Yetişir et al., 2007). Hypocotyl thickness at the time of graft; it may vary depending on the grafting time of the rootstock, the grafting method, the light and temperature values of the environment where the seedlings are grown (Yıldız & Balkaya, 2016). Since the first true leaf is removed in the single cotyledon grafting method, the carbohydrate reserve of the first true leaf cannot be utilized. Therefore, the over development of the true leaves of the rootstock seedlings before grafting may adversely affect the success of the graft. By applying sucrose solution to rootstock seedlings before grafting, the carbohydrate level in rootstock hypocotyl can also be increased, resulting in increased success of watermelon grafting (Dabirian & Miles, 2017). According to Devi et al. (2020) reported that the grafting success of plants applied sucrose and antitranspirant solutions before grafting to rootstock seedlings was 91%, 2% sucrose application alone was 67%, and plants irrigated with only water were 25%. Researchers also reported that the success of the graft was 70% when they applied a different sucrose-free antitranspirant solution to the plants. Antitranspirant products containing abscisic acid (ABA) enter the leaf, manipulate the ABA signaling pathway and cause stomatal closure. As a result, scion-rootstock transpiration is reduced (Nitzsche et al., 1991; Grill & Ziegler, 1998; Hetherington, 1998). In addition to carbohydrates and ABA, other phytohormones play an important role in graft success (Kümpers & Bishopp, 2015; Melnyk, 2017). Previous studies have shown that phytohormones such as auxin and cytokinin stimulate cell division and/or cell differentiation, triggering the proliferation of callus and new vascular tissues (Salisbury & Ross, 1992; Preece & Read, 2005). The cotyledons are an important source of auxin, and auxin derived from the cotyledon promotes graft union in young plants (Bhalerao et al., 2002; Nanda & Melnyk, 2018). Since scar tissue is opened in plants during grafting, external auxin application to the plant increases the rate of cell division and supports graft union (Procko et al., 2014).

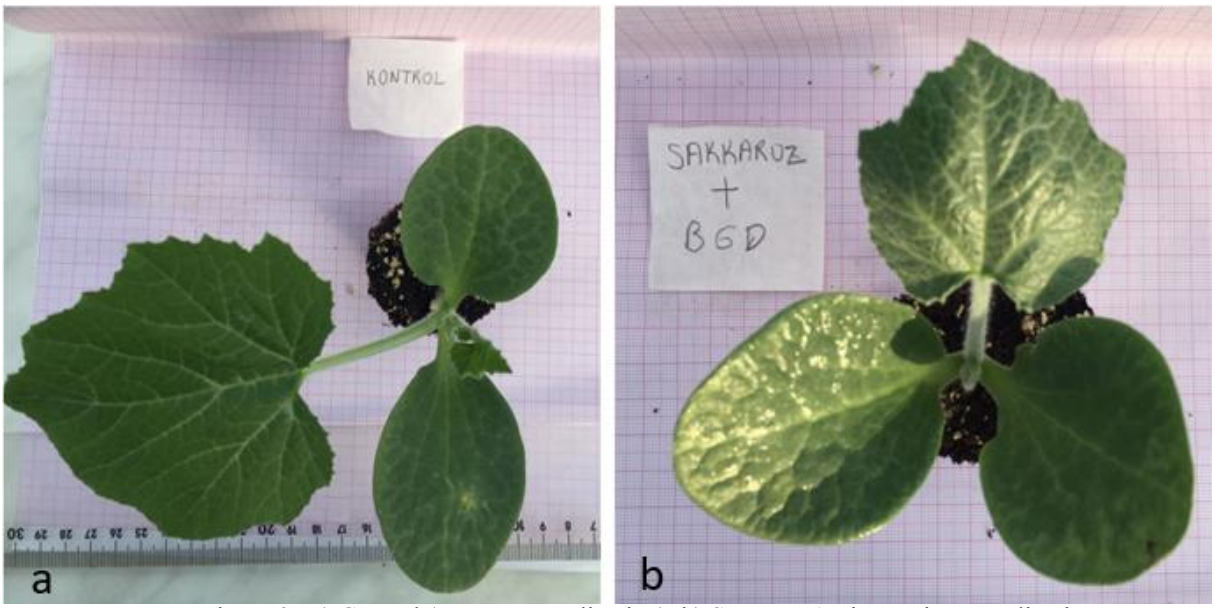

Figure 2. a) Control (tap water application), b) Sucrose+Antitranspirant application

In the study, the highest graft success was obtained in sucrose + antitranspirant and sucrose + antitranspirant + IBA applications. The reason for this is that the cotyledon widths of the rootstock with sucrose and antitranspirant application are higher than the other applications and the first true leaves are small. The width and length of the cotyledon leaves increased the success of graft union, since cotyledons are the carbohydrate storage of the plant and grafting was done using a single cotyledon in the single cotyledon grafting method.

Table 4. Experiment III, Effects of treatments on hypocotyl thickness, hypocotyl length, cotyledon width, cotyledon length, first true leaf width and graft success rate (%)

Treatment	Hypocotyl thickness (mm)	Hypocotyl length (mm)	Cotyledon width (mm)	Cotyledon length (mm)	First true leaf width (mm)	Graft success rate (%)
Rootstocks Kublai Control(Tap Water)	3.69d	40.91a	39.65cd	54.60	67.60b	78.24e
Sucrose	3.66d	35.70ac	41.84a-c	50.29	52.88c	85.29d
IBA	4.61a	41.25a	38.37d	53.54	80.05a	85.88d
Antitranspirant	3.87bcd	31,89c	42,79bd	58.32	65.81b	74.86f
S+IBA	3.67d	38,40ab	40,63a-d	51.06	49.12cd	91,43c
S+A	3.73cd	32.99bc	43.69a	55.77	40.78d	98.82a
A +IBA	4.21b	40.81a	42.52ab	57.58	65.04b	93.71b
S+IBA+A	4.07bc	38.49ab	40.56a	54.42	44.52cd	97.65a
F values	**	**	**	N.S.	**	**

F values: $p < 0.05$ (*), $p < 0.01$ (**) and N.S. Non Significant

The relationships between the measured seedling characteristics and the graft success rate were evaluated in terms of statistical significance based on the error margin of $P \leq 0.05$ and $P \leq 0.01$ (Table 5). A significant positive correlation was determined between hypocotyl thickness and cotyledon length, true leaf width, and graft success rate (correlation coefficients 0.325*, 0.447**, 0.298*, respectively). There was a significant but negative correlation between hypocotyl length and cotyledon width, cotyledon length and graft success rate (correlation coefficients were 0.547**, 0.403**, 0.840**, respectively). A significant positive correlation was found between cotyledon width and cotyledon length and graft success rate (correlation coefficients; 0.456**, 0.889**, respectively). The correlation value between cotyledon length and graft success rate is 0.335*. While a significant positive correlation was observed between the graft success rate and the hypocotyl thickness, cotyledon width and cotyledon length values (correlation coefficients were respectively; 0.298*, 0.889**, 0.335*), the same feature; showed a significant but negative correlation with hypocotyl length (correlation coefficient; 0.840**).

Table 5. Correlation coefficients between seedling characteristics and graft success rate parameters

	Pamaterers	1	2	3	4	5	6
1	Hypocotyl thickness	1	0,169	0,251	0,325*	0,447**	0,298*
2	Hypocotyl length		1	-0,847**	-0,403**	-0,007	-0,840**
3	Cotyledon width			1	0,456**	-0,199	0,889**
4	Cotyledon length				1	0,02	0,335*
5	First true leaf width					1	-0,109
6	Graft success rate						1

F values: $p < 0.05$ (*), $p < 0.01$ (**) Significant

Conclusion

Since the cost of grafted cucumber seedlings is much higher than that of ungrafted seedlings, the survival rate after grafting should be high. The low graft success rate is the biggest barrier to the use of grafted plants in most countries as it exacerbates the high cost. The grafting techniques used, rootstock/scion combination, pre- and post-grafting care conditions affect the success of the graft in cucumber. According to our study results, the highest graft success in cucumber; it was obtained in rooted grafting method with single cotyledon graft and sucrose+antitranspirant and sucrose + antitranspirant + IBA applications to rootstocks before grafting. In the future, studies should be carried out to increase the carbohydrate reserves in the hypocotyl and cotyledon of cucurbit rootstocks, as well as to improve the optimum environmental conditions such as temperature, relative humidity and light in the post-grafting care room and to increase the rooting rate of rootless grafted plants.

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 they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

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Consent for publication

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Selection for high yield and quality in half-diallel bread wheat F_2 populations (*Triticum aestivum* L.) through heterosis and combining ability analysis

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Abstract

The study was carried out to evaluate the combining ability and heterosis of seven parents and their 21 half-diallel F_2 populations for yield and quality traits during the 2013-14 season in the randomized complete block design with three replications. Significant differences were observed among the genotypes, GCA (general combining ability) and SCA (specific combining ability) effects for all traits, except spike length. The best combiner parents were identified as Esperia and Pehlivan for grain yield, and Flamura85, Aldane and Selimiye for quality traits because of significant GCA and per se performance. The ranges of best parent heterosis were -12.71 to 8.23% for plant height, -15.46 to 8.36% for spike length, -16.62 to 24.80% for number of grains per spike, -23.61 to 36.50% for grain weight per spike, -17.13 to 8.84% for harvest index and -44.26 to 15.83 for grain yield, -17.61 to 8.38% for thousand grain weight, -18.55 to 8.44% for wet gluten content, -33.80 to 24.78% for gluten index, -20.24 to 15.23% for Zeleny sedimentation value, -15.58 to 10.00% for quality index and -8.96 to 6.87 for grain protein content. The three (Slm/Phl, F85/Slm and F85/Esp) and seven (F85/Phl, Sb/Fs, Fs/Slm, Esp/Slm, Fs/Phl, Esp/Sb and Sb/Slm) of F_2 populations are offered a good opportunity in base material for selection of potential because of significant SCA effects and best parent heterosis for grain yield and quality traits respectively.

Keywords

Bread wheat, Combining ability, Heterosis, Grain yield, Quality

Introduction

Wheat is not only called the 'King of Cereals', but also the 'Stuff of Life' due to its worldwide spread, high cultivation area, high productivity and is the most basic food (Sharma et al., 2019). Therefore, it is a staple grain for world food security, poverty reduction and livelihoods (Rahul, 2017).

It is vital to increase wheat production to close the gap between production and consumption in Turkey as well as globally. By diversifying wheat breeding programs and developing new high yielding wheat varieties, large differences in wheat productivity in different regions of the country must be reduced to achieve the anticipated high productivity. In addition, the developed new cultivars should be possessed the functional attributes demanded by producers, processors, and consumers under various agro-climatic conditions. Selection of parents and determination of suitable hybrid combinations are the most important points in the development of new varieties that are superior in terms of yield and quality characteristics in combination breeding. A hybrid that is a cross of two genetically different individuals outstrips the average of the parents (heterosis) or the best parent (heterobelthiosis). This phenomenon has been successfully exploited in fibre, cereals, and oilseed crops (Ahmad et al., 2014). The magnitude of heterosis

helps in determining genetic variability and serves as a guide in the selection of desirable parents. Hybrid combinations should have superiority over the best parent, commercial use of heterosis, and high transgressive segregant formation abilities.

Breeders aim to generate populations containing desired genes and gene complexes to select suitable genotypes. Griffing (1956) developed a diallel method for determining the combining ability and explaining the nature and magnitude of gene action. In general, for plant breeding, hybrid combinations with high specific combining ability (SCA) and those with at least one parent with high general combining ability (GCA) are the most sought after (Paini et al., 1996). Combining ability describes the breeding values of parents to produce hybrids. Additive gene action relative to the average performance of a genotype in a series of hybrid combinations refers to GCA (Griffing, 1956; Singh and Chaudhary, 1985). The performance of one parent genotype in crossing combination with another parent genotype is called SCA (Mandal and Madhuri, 2016).

The main objectives of this study were to evaluate the GCA and SCA of parents and F_2 progeny from a half-diallel crosses and was to examine the heterobeltiosis for yield and quality traits and also to identify the heterotic combination which may be further exploited through heterosis breeding programme.

Material and Methods

Plant material

The genetic material, environment characteristics, field trial procedure and set-up of the experiments, are fully described by Yazıcı and Bilgin (2019). The experiment was set up with twenty-one F_2 combinations and their 7 parents (6 widely used commercial bread wheat cultivars and one advanced line) in randomized complete block design with three replicated in 2013-2014 growing year in Tekirdağ Namık Kemal University, Agricultural Faculty, Field Crops Department research area in Tekirdağ Province of Trakya Region where is located North Part of Turkey. It lies at an altitude of 10m above sea level and at 27º 34' East and 40º 59' North. The Thrace region, a peninsula, is under the typical continental climate. Average rainfall of 476mm was lower than the last fifty-year average of 521mm, most of which falls between November and June. The relatively low rainfall (0.2mm) received in June was a negative effect on the filling period. But irregularities in rainfall amounts according to the month are the most characteristic feature of the region. Temperature in summer rise to the maximum of 28.4 ºC, while winter temperature may reach as low as -0.3 ºC. It has a clayey loamy texture and enters the weak soil group according to soil analysis. Grain yield (GY) and some attributes characteristics such as plant height (PH), spike length (SL), number of grains per spike (NGS), grain weight per spike (GWS), harvest index (HI), and grain quality characters such as thousand grain weight (TGW), wet gluten content (WGC), Zeleny sedimentation value (ZSV), quality index (QI) (expressed as ZSV/GPC) and grain protein content (GPC) (for methods see Yazıcı and Bilgin, 2019) were evaluated in half-diallel crosses F_2 combinations and their parents.

Statistical analysis

The data after compilation were subjected to a simple analysis of variance technique (Steel and Torrie, 1980) using the statistical package 'MSTATC' to see whether significant differences existed among the wheat genotypes for further analysis. An ANOVA was done on the F_2 generation for each characteristic evaluated. For features where significant differences were identified, the combining ability analysis was performed in Method 2, Model 1 as proposed by Griffing (1956) using computer software 'AGD-R (2015) Version 2.0' developed by Rodriguez et al. (2015). General combining ability is used to indicate the average performance of a genotype in a hybrid combination, while SCA is used to identify situations where certain combinations perform relatively better or worse than expected, based on the average performance of the genotype concerned (Sprague and Tatum, 1942). The heterosis analysis were performed on the characters showing significant differences. The per cent increase (+) or decrease (-) of F_2 hybrids over mid as well as a best parent was calculated to estimate possible heterotic effects for the characters by using the formula of Fonseca and Patterson (1968) as:

$$Ht(\%)= \frac{F_2 - MP}{MP} x100$$

$$Hb(\%)= \frac{F_2 - BP}{BP} x100$$

Where,

Ht=Heterosis, Hbt=Heterobelthiosis, MP=Mid parent and BP=Best parent value. The "t" test was computed to determine whether F_2 hybrid means were statistically significant from mid parent and best parent means as follows (Wynne et al., 1970).

$$tij= F_2ij - MPij \Big/ \sqrt{\frac{3}{8} EMS}$$

$$tij= F_2ij - BPij \Big/ \sqrt{\frac{1}{2} EMS}$$

Where,

F_2ij=The mean of the ij^{th} F_2 cross, MPij=The mid parent for ij^{th} cross, BPij=The best parent value for ij^{th} cross, EMS=Error mean square.

Results and Discussion

The results of preliminary, GCA and SCA of ANOVA are given in Table 1. The preliminary analysis results revealed that significant ($p \leq 0.01$) differences were observed among the parental genotypes and their F_2 populations for all the traits. While the GCA mean squares of the genotypes were very important for all traits examined, the SCA mean squares were found to be insignificant only for the spike length (Table 1). The extensive genetic variation of the breeding material allows for further evaluation for GCA and SCA effects (Kempthorne, 1957). Singh et al. (2013), Verma et al. (2016) and Rajput and Kandalkar (2018) reported similar results.

Table 1. Preliminary analysis of variance of yield and quality traits for parents and F_2 progeny

SOV	Replication (2)	Genotype (27)	Error (54)	σ^2_{GCA}	σ^2_{SCA}	$\sigma^2_{GCA}/\sigma^2_{SCA}$
PH	1.17	307.66**	44.48	24.25**	1.97**	12.31
SL	0.40	1.14**	0.49	6.07**	1.23	4.94
NGS	23.89	85.80**	28.62	7.21**	1.79*	4.03
GWS	0.03	0.32**	0.09	9.01**	1.82*	4.95
HI	9.51*	64.3**	4.80	76.96**	3.84**	20.04
GY	590.08	9787.85**	1192.45	24.45**	3.57**	6.85
TGW	0.84	65.31**	6.14	39.94**	2.28**	17.52
WG	0.26	20.11**	3.26	19.50**	2.36**	8.26
GI	14.71	524.77**	14.15	117.33**	14.17**	8.28
ZSV	5.14	145.11**	7.76	76.96**	3.84**	20.04
QI	0.07	0.57**	0.04	51.51**	3.00**	17.17
GPC	0.11	0.89**	0.11	24.92**	3.81**	6.54

*significant at the %5 level, **significant at the 1% level

The genetic component of the variances can be explained through estimates of the GCA and SCA variances. It is assumed that the additive genetic variance is equal to the GCA variance and the SCA variance is the dominance variance. In our study, the magnitudes of the genetic component, the total components of the variances for all characters were found to be higher than the dominance components of the variances.

The ratio of genetic components "σ^2gca/σ^2sca" also showed more than one, indicating a predominance of additive variances for almost all characters (Table 1). The present finding is confirmed by Kandil et al. (2016), Rahul (2017) and Ali et al. (2018), were recorded the predominance of additive variances for all the traits.

Combining ability

The combining abilities of parents play a key role in the evaluation of breeding traits and assist in deciding the effective breeding method in segregation generations (Griffing, 1956; Singh and Chaudhary, 1985)

Mean performance of parents and General combining ability effects

Evaluation of GCA effects for yield components and quality traits together with average performances is of great importance in selecting parents for yield and quality improvement. The evaluated parental genotypes and their F_2 crosses exhibited a wide variation for all assessed traits. The parents' Esperia and Pehlivan exhibited the good yielding characteristics, while Flamura85, Aldane and Selimiye have been involved as a parent in most of the best performing for quality traits F_2 combinations (Table 2). Estimates of GCA effects for each parent are presented in Table 2.

The results indicated that advanced line Fs showed significant negative GCA for all studied characters except GPC. This means that Fs can be a good combiner for the sole purpose of shortening PH. Another parent that could be a good combiner for shortening PH was Saraybosna. Flamura85 showed also positive GCA for SL, TGW, GI, ZSV and QI. Esperia showed significant GCA effects for NGS and heavy GWS and TGW and more GY. Moreover, the cultivar Pehlivan showed positive GCA effects for PH, SL, GWS, HI, GY and TGW. Aldane cultivar showed significant GCA effects for grain quality traits such as TGW, GI, ZSV, QI and GPC, while these effects were negative for grain yield traits other than PH. Parental genotypes with desirable GCA effects are considered to be the best parental genotypes and good general combiners that can be exploited to improve the trait in wheat breeding (Afridi et al., 2017; Parveen et al., 2019).

Table 2. The averages and GCA effects of the parental genotypes

Crosses	Flamura85 (F85)		Esperia (Esp)		Saraybosna (Sb)		Aldane (Ald)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
PH	86.4	-0.18	82.5	-1. 28	82.3	-7. 26**	102.7	6. 25**
SL	8.37	0.33*	8.42	-0.27*	8.68	-0.04	8.81	-0.07
NGS	36.3	0.46	45.3	2.39*	50.1	2.98**	39.2	-5.13**
GWS	1.78	0.05	2.35	0.17**	2.43	0.08	1.95	-0.30**
HI	38.00	-1.68**	44.33	0.58	43.33	2.18**	36.33	-3.38**
GY	405	-22.39**	638	12.50*	453	-8.06	553	-1.10
TGW	48.03	1.84**	48.77	0.99*	40.33	-3.25**	48.47	1.12*
GI	92.67	4.60**	85.33	7.64**	55.33	-8.51**	88.67	9.19**
ZSV	55.33	1.69**	44.00	-3.27**	43.33	-2.01**	67.67	8.66**
QI	4.18	0.15**	3.51	-0.10**	3.37	-0.18**	4.66	0.49**
GPC	13.23	-0.07	12.53	-0.53**	12.87	0.11	14.53	0.51**

PH: plant height, SL: spike length, NGS: number of grain per spike, GWS: grain weight per spike, HI: harvest index, GY: grain yield, TGW: thousand grain weight, GI: gluten index, ZSV: Zeleny sedimentation value, QI: quality index, GPC: grain protein content

Table 2. The averages and GCA effects of the parental genotypes (continuation)

Crosses	F/S (Fs)		Selimiye (Slm)		Pehlivan (Phl)	
	Mean	GCA	Mean	GCA	Mean	GCA
PH	83.3	-8. 98**	103.5	5. 61**	100.4	5.84**
SL	9.08	-0.51**	9.06	0.12	8.66	0.45**
NGS	38.7	-1.38	44.3	1.50	42.6	-0.81
GWS	1.95	-0.20**	2.58	0.13*	2.23	0.07
HI	39.67	2.21**	40.33	-0.49	43.67	0.58
GY	325	-49.13**	685	11.53	645	56.65**
TGW	35.53	-5.27**	51.10	2.03**	50.13	2.53**
GI	59.33	-7.88**	84.00	2.86**	58.67	-7.88**
ZSV	43.33	-2.86**	53.67	2.25**	43.33	-4.46**
QI	3.30	-0.24**	4.06	0.17**	3.22	-0.30**
GPC	13.14	0.10	13.23	0.01	13.50	-0.13*

PH: plant height, SL: spike length, NGS: number of grain per spike, GWS: grain weight per spike, HI: harvest index, GY: grain yield, TGW: thousand grain weight, GI: gluten index, ZSV: Zeleny sedimentation value, QI: quality index, GPC: grain protein content

These results show that Esperia and Pehlivan varieties were found to be good combiners, while Selimiye and Flamura85 were average and Saraybosna, Aldane and Fs were poor combiners to increase grain yield. Although Flamura85, Aldane and Selimiye varieties are appropriate parents that can be used to increase grain quality, Esperia, Saraybosna, Fs and Pehlivan were poor combiners to increase grain quality. Consequently, our results on average performance and GCA effects for the respective characters are in agreement with those reported by Ismail (2015), Joshi et al (2020), and Abro et al (2021).

Specific Combining Ability Effects and Heterotic Performances

The SCA effect is an indication of the heterosis (interaction) for a specific trait. Heterosis is the process by which the performance of an F_1 is superior to that of the mean of the crossed parents. Generally, heterosis is manifested in a positive direction for some characters such as adaptation, yield, quality, and general vigour over its parents and in some cases it is in a negative direction for some characters such as plant height, maturity duration, earliness and toxic substances which is also desirable (Chaudhary et al., 2018). Heterosis helps the plant breeders eliminate unproductive crosses in early generations and is of considerable importance to evaluate as means of increasing the productivity of crop plants. On the other hand, Singh et al. (2004) and Zaazaa et al. (2012) especially emphasized that the superiority of heterosis hybrids over the high parent is more appropriate for commercial use, and explained that the parent combinations achieve the ability to produce the highest level of transgressive segregants in this case.

Tables 3 and 4 give the SCA effects and heterosis estimates of the grain yield and quality traits for all the crosses. Although Paroda and Joshi (1970) and Morojele and Labuschagne (2013) stated that a marked decrease in the magnitude of the SCA in the F_2 population was found, good results could still be obtained in F_2. The results of SCA effects (Tables 3 and 4) of different crosses revealed that none of the crosses showed consistently significant positive or negative and desirable SCA effects for all the characters. Data presented in Table 3 revealed that SCA effects of F_2 hybrids ranged between -7.196 and 7.537 for PH, -0.592 and 0.551 for SL, -6.603 and 5.797 for NGS, -0.311 and 0.324 for GWS, -3.213 and 3.157 for HI and -65.694 and 54.417 for GY, and 14 crosses showed a, thereby, indicating good specific combinations for grain yield and other attributing traits. SCA effect of SL were not found significant. Crosses viz., Esp/Slm, Slm/Phl and Esp/Phl registered the best specific combiner for grain yield characteristics because these crosses were the results of good x good general combiners and reported significant positive SCA effects. Crosses Esp/Ald, Esp/Fs, Esp/Sb, F85/Esp, F85/Phl, F85/Slm, Fs/Phl and Sb/Slm were the result of good x poor general combiner and also reported significant positive SCA effect for grain yield characters. The rest of the crosses viz., Sb/Fs, Ald/Fs and Sb/Ald were the result of poor x poor general combiner but exhibited significant positive SCA effect for grain/yield, thereby, suggesting good specific combiner for these traits. The estimated value of heterosis showed that the degree and direction of heterosis varied not from trait to trait but also from cross to cross. The ranges of best parent heterosis were -12.71 to 8.23% for PH, -15.46 to 8.36% for SL, -16.62 to 24.80% for NGS, -23.61 to 36.50% for GWS, -17.13 to 8.84% for HI and -45.65 to 15.83 for GY (Table 3).

Shah et al (2018), as a result of their study, emphasized that 10.0 per cent or more heterosis over the best parent in F_2 can be considered good for all of the characteristics they examined in wheat (except for the maturity characteristics of 5.0 per cent or more). There is a possibility of obtaining a low heterosis cross from high performing parents, as well as a high heterosis percentage crossing from low per se performing parents (Kumar et al., 2015). Since the SCA effect is accepted as an indicator of heterosis (interaction) for a particular trait, examining them together will allow more appropriate inferences to be made (Ceyhan and Avci, 2005). A comparative study of promising crosses identified based on heterosis and combining ability (Table 3) revealed that hybrid viz., Slm/Phl, F85/Slm and F85/Esp showed positive significant heterosis and heterobelthiosis for NGS, GWS, HI and GY. None of the crosses exhibited significant positive heterosis over the best parent for PH and SL. These results are in

accordance with those recounted by Patel et al. (2019), Sharma and Jaiswal (2020), Fleitas et al. (2020) and Abro et al. (2021). The crosses with SCA effects and heterotic performance for seed quality traits have been depicted in Table 4. The crosses having higher SCA effects in a desirable direction for different traits can be utilized to produce superior transgressive segregants for these traits. The involvement of parents with good GCA for specific traits in certain crosses can be attributed to superior SCA effects of these crosses for these traits. The mean of SCA effects changed from -2.708 to 4.962 for TGW, -2.801 to 2.910 for WGC, -17.426 to 11.982 for GI, -4.556 to 7.333 for ZSV, -0.274 to 0.491 for QI and -0.380 to 0.657 for GPC in F_2 hybrids. A total of 14 crosses showed good specific combinations for grain quality traits.

Table 3. SCA effects, heterosis and heterobelthiosis estimates for grain yield associated characters

Crosses	PH			SL			NGS		
	SCA	H_t (%)	H_b (%)	SCA	H_t (%)	H_b (%)	SCA	H_t (%)	H_b (%)
F85/Esp	-7.196**	-4.51	-4.51	0.053	2.80	0.59	1.345	18.27*	12.40
F85/Sb	-1. 422	0.86	-4.75	0.079	3.21	2.84	5.556*	24.93**	14.38
F85/Ald	-5. 467**	11.51*	5.12	0.236	5.89	5.13	2.764	11.36	7.98
F85/Fs	1. 367	8.04	-3.59	0.551	20.77**	8.36	-1.425	4.59	2.65
F85/Slm	6. 948**	13.49**	7.81	0.306	5.96	3.78	1.271	20.38*	19.08
F85/Phl	3. 644**	11.19*	6.70	-0.431	-1.81	-6.48	1.879	14.82	12.40
Esp/Sb	0. 619	1.96	-3.70	-0.275	-6.08	-8.42	5.797*	19.23*	14.61
Esp/Ald	3. 374**	8.04	1.84	0.419	3.07	1.45	-1.862	-1.88	-9.42
Esp/Fs	3. 507**	9.47	-2.32	0.174	4.91	-3.99	0.249	8.46	4.96
Esp/Slm	2. 756**	7.68	2.29	-0.254	-5.73	-9.62	1.312	19.32*	14.88
Esp/Phl	1. 319	7.42	3.08	-0.517	-8.92	-15.02**	4.186	19.69*	16.12
Sb/Ald	-4. 585**	-2.06	-12.49*	-0.592	-8.87	-9.84	-1.384	-3.84	-14.38
Sb/Fs	-4. 585**	-2.77	-8.46	0.113	4.24	-6.76	-1.140	1.71	-5.25
Sb/Slm	7. 130**	11.81*	0.63	-0.218	-4.89	-6.52	4.156	22.71**	13.47
Sb/Phl	2. 393*	7.72	-2.13	0.485	4.07	-0.54	-6.603*	-10.51	-2.91
Ald/Fs	7.537**	15.96**	-1.84	0.203	6.30	-3.99	-0.999	-6.40	-10.87
Ald/Slm	-1. 548	4.64	3.79	-0.148	-3.41	-6.07	-1.569	0.56	-3.49
Ald/Phl	1.615	9.28*	7.27	-0.301	-4.22	-9.40	-3.529	-12.22	-16.62
Fs/Slm	-3. 981**	2.32	-12.71*	-0.530	-4.03	-15.46**	2.908	17.60*	16.97
Fs/Phl	7. 015**	17.43**	0.96	0.320	7.67	7.45	1.582	7.14	6.86
Slm/Phl	1. 363	9.25*	8.23	0.446	3.55	0.64	5.579*	25.79**	24.80*

PH: plant height, SL: spike length, NGS: number of grain per spike, GWS: grain weight per spike, HI: harvest index, GY: grain yield

Table 3. SCA effects, heterosis and heterobelthiosis estimates for grain yield associated characters (continuation)

Crosses	HI			GY		
	SCA	H_t (%)	H_b (%)	SCA	H_t (%)	H_b (%)
F85/Esp	3.083**	12.72**	8.84*	34.120	29.94**	10.59
F85/Sb	0.491	2.36	-7.28*	-18.991	-14.42	-30.73**
F85/Ald	-0.954	-3.20	-4.47	13.713	11.63	-5.56
F85/Fs	-3.213**	-5.47	-13.69**	-28.917	-19.60	-19.60
F85/Slm	0.157	-0.98	-6.92	54.417**	34.31**	11.24
F85/Phl	2.417*	7.10*	0.00	-6.361	-5.10	-32.50**
Esp/Sb	0.231	3.66	-2.99	-39.546*	-21.76*	-25.25**
Esp/Ald	0.787	3.86	-0.98	-9.843	0.00	-0.72
Esp/Fs	-0.139	3.92	-2.17	51.861**	25.35**	6.68
Esp/Slm	-1.435	-2.38	-5.31	30.861	19.72*	15.83
Esp/Phl	3.157**	10.66**	6.86	-17.250	-7.89	-26.23**
Sb/Ald	-2.472*	-7.41*	-17.13**	40.713*	5.85	0.26
Sb/Fs	2.602*	6.47*	5.56	-31.250	-32.86**	-45.65**
Sb/Slm	0.306	-1.55	-5.13	20.750	0.35	-2.52
Sb/Phl	-1.102	-2.65	-5.78	-65.694**	-33.75**	-44.26**
Ald/Fs	2.491*	5.30	-5.00	-5.213	-11.48	-25.11*
Ald/Slm	-0.806	-6.21	-12.93**	-59.880**	-24.58**	-26.51**
Ald/Phl	-1.880	-6.45	-13.72**	9.676	-4.05	-22.73**
Fs/Slm	0.269	-0.89	-3.69	-39.509*	-24.85**	-37.75**
Fs/Phl	-0.472	-0.22	-2.82	-5.954	-14.76	-39.37**
Slm/Phl	-2.435*	-8.04*	-8.46*	7.713	-2.60	-19.96**

PH: plant height, SL: spike length, NGS: number of grain per spike, GWS: grain weight per spike, HI: harvest index, GY: grain yield

Table 4. SCA effects, heterosis and heterobelthiosis estimates for grain quality traits

Crosses	TGW			WGC			GI		
	SCA	H_t (%)	H_b (%)	SCA	H_t (%)	H_b (%)	SCA	H_t (%)	H_b (%)
F85/Esp	-2,708*	-1.86	-2.66	0,429	-0.30	-5.29	2,982	4.61	0.43
F85/Sb	4,962**	15.41**	6.04	-0,345	2.01	-0.56	4,463*	5.95	-15.44**
F85/Ald	1,392	7.26*	7.26	0,888	1.67	-3.69	-9,907**	-9.93**	-11.87**
F85/Fs	-0,419	4.31	-9.37*	-0,645	-1.41	-5.17	2,167	0.92	-17.27**
F85/Slm	0,188	3.83	0.58	0,140	1.67	-3.19	2,093	-1.13	-5.72
F85/Phl	2,581*	10.81**	8.38*	-0,234	-4.12	-10.05**	-13,167**	-18.91**	-33.80**
Esp/Sb	-0,549	0.00	-8.62*	2,166*	7.53*	0.00	0,759	10.38**	-9.02**
Esp/Ald	-0,453	1.03	0.61	-0,568	-4.95	-13.98**	-2,944	5.40	3.38
Esp/Fs	2,203	7.36*	-7.18*	-2,801**	-10.38**	-17.71**	9,797**	20.74**	2.34
Esp/Slm	2,777*	6.41*	3.91	-0,749	-2.93	-11.96**	6,389**	11.82**	10.90**
Esp/Phl	-0,164	2.63	1.19	-1,157	-8.93**	-18.55**	-1,537	5.70	-10.90**
Sb/Ald	0,584	3.38	-5.37	-1,375	-2.17	-5.01	9,204**	21.83**	-1.12
Sb/Fs	-0,360	1.31	-4.71	2,625**	9.94**	8.44*	-7,722**	-6.45	-9.61*
Sb/Slm	-1,319	-1.96	-12.32**	0,977	6.55*	3.72	4,537*	10.05**	-8.80*
Sb/Phl	-2,294	-1.99	-11.57**	-0,231	-1.34	-5.41	-0,389	7.20	4.09
Ald/Fs	-0,631	1.43	-12.19**	-0,108	-1.34	-2.90	6,574**	15.83**	-3.38
Ald/Slm	0,744	3.01	0.19	-0,423	-1.06	-1.58	2,833	7.30**	4.51
Ald/Phl	0,603	4.87	2.99	-0,197	-4.96	-6.18	9,241**	19.97**	-0.33
Fs/Slm	-1,934	-2.77	-17.61**	2,910**	8.89**	7.44*	-17,426**	-22.76**	-34.16**
Fs/Phl	3,692**	12.85**	-3.59	-1,897*	-8.22**	-10.82**	11,982**	25.63**	24.78**
Slm/Phl	0,132	2.76	1.76	-1,445	-6.28*	-7.73*	0,574	2.80	-12.73**

TGW: thousand grain weight, GI: gluten index, ZSV: Zeleny sedimentation value, QI: quality index, GPC: grain protein content

Table 4. SCA effects, heterosis and heterobelthiosis estimates for grain quality traits (continuation)

Crosses	ZSV			QI			GPC		
	SCA	H_t (%)	H_b (%)	SCA	H_t (%)	H_b (%)	SCA	H_t (%)	H_b (%)
F85/Esp	1,482	2.01	-8.32*	0,113	3.64	-4.55	0,057	-0.78	-3.78*
F85/Sb	-1,778	-1.22	-11.93**	-0,085	-1.85	-11.27**	-0,183	0.76	-0.75
F85/Ald	0,222	-0.33	-9.45**	0,018	1.36	-4.07	-0,013	-0.72	-5.51**
F85/Fs	0,074	0.81	-10.13**	-0,079	-2.14	-12.47**	0,294	3.81*	3.03
F85/Slm	0,963	2.20	0.72	0,069	2.18	0.71	0,028	0.00	0.00
F85/Phl	-3,333*	-9.33**	-19.17**	-0,154	-4.60	-15.58**	-0,380*	-4.51**	-5.92**
Esp/Sb	5,185**	16.02**	15.23**	0,265*	10.75**	8.54*	0,443*	5.55**	3.90*
Esp/Ald	-0,815	-1.07	-18.32**	-0,016	2.69	-10.08**	-0,054	-2.22	-8.96**
Esp/Fs	-1,296	-0.92	-1.59	-0,006	2.05	-1.13	-0,380*	-3.12*	-5.34**
Esp/Slm	-4,074**	-6.54	-14.90**	-0,198	-2.38	-8.88*	-0,380*	-3.90*	-6.81**
Esp/Phl	-0,037	-1.60	-2.27	0,070	3.86	-0.56	-0,320	-5.38**	-8.88**
Sb/Ald	-3,407*	-2.70	-20.24**	-0,234*	-2.99	-16.52**	-0,028	1.47	-4.82**
Sb/Fs	-0,889	3.93	3.93	-0,188	-3.60	-4.74	0,513**	8.52**	6.87**
Sb/Slm	7,333**	20.21**	8.57*	0,491**	15.90**	6.17	0,180	3.84*	2.27
Sb/Phl	0,370	3.23	3.23	-0,062	-0.30	0.30	0,339*	3.81*	0.74
Ald/Fs	1,111	3.96	-14.77**	0,142	5.77	-9.65**	-0,183	-0.72	-5.51**
Ald/Slm	3.000*	6.59*	-4.43	0,254*	20.35**	1.50	-0,117	-1.44	-6.20**
Ald/Phl	0,704	0.36	-17.73**	0,098	4.58	-11.80**	-0,091	-3.57*	-6.89**
Fs/Slm	0,519	4.54	-5.59	-0,140	-1.63	-10.86**	0,657**	6.87**	6.06**
Fs/Phl	3,889**	9.24*	9.24*	0,348**	11.69**	10.00*	-0,217	-2.25	-3.70*
Slm/Phl	-4,556**	-9.28*	-18.06**	-0,274*	-5.78	-15.55**	-0,283	-3.75*	-5.18**

TGW: thousand grain weight, GI: gluten index, ZSV: Zeleny sedimentation value, QI: quality index, GPC: grain protein content

Out of the total 21 crosses, 4 (F85/Sb, F85/Phl, Esp/Slm and Fs/Phl), 4 (F85/Sb, F85/Phl, Esp/Slm and Fs/Phl), 8 (F85/Sb, Esp/Fs, Esp/Slm, Sb/Ald, Sb/Slm, Ald/Fs, Ald/Phl and Fs/Phl), 4 (Esp/Sb, Sb/Slm, Ald/Slm and Fs/Phl), 4 (Esp/Sb, Sb/Slm, Ald/Slm and Fs/Phl) and 4 (Esp/Sb, Sb/Fs, Sb/Phl and Fs/Slm) crosses showed significant SCA effects in a desirable direction for TGW, WGC, GI, ZSV, QI and GPC respectively. The GPC is considered one of the most important traits from a breeding point of view as it decides the success of a particular breeding programme. Four crosses namely, Esp/Sb, Sb/Fs, Sb/Phl and Fs/Slm showed significant positive SCA effects for GPC. Crosses with significant and positive SCA effects for

GPC were also identified by Joshi et al. (2020). Cross viz., Ald/Slm registered best good specific combiner for grain quality traits because this cross was the result of good x good general combiners and reported a significant positive SCA effect. Crosses F85/Sb, F85/Phl, Esp/Slm, Sb/Ald, Sb/Slm, Ald/Fs, Ald/Phl and Fs/Slm were the result of good x poor general combiner and also reported significant positive SCA effect for grain quality traits. The rest of the crosses viz., Esp/Sb, Esp/Fs, Sb/Fs, Sb/Phl and Fs/Phl were the result of poor x poor general combiner but exhibited significant positive SCA effect for grain quality traits, thereby, suggesting good specific combiner for these traits. The significant positive and negative estimated value of heterosis over best parent was observed for all quality traits. The ranges of heterosis were -17.61 to 8.38% for TGW, -18.55 to 8.44% for WGC, -34.16 to 24.78% for GI, -20.24 to 15.23% for ZSV, -16.52 to 10.00% for QI and -8.96 to 6.87 for GPC (Table 4). A comparative study of promising crosses identified based on heterosis and combining ability (Table 4) revealed that hybrid viz., F85/Phl, Sb/Fs, Fs/Slm, Esp/Slm, Fs/Phl, Esp/Sb and Sb/Slm showed positive significant heterosis and heterobelthiosis for TGW, WGC, GI, ZSV, QI and GPC. These results, together with significant SCA effect and significant positive heterosis on the best parent, as noted by Singh et al (2004), suggest that commercial use of these hybrids may be more appropriate to improve wheat yield. The present finding is in confirmation by Thorwarth et al. (2018), Boeven and Longin (2019), and Joshi et al. (2020).

Conclusions

The study revealed the existence of a significant amount of variability amongst parental lines and crosses for almost all the traits studied except for SL for which SCA mean squares were insignificant. This shows that the improvement for all the traits except for SL can be achieved through a selection of genotypes with superior traits or by isolation of transgressive segregants. The parents Esperia and Pehlivan exhibited good yielding characteristics and significant positive GCA effects, hence it can be used for the development of lines with high yielding. On the other side, for improvement of grain quality traits such as TGW, WGC, GI, ZSV, QI and GPC, Flamura85, Aldane and Selimiye can be used as one of the parents in the hybridisation programme. A comparative study of promising crosses identified based on heterosis and SCA effects revealed that hybrids namely Slm/Phl, F85/Slm and F85/Esp for yield and yield contributing traits, while F85/Phl, Sb/Fs, Fs/Slm, Esp/Slm, Fs/Phl, Esp/Sb and Sb/Slm were the best hybrids for grain quality traits. These hybrids therefore offer an opportunity for commercial use, either as hybrid varieties or as a base material for the selection of potential homozygous lines from transgressive segregants to improve yield and quality levels of bread wheat.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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

Ethical approval

Ethics committee approval is not required.

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Physico-chemical and cooking quality traits of paddy cultivars of japonica sub-species

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Abstract

Paddy, grown on all continents of the world, is an economical source of food and a strategic cereal. Cooking quality of rice is closely related to physico-chemical characteristics of starch constituting about 90% of rice dry matter. Environmental and genetic factors are effective on rice quality and physico-chemical properties. Knowledge on these traits plays an important role in comprehension the changes encountered during cooking. This study was conducted in 2020 to determine physico-chemical and cooking quality traits of rice grains obtained from 18 paddy cultivars. Physical, chemical and cooking quality traits of 18 rice samples were analyzed in 3 replications. Significant differences were observed in investigated traits of paddy cultivars. The thousand-grain weights varied between 20.32-31.00 g; rice grain lengths between 5.88-7.28 mm; grain elongation ratios between 1.40-2.47%; grain water absorptions between 46.96-71.27%; water uptake ratios between 1.91-3.31%; cooking times between 00:17:19-00:23:28 min; protein contents between 5.45-8.89% and amylose contents varied between 16.91-26.25%. According to the biplot graph, Efe, Kale and Galileo cultivars were found to be prominent for more than one trait. The biplot graph also revealed that cooking time was the most distinctive trait. There were highly significant negative correlations between alkali spreading and cooking time parameters. Although it was banned worldwide in rice codex, different rice cultivars, classes, groups and types are compared and served to markets. Although the constellation plot generated through the use of results for investigated parameters allowed primary separation of paddy cultivars, present parameters were not found to be sufficient for a net separation of the cultivars.

Keywords

Rice, Cooking Time, Elongation Ratios, Amylose, Biplot

Introduction

Paddy constitutes the primary nourishment of more than 3 billion people worldwide. It is also consumed as baby food; thus, it is among the most significant cereal grains constituting a source of nourishment for 505 of the world population and a source of income for a hundred million small farmers (90%). According to genetic information derived from archeological excavations, paddy was cultivated between 6200 - 11500 B.C. (Suh et al., 2010). Paddy, which was started to be consumed by 2500 B.C., is thought to spread to the world from China. It is also estimated that paddy passed to Sri Lanka and India-like countries from China (Kün, 1997). An acquaintance of the western world with paddy coincides with the years 300s B.C. Paddy was thought to reach the continent of America by the end of the 17th century and reached Turkey about 500 years ago. Paddy farming is practiced in 119 countries worldwide over 162 million hectares with an annual production of 744 million tons. More than 75% of this production is realized under an aqueous production system (FAO, 2020).

Including wild species, the genus *Oryza* has 21 species. Of these species, the cultivars of *Oryza Glaberrima* species are cultivated only in West African countries, the cultivars of *Oryza sativa* species are cultivated in entire countries of the world. Traditional cultivars of *Oryza sativa* species inhold three sub-species (*Indica, Japonica* and *Javanica*) in terms of geographical adaptation, morphological, physiological and chemical characteristics. While *Japonica* and *Japonica x Indica* hybrids are generally cultivated in European countries, *Indica* species are mostly cultivated

in South Asian countries. The *Indica* species generally have greater yields than the *Japonica* species (Fan et al., 2017). The paddy grain separated from the hulls, fruit and seed testa is so-called "rice" (Sürek, 2002). Since the quality varies from country to country and region to region, it is quite hard to define the quality of rice. Rice quality is generally designated by genetics and environmental factors (Sezer et al., 2007). While environmental factors largely influence physical quality traits (thousand-grain weight, grain dimensions, head and broken yields), chemical and cooking quality traits (amylose content, gel consistency, gelatinization temperature, water uptake ratio, grain elongation, cooking time) are largely influenced by genetic factors (Bahmaniar and Ranjbar, 2007). However, gelatinization temperature is highly influenced both by genetics and environmental factors (Kishine et al., 2008).

Worldwide, market value, consumer preference and adaptation of new cultivars are the primary factors influencing the quality concept in rice. Physical appearance, physico-chemical characteristics and cooking traits characterize the product and designate direct decisions about the product in consumer markets (Custodio et al., 2009).

In general, rice grain quality could be divided into four categories: nutritional value, industrial quality, compliance with marketing standards, cooking and sensory quality. The factors controlling the cooking quality of rice are mostly related to physico-chemical characteristics of starch constituting about 90% of the dry matter in polished rice grain (Kong et al., 2015). Knowledge of these traits plays significant role in comprehension of the changes encountered during cooking of rice (Custodio et al., 2009). This study was conducted to determine physico-chemical characteristics of nationally and internationally important *japonica* species.

Materials and Methods

In the present study, Edirne, Efe, Gala, Halilbey, Kale, Kızıltan, Osmancık-97, Tosya Güneşi and Yatkın cultivars registered bred by Trakya Agricultural Research Institute and Agusto, Arieti, Baldo, Cammeo, Crono, Galileo, Meco, Nembo and Ronaldo cultivars registered in European countries were used (Table 1).

Table 1. List of tested paddy cultivars (*Oriza sativa L. japonica*)

No	Name	Origin
1	Agusto	Italy
2	Arieti	Italy
3	Baldo	Italy
4	Cammeo	Italy
5	Crono	Italy
6	Edirne	Turkey
7	Efe	Turkey
8	Gala	Turkey
9	Galileo	Italy
10	Halilbey	Turkey
11	Kale	Turkey
12	Kızıltan	Turkey
13	Meco	Italy
14	Nembo	Italy
15	Osmancık-97	Turkey
16	Ronaldo	Italy
17	Tosya Güneşi	Turkey
18	Yatkın	Turkey

Rice samples of the present materials were obtained from the cultivar-yield experiments conducted in Bafra Agricultural Research and Implementation Center of Ondokuz Mayıs University Agricultural Faculty during 2020 paddy growing season (15 May – 20 September). Analyses were conducted in accordance with randomized plots designed with 3 replications. Thousand-grain weight, grain length and grain length/width ratio parameters were determined in accordance with the method specified in Akay (2020). Cooked grain length, grain elongation ratio and cooking time (min) were determined in accordance with the method specified in Simonelli et al. (2017); gelatinization temperature was determined with the method Oko et al. (2012) and amylose content was determined with the method Bergman (2019). Experimental results were subjected to ANOVA in accordance with randomized plots design. Principle component analysis was conducted with the use of JMP (2007) statistical software. Significant of differences were identified with F test and means were compared with the use of Tukey's test.

Results and Discussion

Thousand-grain weight is among the most important quality traits of rice. Greater thousand-grain weights indicate larger grains. Large grains are preferred by consumers, thus increasing market value (USDA, 2009; Akay, 2020). There were highly significant differences in thousand-grain weights of 18 paddy cultivars and the values varied between 20.32 g (Crono) – 31.00 g (Baldo). In terms of thousand-grain weight, Baldo (31.00 g) and Edirne (29.63 g) cultivars were in the same statistical group (Table 2). Present findings on thousand-grain weights were similar to the findings of previous studies for Osmancık-97 (25.20 – 27.77 g), Ronaldo (24.70 - 25.38 g), Baldo (31.41 - 32.41 g), Cammeo (29.68- 30.3 g), Efe (27.51- 27.84 g) and Halilbey (27.55 g) cultivars (Akay et al., 2018; Akay, 2020; TTSM,

2015; Simonelli et al., 2016; Yazman, 2014). The thousand-grain weight of rice is affected by environmental factors as well as genetic factors Abiotic stress factors especially in grain-fill period negatively influence thousand-grain weights (Kün, 1997). Thousand-grain weights of rice cultivars were reported as between 15.0 - 22.0 g by Webb et al. (1989), between 23.1 - 23.7 by Khalif et al. (2007), between 13.4 - 18.8 g by Bashir et al. (2010). Thousand-grain weights of different rice genotypes used in paddy breeding programs of Turkey were reported as between 18.3 - 29.9 g (TTSM, 2015) and Safdar et al. (2013) reported thousand-grain weights of paddy cultivars as between 18.0 - 27.0 g. Paddy grain size vary based on being long or short, thin or thick grains and also influence consumer preferences. In Turkey, generally japonica sub-species with long and wide grains are preferred, but indica sub-species with long and thin grains are not preferred (Binodh et al., 2006; Akay, 2020).

Table 2. Mean values for investigated traits of 18 paddy cultivars

Cultivars	TGW **	GL **	GLWR **	CGL **	GER **	RWA **
Agusto	22.51 efg	6.59 cd	2.56 a	13.31 ab	2.02 bc	60.14 b-f
Arieti	23.42 def	6.62 cd	2.52 a	9.26 hı	1.40 g	50.72 fg
Baldo	31.00 a	7.28 a	2.32 bc	12.47 b-d	1.73 c-f	66.88 abc
Cammeo	28.80 b	6.89 bc	2.41 ab	10.78 d-h	1.56 efg	57.37 c-g
Crono	20.32 h	5.98 e	2.27 bcd	8.61 ı	1.44 fg	46.96 g
Edirne	29.63 ab	6.99 ab	2.23 cde	11.95 b-f	1.71 c-f	65.11 a-d
Efe	23.26 def	6.43 d	2.31 bc	11.21 c-g	1.75 cde	57.57 c-f
Gala	22.56 efg	5.96 e	2.23 cde	12.97 abc	2.17 ab	61.77 a-e
Galileo	28.89 b	5.92 e	1.98 f	11.17 c-g	1.88 bcd	57.31 c-g
Halilbey	23.82 de	5.92 e	2.08 ef	11.17 c-g	1.89 bcd	61.77 a-e
Kale	25.34 c	5.94 e	2.11 def	10.20 f-ı	1.72 c-f	59.89 b-f
Kızıltan	21.57 gh	5.94 e	2.22 cde	10.42 e-ı	1.75 cde	56.27 d-g
Meco	23.31 def	5.95 e	2.23 cde	9.72 ghı	1.63 d-g	52.13 efg
Nembo	22.39 efg	5.95 e	2.10 ef	9.97 ghı	1.68 d-g	51.28 fg
Osmancık-97	23.60 def	5.90 e	2.20 cde	12.81 abc	2.17 ab	58.73 b-f
Ronaldo	22.31 fg	5.91 e	2.23 cde	12.25 b-e	2.07 b	61.76 a-e
Tosya Güneşi	23.28 def	5.90 e	2.18 cde	12.64 a-d	2.14 b	71.27 a
Yatkın	24.33 cd	5.88 e	2.12 def	14.52 a	2.47 a	68.84 ab
Ortalama	24.46	6.22	2.24	11.41	1.84	59.21
CV%	2.02	1.72	2.41	5.33	5.49	5.75

**= p< 0.01; Means indicated with the same letter are not significantly different at 5% level. TGW= Thousand-grain weight (g), GL= Grain length (mm), GLWR= Grain length/width ratio (%); CGL= Cooked grain length (mm), GER= Grain elongation ratio (%), RWA= Rice water absorption (%), CV%= Coefficient of variation.

Grain lengths of the investigated cultivars varied between 5.88 (Yatkın) – 7.28 mm (Baldo) with a general mean of 6.22 mm (Akay, 2020; Beşer et al., 2015; Simonelli et al., 2017). According to Tukey's multiple comparison test results, Baldo (7.33 mm) and Edirne (6.99 mm) cultivars had the greatest grain length values (Table 2). Beşer et al. (2015) reported the mean grain length of Baldo cultivar as 6.9 mm. In TTSM (2015) report, grain lengths of Osmacık-97, Halilbey, Ronaldo and Cammeo cultivars were respectively reported as 6.4, 6.4, 6.5 and 7.3 mm. Yazman (2014) reported the grain lengths of Baldo and Osmancık-97 cultivars respectively as 6.33 and 6.96 mm.Grain length is not a sole indicator of quality, but together with length to width ratio, it turns into a significant physical quality parameter for national and international classification.

The length/width ratio is closely related to the appearance of rice grain and significantly influences consumer preferences. High length/width ratios indicate long thin grains and low ratios indicate round rice grains. The rice grains with a length/width ratio of greater than 3 are classified as thin grains, the ones with a length/width ratio of between 2 -3 are classified as medium and the ones with a length/width ratio of lower than 2 are classified as round grains. In Turkey, consumers generally prefer rice grains with a length/width ratio of between 2 - 3. In the present study, length/width ratios of the paddy cultivars varied between 1.98 (Galileo) – 2.56 (Agusto) and differences in length/width ratios of the cultivars were found to be significant (Table 2). In previous studies, length/width ratios of rice grains were reported as between 1.50 - 3.50 (Shilpa and Krihnan, 2010); between 2.22 - 2.27 (Yazman, 2014); between 1.55 - 3.43 (Şişman, 2016) and between 2.11 - 2.44 (Akay, 2020). Grain length/width ratio is closely related to grain shape. Consumer preferences may vary with the shape and appearance of the grains. While some countries prefer short blunt grains, some others prefer medium long or thin rice grains. The cultivars with thin long grains are generally preferred in Southeast Asian countries and the cultivars with short grains are generally preferred in countries of temperate climate zone (Sürek, 2002). In Turkey, cultivars with long-medium length/width ratios (between 2-3) are preferred. Grain length/width ratio is also closely related to paddy species (indica and japonica type), indica-type paddies generally have thin grains with length/width ratios of greater than 3.

Cooked grain length is directly related to water absorption, elongation ratio and water uptake ratio of rice grain. Cooked grain length is influenced by genetic and environmental factors (Şişman, 2016). The present study, cooked grain lengths varied between 8.61 (Crono) and 14.52 mm (Yatkın) with a general mean of

11.41 mm. According to Tukey's multiple comparison test results, Agusto, Gala, Tosya Güneşi and Yatkın cultivars had the greatest cooked grain length (Table 2).

There were significant differences in grain elongation ratios of the paddy cultivars. The greatest value was observed in Yatkın (2.47%) cultivar and the lowest in Arieti (1.40%) cultivar (Table 2). Similar to the present findings, Akay et al. (2018) reported grain elongation ratios as between 1.55 - 2.02% and Akay (2020) as between 1.58 - 1.99%. According to Tukey's multiple comparison test results on grain elongation ratios, Yatkın, Osmancık-97 and Gala cultivars were placed into the first group. Danbaba et al. (2011) indicated that grain elongation ratios were influenced by amylose content of the cultivars. Flexibility of amylopectin bonds indicates high water uptake capacity of the cultivars (Sürek, 2002; Şişman, 2016; Akay, 2020).

Water absorption ratios of the rice samples varied between 46.96% (Crono) and 71.27% (Tosya Güneşi) and differences in water absorption ratios of the cultivars were found to be significant (Table 2). Similar to the present findings, Şişman (2016) reported that water absorption ratios of paddy cultivars as between 44.37 - 71.07%.

Table 3. Mean values for investigated traits of 18 paddy cultivars

Cultivars	WRR **	CT **	PC **	AC **		DA
Agusto V1	2.57 cde	00:18:36 def	7.55 fg	25.26 ab	High	<69.5 °C
Arieti V2	2.05 ef	00:20:47 b	6.66 ı	26.25 a	High	<69.5 °C
Baldo V3	3.05 abc	00:17:57 e-h	6.90 hı	20.84cde	Moderate	<69.5 °C
Cammeo V4	2.34 def	00:18:31 d-g	7.56 fg	24.04abc	Moderate	<69.5 °C
Crono V5	1.91 f	00:20:16 bc	8.02 de	27.40 a	High	<69.5 °C
Edirne V6	2.89 a-d	00:17:29 fgh	8.33 cd	19.63 def	Low	<69.5 °C
Efe V7	2.40 def	00:18:47 de	8.00 de	19.60 def	Low	<69.5 °C
Gala V8	2.68 a-e	00:17:19 h	8.52 abc	17.98 ef	Low	<69.5 °C
Galileo V9	2.36 def	00:19:23 cd	8.36 cd	21.47cde	Moderate	<69.5 °C
Halilbey V10	2.63 b-e	00:17:32 fgh	8.03 de	19.72 def	Low	<69.5 °C
Kale V11	2.53 c-f	00:17:13 h	5.45 j	19.07 def	Low	<69.5 °C
Kızıltan V12	2.33 def	00:17:23 gh	8.49 bc	19.60 def	Low	<69.5 °C
Meco V13	2.12 ef	00:17:38 fgh	7.21 gh	21.76bcd	Moderate	<69.5 °C
Nembo V14	2.09 ef	00:17:51 e-h	8.78 ab	20.73cde	Moderate	<69.5 °C
Osmancık-97 V15	2.39 def	00:17:31 fgh	7.72 ef	16.91 f	Low	<69.5 °C
Ronaldo V16	2.64 b-e	00:23:28 a	7.92 ef	21.29cde	Moderate	>74 °C
Tosya GüneşiV17	3.31 a	00:18:28 d-g	7.20 gh	25.91 a	High	<69.5 °C
Yatkın V18	3.22 ab	00:19:26 cd	8.89 a	20.13 def	Moderate	<69.5 °C
Ortalama	2.53	00:18:39	7.76	21.53		
CV%	8.22	1.98	1.62	5.52		

**= p< 0.01; Means indicated with the same letter are not significantly different at 5% level. WRR= Water uptake ratio (%), CT= Cooking time (min), PC= Protein content (%), AC= Amylose content; DA= Alkali spreading (gelatinization temperature), CV%= Coefficient of variation.

Differences in water uptake ratios of the cultivars were found to be significant ($p<0.01$) (Table 3). The water absorption ratios of the cultivars varied between 1.91% (Crono) and 3.31% (Tosya Güneşi). Complying with the present findings, Anıl and Koca (2006) reported water uptake ratios of the paddy cultivars as between 1.75 - 1.98% and Danbaba et al. (2011) reported water uptakes of rice grains as between 1.74 - 2.98%.

The cultivars with shorter cooking times are generally preferred by the consumers. Cooking time was reported to have positive correlations with amylose content and alkali spreading (gelatinization temperature) (Akay, 2020). The cooking time of the present cultivars varied between 00:17:19 (Gala) – 00:23:28 min (Ronaldo). The Baldo, Edirne, Gala, Halilbey, Kale, Kızıltan, Meco, Nembo and Osmancık-97 cultivars had the shortest cooking times (Table 3). Present findings on cooking times comply with the findings of previous studies (Akay et al., 2018; Akay, 2020; Danbaba et al., 2011; Fofana et al., 2011; Thomas et al., 2013; Şişman, 2016).

Protein contents of rice samples varied between 5.45% (Kale) and 8.89% (Yatkın) with a mean value of 7.76% (Table 3). Present findings on grain protein contents comply with the findings of earlier studies (Koca and Anıl, 1997; Thomas et al., 2013).

Amylose content significantly influences cooking time and eating quality of rice. The grains of rice with high amylose contents (25-33%) are hard and dry cooking tendency. The ones with moderate amylose contents (20-25%) have a softer and sticky cooking tendency and the ones with low amylose contents (< %20; 12 -13%) are quite soft and sticky. The *Japonica* sub-species generally have moderate and low amylose content (Juliano et al., 1981; Bao et al., 2006; Hossaina et al., 2009; Akay, 2020; Danbaba et al., 2011). Present amylose contents varied between 16.91% (Osmancık-97) and 26.25% (Arieti) and the differences in amylose contents of the cultivars were found to be significant (Table 3). Cultivars were classified based on amylose content with the use of an internationally recognized scale: Agusto, Arieti, Crono and Tosya Güneşi cultivars as high amylose containing cultivars, Baldo, Cammeo, Galileo, Meco, Nembo, Ronaldo and Yatkın as moderate amylose containing cultivars and the rest as low amylose containing cultivars (Table 3) (Cruz and

Khush, 2000; Kasai et al., 2007). Present amylose contents comply with the findings of previous studies (Akay et al., 2018; Akay, 2020; Anıl and Koca, 2006; Donduran, 2014; Simonelli et al., 2016; Şişman, 2016; Thomas et al., 2013).

Gelatinization temperature is a temperature at which rice starch started to swell and lose crystallinity in an irreversible fashion. In other words, it is a physicochemical characteristic of the starch (Sürek, 2002). Rice starch generally gelatinizes between 65 - 85 °C temperatures (Bakshi and Singh, 2019). High gelatinization temperatures result in softening of rice when cooked. In Turkey, cultivars with low gelatinization temperatures are generally preferred (Akay, 2020). Rice gelatinization temperatures are classified as follows: <69.5 °C (tannins are all disintegrated), 70-74 °C (4-5 grains are disintegrated) and >74 °C (3 and more grains are disintegrated) (Juliano, 1979; 1985). In terms of gelatinization temperatures of the present cultivars, only the Ronaldo cultivar is classified as >74 °C and the rest as <69.5 °C (Table 3).

Correlation coefficients for the relationships between investigated physicochemical parameters are provided in Table 4. There were highly significant correlations between thousand-grain weight and grain length (r = 0.66**); between grain length and length/width ratio (r = 0.63**); rice grain length/width ratio and amylose content (r = 0.51*); between rice water absorption and cooked grain length (r=0.83**), grain elongation ratio (r=0.71**), water uptake ratio (r=0.99**); between cooked grain length and grain elongation ratio (r = 0.89**), water uptake ratio (r=0.81**); between grain elongation ratio and water uptake ratio (r = 0.69**). On the other hand, there were highly significant negative correlations between cooking time and alkali spreading (r = -0.76**). In previous studies, positive correlations were reported between amylose content and rice grain length/width ratio (Akay, 2020; Julinao and Villareal, 1993; Khatun et al., 2003; Sheng et al., 2015). Positive relationships were also reported between cooked grain length and elongation ratio (Akhter et al., 2017; Akay, 2020).

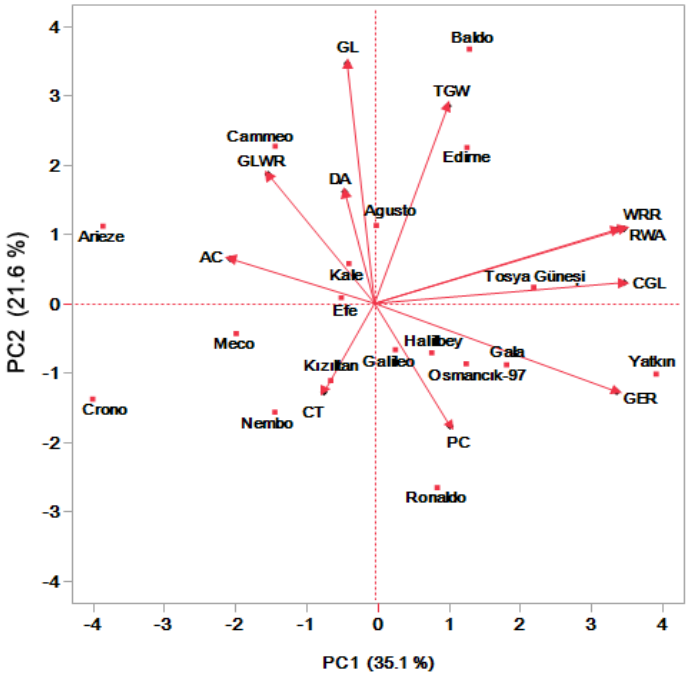
Table 4. Correlations coefficients between the investigated traits

	TGW	*GL*	*GLWR*	*RWA*	*CGL*	*GER*	*WRR*	*CT*	*PC*	*AC*
GL	0.66**									
GLWR	-0.07	0.63**								
RWA	0.39	0.14	-0.19							
CGL	0.19	0.05	-0.04	0.83**						
GER	-0.12	-0.40	-0.32	0.71**	0.89**					
WRR	0.36	0.14	-0.17	0.99**	0.81**	0.69**				
CT	-0.22	-0.07	0.21	-0.13	-0.05	0.01	-0.11			
PC	-0.18	-0.23	-0.29	0.01	0.27	0.35	0.03	0.05		
AC	-0.17	0.19	0.51*	-0.27	-0.34	-0.40	-0.20	0.45	-0.22	
DA	0.18	0.17	0.02	-0.10	-0.13	-0.20	-0.07	-0.76**	-0.05	0.02

*= p< 0.05, **= p< 0.01; TGW= Thousand-grain weight (g), GL= Grain length (mm), GLWR= Grain length/width ratio (%), RWA= Rice water absorption (%), CGL= Cooked grain length (mm), WRR= Water uptake ratio (%), CT= Cooking time (dk), PC= Protein content (%), AC= Amylose content; DA= Alkali spreading (gelatinization temperature).

Biplot graph was generated for a better visual assessment of the relationships between investigated physicochemical parameters of the cultivars (Figure 1). While correlations coefficients present the relationships between two parameters, biplot allows an overall assessment of the relationship among the entire parameters (Sharifi and Ebadi, 2018). According to biplot analysis, the first principal component explained 35.1% and the second principal component explained 21.6% of total variation (both explained 56.7%) (Figure 1). Besides conventional data analysis approach, ANOVA and multiple comparison tests for single variables and correlations analysis for relationships between two variables of agricultural experiments, biplot analysis method and constellation graph allowing multivariate analysis of several physicochemical parameters were also used in present study. Such analyses were thought to contribute overall assessment of concrete outcomes.

Thousand-grain weight, water uptake ratio, water absorption and cooked grain length vectors were placed in the upper-right section of the biplot group and vector angles between these traits were smaller than 90° indicating significant positive relationships among thousand-grain weight, water uptake ratio, water absorption and cooked grain length. These traits were seen to act in reverse direction of cooking time vector, which was placed in lower-left section of the biplot graph. Grain length, length/width ratio, alkali spreading and amylose content vectors were placed in upper-left section of the biplot graph indicating positive relationships among them. The traits with negative relationships with these parameters (grain elongation ratio and protein content) were placed in lower-right section of the biplot graph indicating positive relationships between them. Since cooking time had the shortest vector length, it was identified as the most distinctive trait. The cultivars placed close to origin of the biplot graph were prominent for more than one traits. Kale cultivar was prominent for grain length/width ratio; Efe cultivar for amylose content; Kızıltan cultivar for cooking time; Galileo cultivar for protein content; Gala cultivar for grain elongation ratio; Tosya Güneşi cultivar for cooked grain length; Baldo cultivar for thousand-grain weight and Agusto cultivar was prominent for grain length (Figure 1).



* TGW= Thousand-grain weight (g), GL= Grain length (mm), GLWR= Grain length/width ratio (%), RWA= Rice water absorption (%), CGL= Cooked grain length (mm), WRR= Water uptake ratio (%), CT= Cooking time (dk), PC= Protein content (%), AC= Amylose content; DA= Alkali spreading (gelatinization temperature).

Figure 1. Biplot grouping of investigated traits relations of paddy cultivars with investigated traits.

In the constellation plot, present cultivars were divided into 2 main groups and 8 sub-groups (Figure 2). Osmancık-97, Gala, Halilbey and Galileo cultivars were placed into the 1st group; Meco, Efe, Nembo and Kızıltan cultivars into the 2nd group; Kale cultivar into the 3rd group; Agusto cultivar into the 4th group; Cammeo, Edirne and Baldo cultivars into the 5th group; Arieti and Crono cultivars into the 6th group; Tosya Güneşi and Yatkın cultivars into the 7th group and Ronaldo cultivar was placed into the 8th group(Figure 2; Table 1; 2).

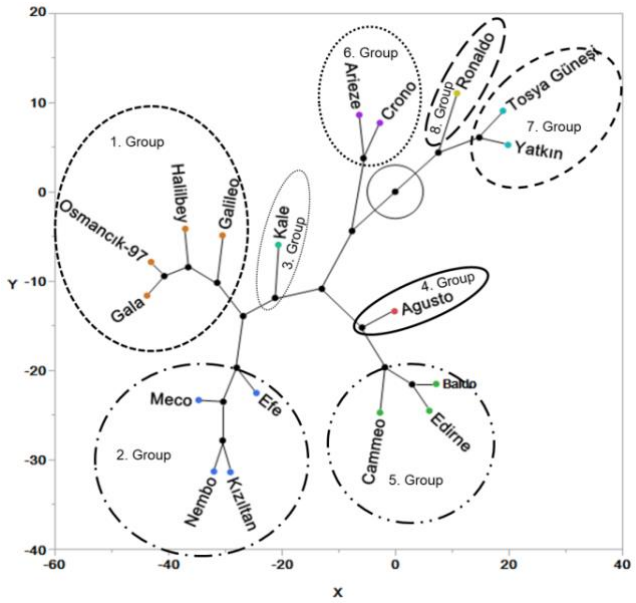


Figure 2. Constellation plot graph of cultivars based on investigated traits.

While the first group of cultivars (Osmancık-97, Gala, Halilbey and Galileo) had high protein contents, the second group of cultivars (Meco, Efe, Nembo and Kızıltan) had the lowest grain length values. The 3rd group of cultivars (Kale) had lower cooking time and grain protein content than the other cultivars. The 4th group of cultivars (Agusto) had greater grain length/width ratio than the other cultivars. The 5th group of cultivars (Cammeo, Edirne and Baldo) had the greatest thousand-grain weight values. The 6th group of cultivars (Arieti and Crono) had the lowest water absorption, cooked grain length, grain elongation ratio and water uptake ratios. The 7th group of cultivars (Tosya Güneşi and Yatkın) had the greatest water absorption, cooked grain length, grain elongation ratio and water uptake ratio. The 8th group of cultivars (Ronaldo) had the

greatest cooking time and the greatest alkali spreading temperature (Figure 2; Table 1; 2).

Conclusion

Although it is inconvenient to compare both physical and chemical quality traits of the rice grains obtained from different cultivars, the physically-close rice grains are generally compared with each other. Comparison of highly different cultivars was banned in both national and international rice codex. It is quite hard to separate such a physical mixture. Therefore, physicochemical characteristics are used for separation of cultivars. In present study, some physical and chemical characteristics and cooking parameters of 18 paddy cultivars belonging to Japonica sub-species and registered in Turkey and European countries were investigated. There were highly significant negative correlations between alkali spreading and cooking time. The Efe, Kale and Galileo cultivars approaching to the origin of biplot graph were found to be prominent for more than one trait. According to biplot graph, cooking time was identified as the least distinctive trait. While Ronaldo, Tosya Güneşi and Yatkın cultivars were placed into one main group in terms of some physical and chemical characteristics, the other cultivars were all placed into the other main group. But that main group had 8 sub-groups. The cultivars constituting the sub-groups were close to each other in terms of several traits. Present groupings revealed that investigated cultivars had close physicochemical characteristics to each other and it is possible to separate the cultivars of the same with the use of genetic markers.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

All the authors read and approved the final manuscript.

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

Ethical approval

Ethics committee approval is not required.

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Assessing the potential of earthing up and integrated nutrient management on Irish potato (*Solanum tuberosum* L.) productivity in smallholder farming systems

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Abstract

Irish potato (*Solanum tuberosum* L.) is a strategic food security crop in Zimbabwe which is a good source of carbohydrates. This has created the need to increase research so as to increase potato productivity. The aim of the study was to assess the effects of earthing up and nutrient management on potato growth and yield. The study was carried out in Masvingo district of Masvingo Province. The experiment was laid out as a 2*3*3 factorial design with 18 treatments replicated three times to give 54 plots. Days to maturity were observed when potato plant leaves turned yellow and 85% of the tubers were ready for harvest at physiological maturity. Results show that plant height was not significantly affected ($p<0.05$) by earthing up. Nutrient management significantly influenced ($p<0.05$) plant height with highest (31.2 ± 0.97 cm) observed from 5 t ha^{-1} cattle manure + 50 kg ha^{-1} potassium fertiliser. Days to maturity were statistically affected ($p<0.05$) by tillage type and nutrient management. Days to maturity increased statistically ($p<0.05$) with increase in application rates of cattle manure + potassium fertiliser irregardless of tillage type. Earthing up considerably influenced potato yield, with highest yields obtained from earthing up treatments amended with cattle manure + potassium fertiliser. Combining 5 t ha^{-1} cattle manure and + 50 kg ha^{-1} potassium fertiliser have the potential to improve potato growth and yields. It can be concluded that farmers adopt the use of earthing up integrated with cattle manure + potassium fertiliser at a rate of 5 t ha^{-1} + 50 kg ha^{-1} can be a better option to improve potato production in smallholder farming systems.

Keywords

Cattle manure, Earthing up, Nutrient management, Potato, Smallholder, Farming systems

Introduction

Potato (*Solanum tuberosum*. L), is an edible plant which belongs to family Solanaceae and originated from South America where it was first grown in Peru (Quin, 2011; Sakadzo *et al*., 2020). Potatoes are used as food by many people world-wide because of its high carbohydrate content and edibility (Muhammad *et al*., 2013; Svubure *et al*., 2015). The plant produces a range of flowers which include white, pink, and red, blue or purple (Amador *et al*., 2003). Potatoes with white flowers produce tubers with white skin (Winch, 2006). When young potato tubers are green, they are like young tomatoes (Karam*et al*., 2009). Propagation of potatoes is mainly done with tuber seeds (Amador*et al*., 2003). The plant has capacity to grow up to 1m or above depending on soil fertility (Gusha, 2014). Maturity of potatoes depends on variety with many varieties maturing 120 days after planting (Kugedera, 2019).

Low soil fertility and moisture stress has affected potato production in most dry regions in Zimbabwe. Most farmers grow potato in home gardens which have poor soils due to monoculture and continual mining of

nutrients by crops grown in these gardens. Integrated nutrient management is one of the options which can be adopted by farmers (Vanlauwe *et al*., 2015; Nyambati *et al*., 2020) to improve productivity. Nutrient management has been seen as an option to increase nutrient availability, increase crop yields and reduce leaching in most arable lands (Shumba *et al*., 2020). It has been highly appreciated that the use of nutrient management through combining mineral fertilisers and cattle manure can improve soil fertility (Shumba *et al*., 2020) but soil fertility improvement alone cannot achieve higher yields. There is need to combine nutrient management with water and soil conservation techniques such as earthing up (Masvodza, 2015) to increase potato production. Potassium has been seen as one of the major nutrient needed in potato production (Kumar *et al*., 2012) to increase yield and quality of tubers. Potato production has been also decreasing due to reduced use of potassium fertiliser by poor resources farmers in many countries in Africa (Karam *et al*., 2009). Balancing potassium with other nutrients in potato production has been noted to increase growth, yield and quality tubers (Winch, 2006; Kumar *et al*., 2012).

Lack of knowledge about earthing up of potatoes (Sakadzo *et al*., 2020) and inadequate use of mineral and organic fertiliser has contributed to low potato yields in smallholder farming systems (Masvodza, 2015). There is need for proper dissemination information about these technologies to smallholder farmers as means of improving potato tuber yields. Zimbabwe has the capacity to produce higher yields of potatoes if smallholder farmers are equipped with knowledge and resources (Vita, 2015). The major objective of the study was to assess the effects of earthing up and nutrient management on potato growth and yield.

Methodology

Description of the Study Area

The study was carried out in ward 18 of Masvingo district which lies between 6°35'18" to 6°56'37"N and 38°35'60" to 38°53'36"E. The experimental site is 64 km south east of Masvingo town at geographic coordinate of 06041' N and 38043' E with elevation of 2521 masl. On average the areas receives 420 mm rainfall per annum with high rates of evapotranspiration due to high temperatures which ranges from 18 ºC (minimum) to 32 ºC (maximum). The study area is characterised by sandy loam soils which are infertile (FAO, 2016). Soil characterisation indicated that the soil had 80 % sand, 9 % clay and 11 % silt with a pH of 5.3. The soil also had 1.4 % organic carbon, 0.15 % total N and 3.87 mg kg^{-1} available phosphorous. Major crops grown include sorghum, groundnuts, maize, Bambara nuts, cowpeas, sweet potatoes, and finger millet. Nutrition gardens are also common with farmers growing crops such as cabbage, tomatoes, onion, covo, rape, sugar beans and potatoes.

Experimental design

The experiment was arranged as a 2*3*3 factorial design with two main factors which were type of tillage and nutrient management. Tillage method was divided into earthing-up and flat bed. Nutrient management was subdivided into cattle manure (0, 2.5 and 5 t/ha) and potassium fertiliser (0, 25 and 50 kg/ha). A total of 18 treatments were used, which were each replicated three times. Treatment combinations are shown in Table 1. Cattle manure used had the following properties; 1.09% total N, 0.23 % P, 0.38% K and 18.2% organic carbon as well as 18.6 % moisture content.

Table 1. Treatment combinations

Treatment No.	Treatments	Combinations
1.	earthing up	E
2.	flat bed	F
3.	2.t ha^{-1} cattle manure +earthing up	$CM_{2.5}E$
4.	5 t ha^{-1} cattle manure +earthing up	CM_5E
5.	25 kg ha^{-1} potassium +earthing up	$25KO_2E$
6.	50 kg ha^{-1} potassium +earthing up	$50KO_2E$
7.	2.5 t ha^{-1} cattle manure +25 kg ha^{-1} potassium + earthing up	$CM_{2.5}25KO_2E$
8.	2.5 t ha^{-1} cattle manure+ 50 kg ha^{-1} potassium +earthing up	$CM_{2.5}50KO_2E$
9.	5 t ha^{-1} cattle manure +25 kg ha^{-1} potassium + earthing up	CM_525KO_2E
10.	5 t ha^{-1} cattle manure + 50 kg ha^{-1} potassium + earthing	CM_550KO_2E
11.	2.5 t ha^{-1} cattle manure + flat bed	$CM_{2.5}F$
12.	5 t ha^{-1} cattle manure + flat bed	CM_5F
13.	25 kg ha^{-1} potassium + flat bed	$25KO_2F$
14.	50 kg ha^{-1} potassium +flat	$50KO_2F$
15.	2.5 t ha^{-1} cattle manure + 25 kg ha^{-1} potassium + flat bed	$CM_{2.5}25KO_2F$
16.	2.5 t ha^{-1} cattle manure +50 kg ha^{-1} potassium + flat bed	$CM_{2.5}50KO_2F$
17.	5 t ha^{-1} cattle manure + 25 kg ha^{-1} potassium + flat bed	CM_525KO_2F
18.	5 t ha^{-1} cattle manure + 50 kg ha^{-1} potassium + flat bed	CM_550KO_2F

Experimental plot layout

Each plot was measuring 3 m by 4 m and planting spacing of 0.3m x 0.9 m was used and the distance between two plots was 1.5m. In each plot, 44 tubers were sown. A total of 54 plots were used. Experimental site was ploughed using oxen drawn plough to a depth of 30 cm and seed tuber were placed to a depth of 5 cm in the soil in each plot at a spacing of 0.3 m between each seed tuber and 0.9 m between rows in same plot. Mineral fertiliser, potassium (KO_2) was applied using banding methods at full dose and variations according to treatments at plant and covered with soil before seed tuber was placed. Cattle manure was applied at full dose and variations according to treatments were used

two weeks before planting. Cattle manure was incorporated and mixed well with soil to give it time to decompose. Furrow irrigation was done from planting to harvesting and all other agronomic activities were observed during the growing season.

Data Collection

Data collected was based on objectives of the study. Growth parameters collected was plant height which was measured in cm 30 days after sowing (DAS), 60 DA, 90 DAS and at harvesting using ruler and tape measure. Plant height was measured from ground level to the tip of a plant. Twelve plants were randomly selected and marked from a net plot measured 2 m by 3 m. These plants were measured in each plot and mean calculated and used for analysis. Days to maturity were observed when potato plant leaves turned yellow and 85% of the tubers were for harvest at physiological maturity. Yield parameters were measured after harvesting each plot. Total yield was recorded for each plot and tubers were sorted into marketable and unmarketable tubers. Total yield was converted from kilogrammes to tonnes per hectare using the formula below:

$$Tuber\ yield\ (t\ ha^{-1} = \frac{yield\ (kg)}{6m^2}\ x\ 10000m^2$$

Where $6m^2$, the area of net is plot and $10000m^2$ is the area of 1 ha.

Data analysis

Collected data was recorded and processed using Ms Excel and data was statistically analysed for Analysis of variance (ANOVA) using IBM Statistical Package for Social Sciences (SPSS) version 25. Significant means were separated using least significant different (LSD) at 0.05 level of significance.

Results

Effects of nutrient management and earthing up on potato height

Results show that plant height was not significantly ($p>0.05$) influenced by earthing up. Plant height during 30 days after sowing (DAS) was significantly influenced ($p<0.05$) by earthing up and nutrient management (Figure 1). Plant height increased with increase in application rates of cattle manure and potassium fertiliser irregardless of tillage system. All treatments with cattle manure and earthing up recorded higher plant heights which were significantly different ($p <0.05$) from treatments with flat beds and cattle manure (Figure 1). Results show no significant effects ($p>0.05$) on plant height from all treatments at 90 DAS and harvesting stage (Figure 1). Plant height did not increase statistically ($p>0.05$) from 90 DAS to harvesting stage.

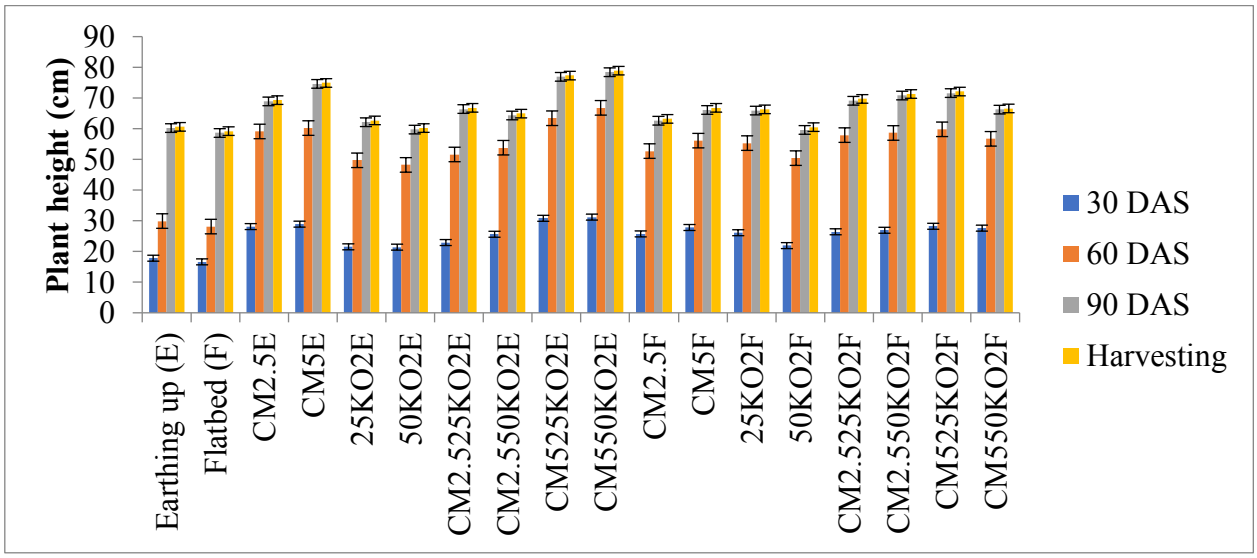

Figure 1. Effects of tillage systems and nutrient management and its interaction on plant height

Effects of earthing up and integrated nutrient management on days to maturity of potatoes

Results show that days to maturity were significantly affected ($p<0.05$) by main treatment factors. Earthing up had longer days tom maturity which was significantly different ($p<0.05$) from potatoes grown in flat beds (Table 2). Days to maturity increased with increase in application rates of nutrient management sources. Potatoes grown under cattle manure treatments had considerably ($p<0.05$) higher days to maturity compared to those grown under potassium fertiliser (Table 2).

Interactive effects of cattle manure and earthing up significantly affected ($p<0.05$) days to maturity but did not show significant difference ($p>0.05$) with interaction of flat beds and cattle manure at any application rate. Interaction of potassium fertiliser with earthing up and flat beds did not show any significant difference ($p>0.05$) but show significant effects ($p<0.05$) among treatment levels. Treatments without any nutrient amendments had the lowest days to maturity which was significantly different ($p<0.05$) between tillage systems (Figure 2). Results also show that application rate of 2.5 t ha^{-1} and 5 t ha^{-1} cattle manure did not show any significant effects ($p>0.05$) on days to maturity for both tillage systems (Figure 2). Combining cattle manure with potassium fertiliser show significant effects ($p<0.05$) on days to maturity even when combined with earthing up and flat beds.

Table 2. Effects of earthing up and integrated nutrient management on days to maturity of potatoes

Treatments	Mean ± SE days to maturity
Tillage practice	
Earthing up	115.53 ± 0.449[a]
Flat	115.27 ± 0.611[b]
P-value	<0.05
Cattle manure (t ha^{-1})	
0	112.19 ± 0.601[a]
2.5	116.74 ± 0.13[b]
5	117.27 ± 0.162[c]
P-value	<0.05
Potassium Fertiliser (KO_2) (kg ha^{-1})	
0	113.78 ± 0.864[a]
25	116.16 ± 0.412[b]
50	116.26 ± 0.417[c]
P-value	<0.05

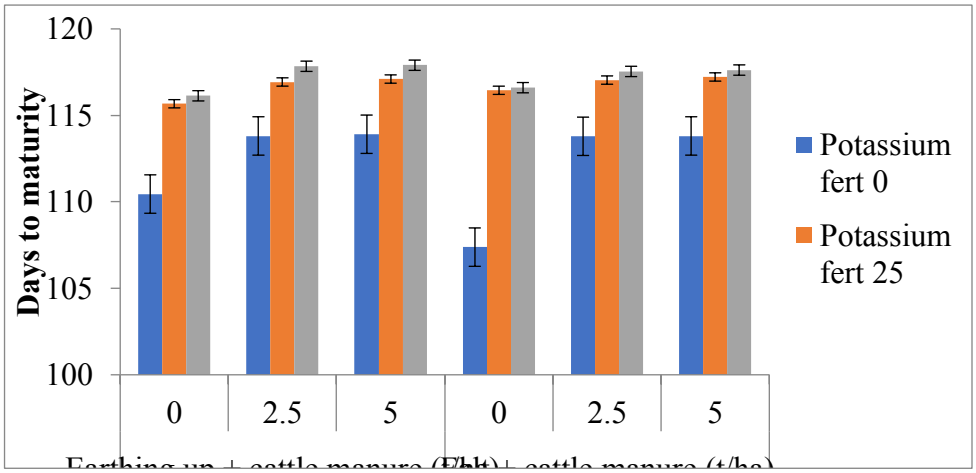

Figure 2. Interactive effects of tillage systems and nutrient management on days to maturity of potatoes.

Effects of earthing up and integrated nutrient management on days to tuber yield

Main treatment factors significantly influenced ($p<0.05$) potato tuber yields. Tuber yield was considerably different between earthing up and flat beds together with cattle manure and potassium fertiliser (Table 3). Tuber yield was highest from treatments applied 5 t ha^{-1} cattle manure which was significantly different ($p<0.05$) from all other treatments (Table 3). Increasing application rate of cattle manure and potassium fertiliser statistically increased tuber yield by 12.5 % and 6.3 % respectively. Tuber yield difference between earthing up and flat beds was 1.79 t ha^{-1} (8.6 %) higher from earthing up treatments.

Table 3. Effects of earthing up, flatbed, cattle manure and KO_2 on potato tuber yields

Treatments	Mean ± SE tuber yield
Tillage practice	
Earthing up	20.91 ± 0.953[a]
Flat	19.12 ± 1.02[b]
P-value	<0.05
Cattle manure (t/ha)	
0	14.73 ± 0.774[a]
2.5	21 ± 0.683[b]
5	24.83 ± 0.87[c]
P-value	<0.05
Potassium Fertiliser (KO_2) (kg/ha)	
0	15.8 ± 0.973[a]
25	21.41 ± 1.02[b]
50	22.84 ± 0.996[c]
P-value	<0.05

Results show that earthing up statistically influenced ($p<0.05$) tuber yield when integrated with cattle manure and potassium fertiliser compared to flat beds (Figure 3). Yield increased significantly with increase in cattle manure + potassium fertiliser. Higher yields were observed from all treatments with cattle

manure + potassium fertiliser and earthing up compared to same treatments under flat beds. Tuber yield at 5 t ha^{-1} cattle manure combined with 50 kg ha^{-1} did not show significant differences ($p>0.05$) between tillage systems (Figure 3). Application of 2.5 t ha $^{-1}$ cattle manure + 25 kg ha^{-1} potassium fertiliser significantly influenced ($p<0.05$) tuber yield (Figure 3).

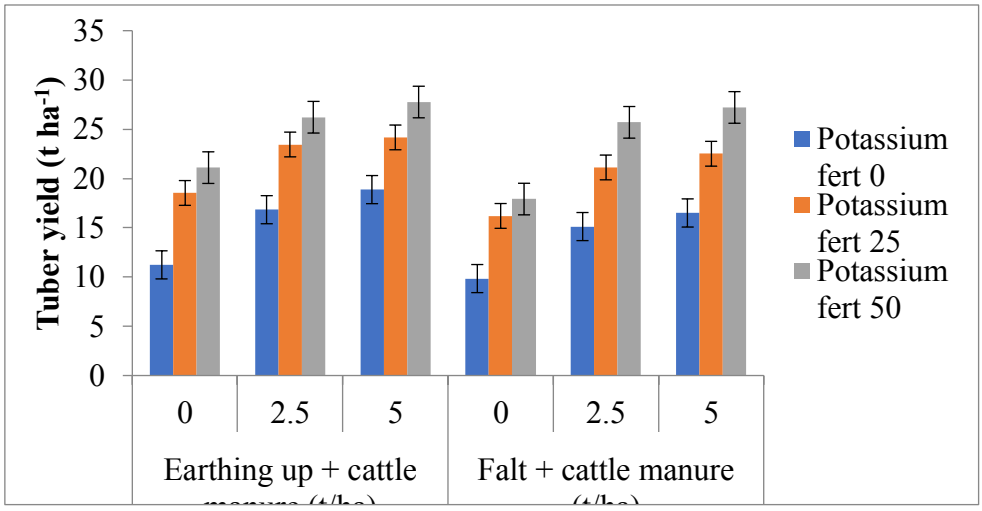

Figure 3. Interactive effects of tillage systems and nutrient management on potato tuber yields

Discussion

Effects of nutrient management and earthing up on potato height

Plant heights were low from control treatments because potato is one of the most sensitive crop to nutrient stress (Joergensen, 2012). Higher plant heights were recorded from treatments which were added cattle manure and potassium fertiliser and grown on earthing up lands. These results coincides with results by Geremew *et al*. (2007) who reported that addition of organic and mineral fertiliser stimulates vegetative growth of potatoes. Cattle manure also improves soil structure that reducing leaching of nutrients allowing potato plant to absorb maximum nutrient for growth and other processes (Israel *et al.*, 2012; Israel *et al*., 2018; Kugedera, 2019). The results also coincides with results by Suh *et al.* (2015) who reported that potato growth rate and plant height is significantly influenced by application of high quantities of cow dung and mineral fertiliser. The results also concurs with results by Najm *et al*. (2013) who reported that combining cattle manure and mineral fertiliser increases concentration of both major and trace elements which are required for metabolic and physiological processes which increases plant growth rates. Combining cattle manure and inorganic fertiliser increases nutrient availability which promotes plant growth rates leading to increased plant height (Kumar *et al*., 2012). Earthing up also increased soil depth which allows plants to spread their roots freely and go deep absorbing more nutrients and take up more water as earthing up conserves moisture and reduces leaching of nutrients especially where cattle manure is applied (Sakadzo *et al*., 2020).

Effects of earthing up and nutrient management options on days to maturity and potato yield

Day to maturity were significantly influences by nutrient management options and earthing up. Potatoes grown on earthing up beds amended with cattle manure in combination with potassium fertiliser mature late. Early maturity was recorded from control treatments on flat beds and earthing up but flatbed recorded the lowest days to maturity. These results overlaps with results by Zelalem *et al.* (2009) who reported that N and P nutrients delay maturity as this promotes growth and increase in number of main stems. The results also matches with results by Nebret (2012) who reported that organic and mineral fertilisers delay physiological maturity. Cattle manure also delays physiological maturity as it improves soil structure leading to improved nutrient availability, stimulates nutrient uptake and promotes plant growth. This accords with results by Kugedera (2019) who reported increased days to maturity after using cattle manure and reduced rates of mineral fertiliser. The results also agree with work done by Amin (2018) who reported increased days to maturity with the use of cattle manure and mineral fertiliser on potatoes.

Effects of earthing up and nutrient management options on potato yield

Treatments with cattle manure, potassium fertiliser and earthing up recorded tuber yield larger than those without cattle manure. These results corresponds with results by Taheri *et al*. (2010) who reported highest tuber yield of 20 t/ha of cattle manure was applied in combination with mineral fertiliser same as the results from this study were highest tuber yield was recorded from treatments with 5 t/ha cattle manure amended with 50 kg potassium fertiliser. The results also coincides with results by Mohammad *et al*. (2013) who reported that cattle manure application balances nutrients in the soil which promotes growth rate, metabolic reactions and other processes which leads higher tuber yields produced. The use of cattle manure increases soil water retention, nutrient availability and regulates soil pH as well as temperature which delays maturity hence more days were observed. This concurs with findings by Kugedera (2019) in Zimbabwe who reported increased tuber yields after using cattle manure and reduced rates of mineral fertiliser. The results also agree with work by Amin (2018) in

Ethiopia who reported increased tuber yields with the use of cattle manure and mineral fertiliser on potatoes. Results from this project were in support of results by Sakadzo *et al*. (2020) who reported increased tuber yields with the use of earthing up potatoes in Zimbabwe (Zaka district). Results from this experiment corroborate with report by Masvodza (2015) who indicated that earthing up increased tuber yields if done on right time.

Conclusion

The results from the study indicated that plant heights were higher from treatments with a combination of cattle manure and potassium fertiliser applied to potatoes grown on earthing up. Higher rates of cattle manure and potassium fertilisers had significant effect on potato plant heights. Earthing up also significantly influenced plant growth rates and plant heights. Potatoes grown on flatbed recorded lower plant heights compared to those grown on earthing up. Days to maturity were also influenced by combination of cattle manure, potassium fertiliser and earthing up. Potatoes grown on earthing up mature late compared to potatoes grown on flatbed. The results also show that days to maturity were influenced by nutrient management and earthing up which also had a significant effect on tuber yields. Potato plants which mature late had the highest tuber yield compared to those which mature earlier. Application of cattle manure in combination with potassium fertiliser also significantly increased tuber yields irregardless of being grown on earthing up or flat beds.

Recommendations

Farmers are recommended to grow potatoes on earthing up beds and use a combination of cattle manure and potassium fertilisers to maximise yields but the use of larger quantities of mineral fertiliser are not recommended as they cause soil acidity. Farmers are recommended to use 5 t/ha cattle manure or more as cattle manure controls land degradation and improves soil biophysio-chemical properties.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

All the authors read and approved the final manuscript.

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

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Functional and numerical response of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) on *Macrosiphum rosae* (L.) (Hemiptera: Aphididae)

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Abstract
In this study, functional and numerical response tests, which are important components in the selection of biological control agent, were carried out. In functional response trials, the amount of food consumed, attack rate (a) and handling time (Th) were calculated for each developmental period, depending on the number of preys given after 24 hours. The obtained results were evaluated with the Holling. In numerical response experiments, the development of the predator insect was examined depending on the number of preys given in certain numbers (5, 10, 20, 40 and 80) and the data were recorded. This phase of the trials continued until the individuals died. At this stage of the trials, the reproductive response of the predator on the aphid and the prey use efficiency were calculated. All of the productions and trials were carried out in climate rooms with a temperature of 27±1 °C, 65±5% RH. Experiments were carried out with 50 replications for each growth period and prey densities. According to the results obtained in the functional response trials, it was determined that the development periods with the highest productivity were the fourth larval and adult stages. When the results obtained from the numerical response experiments were evaluated, it was observed that the reproductive response increased with the increase of the prey given to *H. variegata*. Considering all the data obtained as a result of the study, it was concluded that *H. variegata* is effective on *M. rosae* and can be used in the control of this pest.

Keywords
Biological control, Coccinellids, Reproductive response, Attack rate, Handling time

Introduction
Rosa genus (Rosales, Rosaceae), known as rose, is one of the important plant species in the world for humans. This genus is distributed in the Holarctic and contains more than 250 species. The members of this genus, which are found naturally in almost all parts of Turkey, are distributed in a wide area including Central and Western Asia, the Caucasia, Europe, Northwest Africa and North America (Cairns, 2003; Ekincialp et al., 2007; Özçelik, 2013). According to some studies, it is estimated that there are around 500 local genotypes depending on 70 species in Turkey (Özçelik, 2010; Özçelik and Özçelik Doğan, 2018). These plants are especially used by people for decorative purposes. In addition, rose oil obtained from its leaves is used in the perfumery industry (Seneta and Dolatowski, 2003; Jaskiewicz, 2000; 2006). Considering some studies conducted around the world, it has been reported that over a hundred aphid species are harmful on roses (Blackman and Eastop, 2006; Holman, 2009; Blackman and Eastop, 2021). Among these species, *Macrosiphum rosae* (Linnaeus, 1758) is one of the most important rose pests worldwide (Blackman and Eastop, 2021). *M. rosae* damages the young leaves, upper parts of the shoots and buds of plants. They feed

by sucking the plant sap, and if their population is high, conditions such as downward bent stems, weak leaves and premature leaf fall occur in the plant. In addition, it creates sooty mold on the flowers and leaf surface due to a substance it secretes (Mehrparvar and Hatami, 2007). It has been reported that this pest mostly causes damage to 1 year and 2 years old rose shoots (Margina et al., 1999). Different strategies are being developed to keep aphid populations below the economic damage threshold (Holtzer et al., 1996). It has been observed that when chemical control is preferred against these pests, aphids develop resistance, negative effects on human and environmental health occur, and the populations of natural enemies decrease (Bielza, 2008; Saleem et al., 2008). Researchers and manufacturers focus on different alternative methods in order to prevent such negativities (Lacey et al., 2001). Coccinellidae is a family that is used in biological control studies and has 5200 species worldwide (Khan et al., 2007). A species belonging to this family, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) is a polyphagous and is widely found in the Palearctic region. This species is effective on different pests, including aphids, in greenhouses and open fields due to its high nutritional capacity (Franzman, 2002; Aslan and Uygun, 2005; Elekçioğlu and Şenal, 2007).

Functional response, which is one of the important components in the selection of biological control agents, is used in models of the predator-prey relationship (Jeschke et al., 2002; Lester and Harmsen, 2002). The functional response of a predator insect indicates the rate of prey consumed by the predator at varying prey densities and its effectiveness in preventing pest populations (Murdoch and Oaten, 1975). The numerical response is known as an indicator of the reproductive abilities of the predator insect at varying prey concentrations. In addition, species with a high numerical response are known to be able to control growing prey populations (Davis et al., 1976). In this study, experiments were carried out on the functional and numerical response of *H. variegata* on *M. rosae*.

Materials and Methods

The main materials of this study are rose seedlings, rose aphid (*M. rosae*) and predator insect *H. variegata*.

Supply of rose seedlings

The rose seedlings were obtained commercially and transferred to 10 liter pots containing a 1:1:1 soil:peat:perlite mixture. The seedlings transferred to the pots were left in a climate room with 27±1 °C temperature and 65±5% RH and long daylight (16:8/L:D) conditions.

Production of *Macrosiphum rosae*

The first individuals of *M. rosae* were obtained from mass production in the laboratory. Individuals of *M. rosae* were transferred to plants grown in the laboratory with the help of a sable brush and were taken to separate climate rooms to be used in the experiments. New plants were transferred to the environment and the production of pests continued periodically until the number of aphids was sufficient. All aphid productions were carried out in climate rooms with 27±1 °C temperature and 65±5% RH and long daylight (16:8/ L:D).

Production of *Hippodamia variegata*

Hippodamia variegata individuals were collected from field conditions and brought to the laboratory and then diagnosed. In the experiments, predatory insects were fed on *M. rosae* for a while (one generation). In this way, food-borne trial errors were prevented. In mass production, cages (50x50x50 cm) made of plexiglass material covered with tulle were used. The individuals used in the experiments were also obtained from this mass production. Predator production was carried out in climate rooms with 27±1°C temperature, 65±5% RH and long daylight (16:8/ L:D).

Establishment of Trial

Functional Response Experiments

At this stage of the experiments, as soon as the larvae (L1) emerged from the eggs of the predatory insects in the mass production dishes, they were taken into separate petri dishes. After this process, the larvae were starved for 24 hours and the next day, a certain number of (5, 10, 20, 40 and 80) 2nd and 3rd instar *M. rosae* were given to each larva and adults. After the prey was given, it was waited for 24 hours, and then the amount of prey consumed by the larvae and adults were counted and recorded. All of these procedures were performed for all larval stages (L2, L3 and L4) and adult individuals (female and male). These trials started with 50 eggs separately for each development period (*H. variegata*). These experiments were carried out in a climate room with a temperature of 27±1°C and 65±5% RH with long daylight (16:8/L:D). The functional response of the predator insect was calculated by the formula used by Holling (1959).

Na=TPaN/(1+aThN)

(Na: Number of prey consumed, T: Duration of keeping the predator and prey together, P: Number of predators, N: Prey density per unit area, a: Attack rate of the predator, Th: Handling time of each prey)

Numerical Response Experiments

At this stage, as soon as the larvae (L1) hatched from the eggs of *H. variegata*, they were taken into separate petri dishes. Certain numbers (5, 10, 20, 40 and 80) of 2nd and 3rd period *M. rosae* were given. All developmental stages of the predator insect were followed (L2, L3, L4, female and male) and the amount of food consumed each day was recorded and the missing ones were added. After the individuals became adults, females and males were brought together and mated. The number of eggs laid by the females was recorded. These processes continued till to the individuals died. Numerical response experiments were carried out with 50 replications. These experiments were carried out in climate rooms with a temperature of 27±1°C, 65±5% RH and long daylight (16:8/ L:D). In calculating the data obtained, the reproductive response (ECI) of female predators at different prey concentrations were calculated using the following formula:

$$\text{ECI (Efficiency of Conversion of Ingested Food)} = \frac{\text{Number of eggs laid}}{\text{Number of consumed food}} \times 100$$ (Omkar and Pervez, 2004).

Analysis of variance (ANOVA) was applied to determine the differences related to the data obtained from the functional and numerical response of *H. variegata*. The level of this significance was determined according to the TUKEY multiple comparison test, if the difference between the means was statistically significant. SPSS (Ver. 17) and Minitab (ver. 16) statistical programs were used in the analysis of the data.

Results and Discussion

Functional response of *Hippodamia variegata*

In the first phase of the study, the functional response of the predatory insect *H. variegata* on *M. rosae* at different densities was determined. Looking at the results, it was determined that the amount of food consumed increased with the development of the larval stages of *H. variegata*. It is seen that the amount of food consumed after the individuals become adults decreases compared to the fourth larval stage, and the amount consumed by male and female individuals is close (Figure 1).

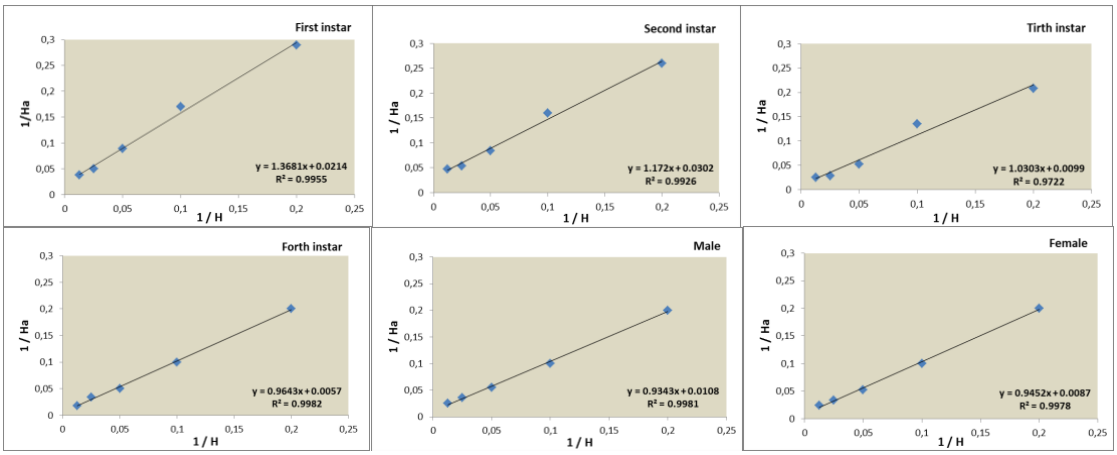

Figure 1. Functional response of *Hippodamia variegata* on *Macrosiphum rosae* at different densities

The hunting rates of *H. variegata* were also calculated. Accordingly, it has been observed that *H. variegata* individuals consume low-density foods to a large extent. Considering the data obtained, it was determined that the consumption decreased as the prey density increased. The proportional data of this are also given in Figure 2.

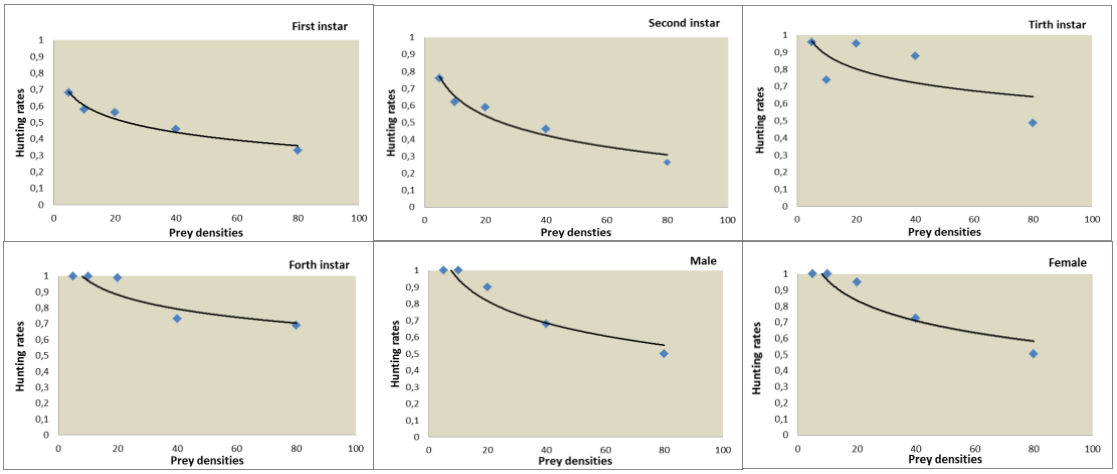

Figure 2. Hunting rates of *Hippodamia variegata* on *Macrosiphum rosae* at different densities

Using the data obtained as a result of the study, the attack rate (a) and catching times (Th) of predatory insects on different foods were calculated according to Holling (1959). Accordingly, the attack rates increased as the developmental stages of the predator insect improved. The highest value was observed in the fourth instar larva and adult individuals. When the catching time was examined, the lowest catching time was calculated in the fourth instar larva (Table 1).

Table 1. Parameters of functional response (attack rate, handling time) of *Hippodamia variegata* on *Macrosiphum rosae*

Parameters	1st instar	2nd instar	3rd instar	4th instar	Male	Female
Attack rate (*a*)	0.73	0.85	0.97	1.037	1.07	1.06
Handling time (*Th*)	0.51 h	0.72 h	0.2376 h	0.1368 h	0.26 h	0.21 h

Numerical response of *Hippodamia variegata*

In the second stage of the study, the numerical response of *H. variegata* on *M. rosae* at different densities was determined. Prey utilization efficiencies of *H. variegata* were demonstrated depending on aphid amounts at different densities. According to the data obtained, this ratio was observed to be over 90% in low prey densities (10, 20), while the efficiency was calculated as 81.1% in 40 prey densities. In the study, it was determined that the productivity was 53.3% in the highest preferred prey density (80) (Figure 3).

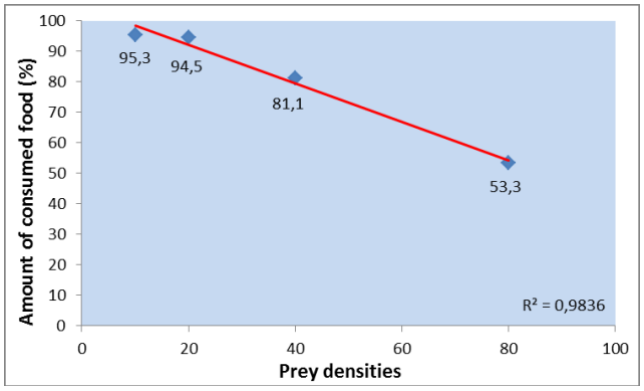

Figure 3. Amount of consumed food at different prey densities

Since the daily developments of the predatory insects were followed in numerical response experiments, the number of eggs laid by female individuals after they became adults was also calculated. Accordingly, the total number of eggs laid by *H. variegata* fed at different prey densities (5, 10, 20, 40 and 80) was calculated as 0, 68.0, 197.9, 643.6, 1224.0, respectively (Figure 4).

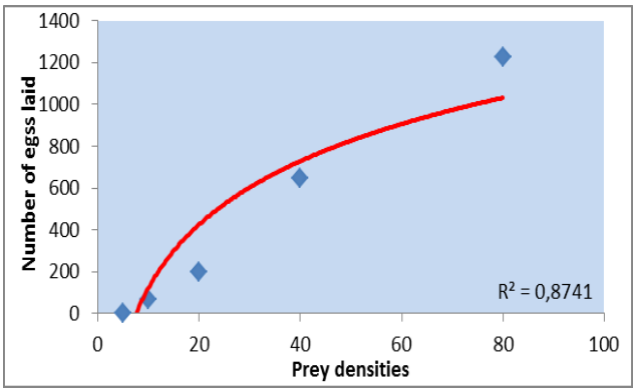

Figure 4. The number of eggs laid by female individuals at different prey densities

According to the data of the study, the reproductive responses (ECI) of female individuals of predatory insects were also determined. Looking at the data obtained, it was determined that adults did not occur at 5 prey density. In other prey densities, the reproductive response also increased as the prey density increased (Figure 5).

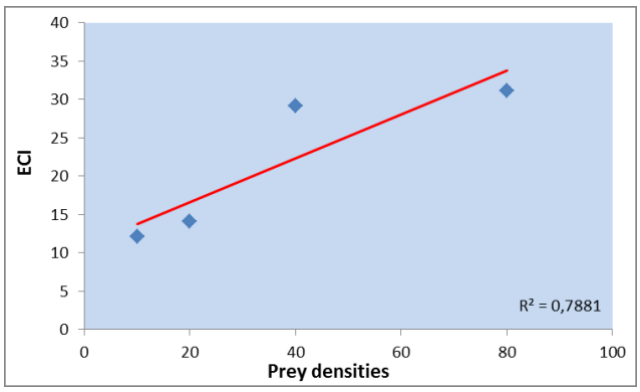

Figure 5. The reproductive responses (ECI) of female individuals of *Hippodamia variegata*

Considering the food experiments on coccinellids, it was observed that especially the last stage larvae consume a high amount of food (Bayoumy, 2011; Lee and Kang, 2014; Moura et al., 2006; Omkar and Pervez, 2004). It is thought that this is due to the need for high energy for development and the weight required for the pupal stage (Hodek and Honěk, 1996). It was reported that the food consumed in the larval stages of *Scymnus apetzi* (Mulsant) (Coleoptera: Coccinellidae) feeding on *Hyalopterus pruni* (Hemiptera: Aphididae) increased depending on the amount of prey in the environment (Kaydan and Yaşar, 1999). In a study on the same food, it was determined that consumption data of *Scymnus subvillosus* (Goeze) (Coleoptera: Coccinellidae) showed Holling's type 2 functional response (Atlıhan and Güldal, 2008). Another study examining the functional response of *Harmonia eucharis* (Mulsant) (Coleoptera: Coccinellidae) on *Aphis pomi* (Hemiptera: Aphididae), it was observed that the response obtained was of the second type. According to the data obtained in the study, it was determined that individuals in the fourth larval stage were hunted in a shorter time and in higher amounts compared to other developmental stages (Khan, 2010). According to Madadi et al. (2011) used pea aphid and cotton aphid in their study with *H. variegata* and reported that the amount of prey consumed decreased due to the intense increase in the population of these pests. Determined that the functional response type of *H. variegata* exhibited Type-II due to the decrease in the amount of food consumed. In a study on third instar nymphs of *Aphis gossypii* (Hemiptera: Aphididae), the functional response of female *H. variegata* was investigated. According to the data obtained in the study, it was reported that the ratio and viability of the remaining *H. variegata* female individuals at the end of the trials depend on the prey density (Dehkordi and Sahragard, 2013). Saleem et al. (2014) investigated the predatory effect of *Menochilus sexmaculatus* (Fab) (Coleoptera: Coccinellidae) on *M. rosae* (rose aphid) in their experiments. According to the data they obtained, it was determined that the amount consumed by the late stage larvae was higher than the other developmental stages. Zarghami et al. (2014) investigated the functional response of *Nephus arcuatus* (Kapur) (Coleoptera: Coccinellidae) on mealybug *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae). It was determined that the data obtained as a result of the study were suitable for the III Type functional response. In a different study, it was tried to determine the preference and hunting potential of *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) on cotton mealybug *Phenacoccus solenopsis* and *A. gossypii*. It has been observed that the predator insect can be effective in the biological control of both pests (El-Zahi, 2017). Bayoumy and Awadalla (2018) investigated the effects of *C. septempuctata* and *H. variegata* in the fourth period on two different foods [*Myzus persicae* Sulzer and *Aphis craccivora* Koch (Hemiptera: Aphididae)] at different densities. It was observed that the functional response type was Type-II. In a different study on the feeding of *Scymnus syriacus* (Coleoptera: Coccinellidae), the functional response of the predator insect *Aphis spiraecola* and *Aphis gossypii* (Hemiptera: Aphididae) was determined. It was determined that the response type was Type-II. In addition, it has been observed that especially the late larvae and adults of the predator insect have a high attack rate on both foods in a short time (Moradi et al., 2020). In a study on *Bemicia tabaci* (Hemiptera: Aleyrodidae) eggs of different densities, the functional response of *Delphastus catalinae* and *D. pallidus* (Coleoptera: Coccinellidae) was determined. Accordingly, it was determined that both predatory insects showed a Type-II functional response (Kumar et al., 2020). In our study, it was determined that especially the late larvae and adult individuals (male, female) of *H. variegata* reached a high attack rate on M. rosae in a short time. When the data obtained were examined, it was observed that the functional response type was Type-II, as in other predatory insects. When examined in terms of consumption amounts, it was determined that especially low-density foods were consumed quickly. In addition, as in other studies, it was determined that the consumption decreased with the increase in the prey density in the environment.

Kaydan and Yaşar (1999) studied both the numerical and functional responses of *S. apetzi* on *H. pruni*. Accordingly, they determined that the amount of food consumed in the larval stages increased depending on the amount of food in the environment. When the data obtained are examined, it is observed that there is a linear relationship between the number of prey and the amount of food consumed. In a different study, the amount of *Macrosiphum euphorbiae* (Hemiptera: Aphididae) consumed by *C. septempunctata* in larval and adult stages was determined. Accordingly, it has been observed that it consumes a high amount of food, especially in the last

period of larvae and adults (Yoldaş and Sanjrani, 1999). Stathas (2000) investigated the predator's reproductive capacity as well as the daily and total food consumption of *Rhyzobius lophanthae* (Coleoptera: Coccinellidae) feeding on *Aspidiotus nerii* (Hemiptera: Diaspididae). According to the data obtained, it was determined that the consumption amount of the larvae, especially in the last period, increased. In addition, he reported that female individuals consume more food than males. In addition to these studies, the effects of different predator insects that share the same habitat with coccinellids on aphids were determined. Accordingly, it was observed that the amount consumed by predator insects as their larval stages progressed (Atlıhan et al., 2004; Huang and Enkegaard, 2010; Batool et al., 2014; El-Zahi, 2017; Rana et al., 2017; Kayahan, 2020). Considering the results obtained from the numerical response experiments in our study, it was determined that the efficiency of prey consumption was high at low densities, as in the literature, and the consumption increased as the density increased. However, when these data are evaluated in terms of catch use efficiency, the opposite is the case. In other words, it was determined that the consumption efficiency decreased with the increase in the prey density. Yaşar and Özger (2005) determined the development, feeding and reproductive responses of *Adalia fasciatopunctata revelierei* (Mulsant) (Coleoptera: Coccinellidae) on *H. pruni* in their study. When the egg laying numbers of the predator insect were examined, it was determined that this number increased in direct proportion to the prey density. In another study, the effect of *Nipaecoccus viridis* (adult females and 1st, 2nd and 3rd instar nymphs of *N. viridis*) on the numerical response of *Nephus arcuatus* (Coleoptera: Coccinellidae) at different periods and at different intensities was investigated. According to the results they obtained, it was determined that as the prey density increased, the number of eggs laid by the females increased nonlinearly (Ramezani and Zargami, 2017). Considering the studies on different predator insects, it has been determined that there is an increase in the daily and total number of eggs given depending on the increase in the prey density in the environment (Ambrose and Claver, 1997; Atlıhan et al., 2004; El-Zahi, 2017, Fathipour et al., 2020; Kayahan, 2020). In our study, it was observed that the number of eggs laid by *H. variegata* increased with the increase in prey density. One of the results of numerical response studies is the Reproductive Response (ECI). According to the results obtained in our study, it was determined that the reproductive response increased as the prey density increased depending on the number of eggs laid. Considering the studies on aphids, it is reported that there is a linear increase in the reproductive response value, especially depending on the aphid density (Khan and Zaki, 2008; Kayahan, 2020).

Conclusion

When the results obtained from functional and numerical response experiments were examined, it was observed that *H. variegata* was effective on *M. rosae*. Accordingly, it is thought that the predator insect may be effective in controlling the population of this type of pest. However, since this study is a laboratory study, it was concluded that experiments should be carried out in field conditions in order to obtain more efficient results. For this reason, it is very important to reveal the numerical response of the species in determining the impact power of a predator that has an effect on aphids.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Present situation of agricultural information needs and accessibility of women farmers in Imbulpe DS division in Sri Lanka

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Abstract
Agricultural information and accessibility of women farmers are more crucial for enhancing food production. Thus, the main objectives of this research were to study the present situation of the agricultural information needs and accessibility of the women farmers in Imbulpe Divisional Secretariate (DS) Division of the country. Out of the women farmers of the study area, 238 were selected from the simple random sampling method as the sample for the study. A pre-tested, interviewer-administered questionnaire survey was used as the primary data collection method from March to July 2019. Descriptive statistics and chi-square analysis were used as the data analyzing methods. Based on the findings, women farmers showed that, they need more information regarding improved crop varieties, application of agrochemicals, new cropping systems and irrigation systems. Furthermore, most of the respondents showed that the higher level of accessibility to agricultural information on improved crop varieties. However, a lower level of agricultural information accessibility on suitable storage facilities was observed. Extension agents and successful women farmers act as their major sources of agricultural information. Furthermore, ICT equipment act as the least important agricultural information source. Age, marital status, educational level and monthly income were shown as the considerably higher associatiative variables with the agricultural information accessibility than the size of the farmland and farming experience of them. However, farmland size represented a moderate level of statistically significant positive association with the accessibility of agricultural information for the women farmers. Therefore, agricultural information accessibility can be enhanced by organizing awareness programs and extension service for the women farmers. Moreover, enhancement of accessibility for market information through the ICTs and encouragement of women farmers' participation in the farming societies of Imbulpe area will be very important to develop the agricultural information accessibility furthermore.

Keywords
Agricultural information, Imbulpe, Information accessability, Sri Lanka, Women farmers

Introduction
Women constitute about 50% of the global population and they act as the co-builders of civilization. However, still they are underprivileged in many countries in the world, especially in developing countries (World Bank, 2021; Rahman et al., 2007).

In most of the developing countries, women's contribution has been hidden for the development of the country. Based on the sustainable development goals, gender equity or women empowerment was aimed to achieve the sustainable development in developing countries of the globe (United Nations, 2015).

Women farmers perform most of their farming practices on a traditional basis; pre-planting, planting and post-harvest activities due to the increment of men participation in the industrial sector and service sector (International Labor Organization-ILO, 2018;

Ibharhokanrhowa, 2016; Malkanthi, 2016). However, in most of the developing countries, rural women still play a significant role in the agricultural sector. Meanwhile in developed nations, the agricultural operations are mechanized and women farmer's involvement is obtained relatively at a lower level (Ibharhokanrhowa, 2016).

With the development and modernization of societies, male counterpart are having better opportunities in enhancing their capabilities in terms of education, accessibility to modern farming technologies as well as access to agricultural information (FAO, 2018). Because, women farmers have to perform domestic activities, child caring and family welfare activities as a mother. So they have poor level of accessibility to join social networks and lower level of accessibility to monetary facilities than male farmers. Andalso, male farmers have a higher level of accessibility for agricultural information and participation in different extension programs (Bahadurghartimagar, 2011). Gender-differences affect for the use and accessibility of agricultural information sources (Godwin et al., 2018).

Most of the developing countries are suffering from gender inequality which is one of the main factors comes under the sustainable development goals. It causes to increase in the knowledge barrier for the women farmers (Rathnachandra & Malkanthi., 2020; Mojaki & Keregero., 2019). In Sri Lanka, women represent about 14% share of economically active population (Madurawala, 2018; Annual labor Force Reports, 2017). Also, women's contribution to the agricultural sector is gradually increasing in the country. Moreover, most of rural women has contributed their labor in the agricultural sector rather than the industrial sector and service sector (Annual Labor Force Reports, 2017).

Genarally Imbulpe DS Division is a farming area and most of the women are engaging in farming activities more or less similar way to men (Census and Statistics of Agriculture base Report –Rathnapura District, 2013/14). It has 50 GN divisions and is located under the administrative distribution of Sabaragamuwa Province. Based on the statistics of the Imbulpe divisional secretariat office, while the avarage monthly income of most of the people is around LKR 20,000-30,000, a higher percentage of income represent by agricultural activities.

Access to reliable, timely and relevant knowledge and necessary information can help significantly in many ways to reduce farmers' risk and uncertainty, enabling them to make good decisions in farming activities. But, there is a considerable level of knowledge and skill gaps and difficulties obtain in reaching their agricultural information needs. It is clare that accessibility of agricultural information is supported to build up higher women farmer capacity and upward their level of empowerment (Velde et al., 2020). Thus, this study was conducted to assess the women farmers' agricultural information needs and accessibility in Imbulpe DS division and also, the impact on socio-economic factors of the women farmers to the accessibility of agricultural information. The specific objectives were to identify the areas of agricultural information needs, level of agricultural information requirement for the women farmers, to determine the accessibility of required agricultural information by women farmers, to identify the sources of agricultural information and to determine the relationship between socio-economic factors of women farmers and their accessibility for agricultural information within the study area.

Research Methodology

Imbulpe DS Division is a rural farming area, situated in Rathnapura district in Sabaragamuwa province of Sri Lanka. In this area, a considerable percentage of male counter part have moved to urban areas searching for jobs in the industrial sector. Therefore, most of the women have to do both agricultural activities and also household activities simultaneously. Out of the fifty GN divisions of this area, seven GN divisions were purposively selected for the study namely; Halpe, Seelogama, Kinchigune, Puwakgahawela and Muttettuwegama, Imbulpe and Karagastalawa.238 women farmers were randomly selected as the respondents of the study to minimize the samping error, from the women farmers who registered under the Agrarian Service Center of the study area. A pre-tested, interviewer-administered questionnaire survey and focus group discussion was conducted as the method of primary data collection from March to July 2019. Descriptive statistics and chi-square analysis were used for the data analyzing process.

The areas of agricultural information needs were identified through the findings of the piolet study perior to the data collection process. These agricultural information needs were ranked by the respondents while conducting the questionnaire survey. In analyzing, the required level of accessibility of agricultural information was measured by ranking their information needs as high (3), moderate (2) and low (1) based on the study of Ikuakam et al., 2016.The weighted average was calculated for each information needed area, to assess the level of agricultural information needed by women farmers in the study area. Agricultural information accessibility was determined by ranking their requirement of agricultural information needs. In addition to that, the sources of agricultural information were analyzed through the ranking of a provided list of selected agricultural information sources based on the findings of the pilot study. The Chi-square analysis was used to discover the relationship between socio-economic factors of women farmers and accessibility of agricultural information within the study area for the further data analyzing process.

Results and Discussion

Socio-economic factors of the respondents

Important socio-economic factors of women farmers were studied. Findings are presented in Table 1.

Table 1. Socio-economic factors of respondents (n=238)

Factor	Category	Frequency	Percentage (%)
Age (Years)	20-39	40	16.8
	40-59	149	62.6
	60-79	49	20.6
Marital status	Single	09	3.8
	Married	215	90.3
	Other	14	5.9
Educational level	No Primary education	08	3.4
	Primary education	68	28.6
	Junior secondary education (O/L)	153	64.3
	Senior secondary education (A/L)	09	3.8
Monthly income (LKR)	Less than 20,000	61	25.6
	20,001 – 40,000	156	65.5
	40,001 – 60,000	21	8.8
Number of family members	less than 3	79	33.2
	3 - 5	128	53.8
	more than 5	31	13.0
Farmland size (Acre)	0.0 -0.5	17	0.71
	0.5-1.0	158	66.4
	1.0-1.5	50	21.0
	1.5-2.0	13	05.5
Farming experience (Years)	0-5	12	05.4
	5-10	64	26.9
	10-15	140	58.8
	15-20	24	10.1

Source: Field survey March to July 2019

Based on the results of table 1, majority of women farmers (62.6%) was in between 40 -59 years in their age. It is clear that, women farmers in this age category is able to do farming well based on their farming experiences. And also, most of the them were belonged to the economically active population. Furthermore, in this study, 90.3% of women farmers were married and the majority of them (64.3%) have studied upto junior secondary education (GCE Ordinary Level). However, 3.4% of respondents have not primary education either. Among them, only 3.8% share of women farmers have senior secondary education. According to FAO (2014), if women farmers have a considerable level of education, there is a potential to access and adoption of modern farming technologies, access to credit facilities as necessary and also the agricultural information needs and accessibility. Nevertheless, most of the women farmers reported that they have 3-5 members in their families. Moreover, their average farm size was 0.84 acres and the average level of farming experience is 15 years. While 65.5% of women farmers have received LKR 20,001 – 40,000 as their monthly income, 25.6% of them have received only LKR 20,000 as their monthly income. It is a low level of monthly income. So, it expresses the importance of agricultural information needs and accessibility of them within this area.

Agricultural information needs of women farmers in the area

The main areas of agricultural information needed by women farmers were studied well. The findings are presented in Table 2.

According to the findings in table 2, majority of the women farmers (57.7%) mentioned that they need a higher level of information regarding improved crop varieties. And also they informed that information about the application of agrochemicals (55.3%), new cropping systems (47%) and irrigation systems (45.1%) are very important in farming. However, they were less interested in the information on improved livestock varieties (18.6%) and suitable storage facilities (12.7%). Women farmers were showed that they need moerate level of information regarding modern farming technologies and improved market systems. Because, most of the rural farmers are engaged in the small-scale farming rather than large-scale commercial farming operations (Ranachandra & Malkanthi., 2020). In addition to that, they have a moderate level of education and poor awareness regarding using of ICT equipment for the accessibility of agricultural information (Ranachandra, 2020). A similar findings has reported by Rahman et al., 2020 and Chikaire et al.,2015 in their research studies regarding the subsistence farming practices of women farmers and they do not use ICTs to the accessibility of agricultural information due to lower

level of awareness about ICT usage of women farmers. Thus, there is a requirement of a considerable level of agricultural information about modern farming technologies (Rathnachandra, 2020). Murage et al., 2016, have also reported that in their study, if women farmers have poor level of education, it leads to low level of awreness on modern farming technologies. Andalso, women farmers have considerabally lower level of education, poor access to modern farming technologies, constraints to access financial facilities and agricultural information sources (Rathnachandra & Malkanthi, 2020).

Levels of agricultural information accessibility of the women farmers

Required levels of agricultural information needs of women farmers was categorized into three levels namely; high, moderate and low. Findings of the level of agricultural information needed by women farmers in this area are presented in Table 3.

Table 2. Areas of agricultural information needed by the respondents (n = 238).

Areas of information need	Frequency	Percentage (%)
Irrigation methods	114	45.1
Improved market systems	80	33.6
Application of agrochemicals	140	55.3
Improved livestock varieties	44	18.6
Modern farming technologies	54	22.5
New cropping systems	112	47.0
Improved crop varieties	146	57.7
Suitable storage facilities	30	12.7

Source: Field survey March to July 2019

Table 3. Level of agricultural information accessibility by respondents (n=238)

Level of need	Frequency	Percentage (%)
High	144	60.5
Moderate	71	29.8
Low	23	09.7
Total	238	100.0

Source: Field survey March to July 2019
(Categorizations based on the Ikwuakam et al.,2016)

As per the results shown in table 3, most of the respondents (60.5%) indicated that they have higher accessibility of agricultural information on improved crop varieties while 29.8% had a moderate levels of agricultural information access regarding the modern farming technologies and improved market systems in the study area. Lower level (9.2%) of agricultural information access was shown in suitable storage facilities and improved livestock varieties.

Sources of agricultural information used by women farmers

Possible sources of agricultural information in this area were identified and their application by women farmers was studied in detail. Piolet study was indertaken to identify the agricultural information sources: extension agents, ICT equipment, contact farmers and fellow women farmers. Results are presented in Table 4.

Table 4. Sources of agricultural information used by the women farmers (n = 238).

Source	Frequency	Percentage (%)
Extension agents	114	47.9
ICT equipment (phones, internet etc)	12	5.1
Contact farmers	37	15.5
Fellow women farmers	69	29.0
Other sources	06	2.1

Source: Field survey March to July 2019

Based on the results of table 4, the majority of the respondents mentioned that extension agents (47.9%) and fellow women farmers (29%) as their main sources of agricultural information. However, contact farmers and ICT equipment have acted as less important sources of agricultural information within the study area. Newspapers, farming societies and other family members were noted as the other sources (2.1%) of agricultural information needed of the respondents. As Imbulpe area is a rural area, it consists of rural culture

and usage of ICT for getting information regarding the modern farming technologies is at a very low level. They have a better relationship with the extension agents of the area. As there are female extension agents, they try to disseminate agricultural information according to the requirements and empower women farmers to upgrade their capabilities within the agricultural sector.

Relationship between socio-economic factors of women farmers and accessibility for agricultural information

Firstly, a comparition between socio-economic factors and agricultural information accessibility was conducted. The results are presented in Table 5. In order to identify the relationship between socio-economic factors of women farmers and accessibility to agricultural information, a hypothesis was tested using a chi-square analysis.

($H_{0\ a}$) There is no significant relationship between age of the women farmers and the accessibility of agricultural information.

($H_{0\ b}$) There is no significant relationship between marital status of the women farmers and the accessibility of agricultural information.

($H_{0\ c}$) There is no significant relationship between educational level of the women farmers and the accessibility of agricultural information.

($H_{0\ d}$) There is no significant relationship between monthly income of the women farmers and the accessibility of agricultural information.

($H_{0\ e}$) There is no significant relationship between farmland size of the women farmers and the accessibility of agricultural information.

($H_{0\ f}$) There is no significant relationship between farming experience of the women farmers and the accessibility of agricultural information.

The dependent variable was agricultural information accessibility and the selected socio-economic factors of the women farmers were utilized as the independent variables of the study. Agricultural information accessibility was measured through the ranking scale of their access as low, moderate and high. Results are presented in Table 6.

Table 5. Comparison between socio-economic factors and agricultural information accessibility

			Agricultural information accessibility		
Factor	Category	Low	Moderate	High	Total
Socio-economic factors					
Age	20-39 Years	0	0	40	40
	40-59 Years	0	45	104	149
	60-79 Years	23	26	0	49
	Total	23	71	144	238
Marital status	Single	0	0	10	10
	Married	9	71	135	215
	Widowed	13	0	0	13
	Total	23	71	144	238
Educational level	No Primary education	0	8	0	8
	Primary education	0	68	0	68
	Junior secondary education (O/L)	14	71	68	153
	Senior secondary education (A/L)	0	0	9	9
	Total	14	147	77	238
Monthly income (LKR)	Less than 20,000	0	61	0	61
	20,001 – 40,000	2	71	83	156
	40,001 – 60,000	0	0	21	21
	Total	2	132	104	238
Farm land size (acre)	0 - 1	22	65	124	211
	1.1 - 2	1	6	15	22
	2.1 - 3	0	0	5	5
	Total	23	71	144	238
Farming Experience (Years)	5 - 9	0	5	10	15
	10 - 14	0	14	28	42
	15 - 19	23	67	106	181
	Total	23	81	134	238

Source: Field survey March to July 2019

Results of table 5 reveals that women farmers who are in between 20-39 and 40-59 years of age range have a higher level of agricultural information accessibility. While a moderate level of agricultural information accessibility was received by women farmers of 60-79 years. A lower level of information accessibility was received by widowed respondents. Because, most of the widowed respondents were belongs to 60-79 years. Widowed farmers are less aware of the various sources of agricultural knowledge and most of them have not

considerable level of education. However, married women farmers receive both the moderate and higher level of agricultural information accessibility. Because, married women farmers have various responsibilities regarding child caring, domestic activities etc. Therefore, sometimes married women farmers can receive only a moderate level of agricultural information accessibility. In addition to that, most of the married respondents are in 20-59 years age and they have higher level of agricultural information accessibility. However, most of the married women farmers who are under 20-59 years age have an adequate level of education and awareness about the usage of ICT tools and apply them for agricultural purposes. Similar findings have been received by Narmilan et al., 2020 in their research study. Furthemore, women farmers who had education upto senior secondary level, have higher level of agricultural information accessibility. Moreover, moderate level of agricultural information accessibility obtained women farmers who educated up to junior secondary level and respondents who educated below the junior secondary level, showed lower agricultural information accessibility. Furthermore, respondents who have monthly income of LKR 20,001-40,000 showed a higher level of agricultural information accessibility and moderate level of agricultural information accessibility was showed respondents who received below LKR 20,000 as their monthly income. Also, it was shown that when increase the size of the farmland, there was a higher accessibility for agricultural information. Because, higher farmland size represent the commercial scale farming and they have higher agricultural information accessibility than subsistence farmers (Debonne et al., 2021). Higher farming experience was indicated higher agricultural information accessibility and when farming experience obtained at low, their agricultural information accessibility remain as lower.

Chi-square analysis of the socio-economic factors of the women farmers and accessibility of agricultural information

The results of the chi-square analysis of socio-economic factors of the women farmers and accessibility of agricultural information are presented in Table 6.

Table 6. Chi-square analysis of the relationship between socio-economic factors of the respondents and agricultural information accessibility.

Socio-economic factor	Cramer's V value	p value	Contigency co-efficient
Age	0.564	0.00	0.62
Marital status	0.718	0.00	0.78
Educational level	0.741	0.00	0.79
Monthly income	0.721	0.00	0.72
Farm land size	0.498	0.04	0.57
Farming experience	0.190	0.07	0.26

Source: Field survey March to July 2019

As per the results of table 6, age, marital status, educational level and monthly income showed a considerably higher level of cramer's v values and contingency co-efficient values than the size of the farmland and farming experience of the women farmers in the study area. Also, the p values of the above mentioned variables (age, marital status, educational level and monthly income) are less than 0.05. Therefore age, marital status, educational level and monthly income are the strongly associated socio-economic variables with agricultural information accessibility. Moreover, the size of the farmland showed a moderate level of association with the agricultural information accessibility of women farmers in the study area.

Hence,

$H_{0\,a}$ *rejected*

$H_{o\,b}$ *rejected*

$H_{o\,c}$ *rejected*

$H_{o\,d}$ *and* $H_{o\,e}$ *rejected*

Findings of the study of Rehman et al (2013) showed that the educational level of the respondents creates a considerable impact on agricultural information accessibility. However, farming experience have not shown a significant relationship with agricultural information accessibility. According to the findings of the Okwu and Umoru (2019) age, education and income level of the women farmers obtain a significant relationship with the agricultural information accessibility in Nigeria. Based on the findings of the Akinola (2017), age, marital status, gender, educational level, monthly income, farmland size and farming experience show a significant relationship with the agricultural information accessibility.

Conclusion

According to the findings of the study, several conclusions can be drawn. Most of the respondents need the information about improved crop varieties. In addition to that, information related to the application of agrochemicals, new cropping systems and irrigation systems related information are also more important for them.

However, they are less interested related to the information about improved livestock varieties and suitable storage facilities. Most of them are engage in small-scale farming rather than large-scale farming operations.

The majority of the women farmers are willing to get agricultural information from extension agents. Since women extension officers are there, women farmers have a very close contact with them. Fellow women are also doing a great job in this regard. In addition to that, contact with better farmers provides a considerable level of agricultural information. ICT equipment act as a less important agricultural information source in this area. Newspapers, women farmers' husbands, farming societies and other family members are also somewhat important sources of agricultural information in this area.

Age, marital status, educational level and monthly income are showing a considerably strong positive association between agricultural information accessibility of women farmers in the study area. However, farmland size represents a moderate level of statistically significant positive association with the agricultural information accessibility of the women farmers.

Recommendations

Dissemination of timely important agricultural information within the study area and pursuation of them to share the farming experiences with fellow women farmers, contact farmers and other family members.

Motivation of Pursuant women in order to act as agricultural extension agents in order to work with women farmers for efficient information sharing and improving the social networks.

Provision of awareness programs related to use of ICT equipment and also dissemination of the latest information on agriculture for the women farmers

Encouragement of women farmers' participation in the farming societies of this area.

Compliance with Ethical Standards

Conflict of interest

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

Author contribution

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

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

Ethical approval

Ethics committee approval is not required.

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